

CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

April 1, 2011 to March 31, 2012

BREEDING CRISPHEAD AND LEAFY LETTUCE

Richard W. Michelmore

Oswaldo E. Ochoa

María José Truco

The Genome Center and
The Department of Plant Sciences
University of California, Davis

rwmichelmore@ucdavis.edu

mjtruco@ucdavis.edu

oeochoa@ucdavis.edu

SUMMARY

The program continues to emphasize the identification and incorporation of genes for disease resistance, particularly to downy mildew, *Verticillium* and *Fusarium* wilts, corky root, lettuce mosaic virus, and anthracnose, into crisphead and four leaf horticultural types suitable for California. Resistance for downy mildew is being introduced from several new sources and combined with resistance to lettuce mosaic virus and corky root. We have continued to monitor variation in the ability of the downy mildew pathogen to overcome resistance genes. Of the known genes for resistance to downy mildew, only *Dm17* remains effective against all of the California isolates tested. Utilization of multiple new sources will minimize the chances that changes in the pathogen will render all cultivars susceptible simultaneously. Genetic studies are in progress to determine the genetic basis for the resistances and to identify molecular markers to increase the efficiency of generating resistant varieties.

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PROJECT TITLE: **BREEDING CRISPHEAD AND LEAFY LETTUCE**

PRINCIPAL INVESTIGATORS: **Richard W. Michelmore**
Oswaldo E. Ochoa
María José Truco
The Genome Center and
The Department of Plant Sciences
University of California, Davis
rwmichelmore@ucdavis.edu
oeochoa@ucdavis.edu
mjtruco@ucdavis.edu

COOPERATING PERSONNEL: **Miguel Macias Gonzalez**
The Genome Center and
The Department of Plant Sciences
University of California, Davis
Ryan Hayes
Ivan Simko
UDSA-ARS, Salinas
Krishna Subbarao
Thomas Gordon
The Department of Plant Pathology
University of California, Davis
Steve Koike
Richard Smith
Michael Cahn
UC-Cooperative Extension, Monterey County

OBJECTIVES:

- 1) To identify new genes for disease resistance in wild germplasm and incorporate multiple genes from diverse sources into advanced breeding lines to achieve durable resistance.
- 2) To monitor variation in pathogen populations, particularly downy mildew, to facilitate the deployment of effective resistance genes.
- 3) To determine the genetic basis of agriculturally important traits, particularly disease resistance.
- 4) To release advanced breeding lines which have resistance to multiple diseases, superior appearance and quality, high yielding ability, uniform maturity, and are slow bolting.

PROCEDURES AND RESULTS:

Development of Disease Resistant Lines

Breeding Strategy

The strategy used for the UC Crisphead and Leafy Lettuce Program is to initiate crosses and early generations at Davis; later generations are being trialed and selected at several different lettuce-growing areas in collaboration with USDA, Cooperative Extension in Salinas and California growers. Backcross or modified single-seed descent strategies are being employed for most of the early generations. We are selecting for type, color, slow bolting, and yield as well as disease resistance in the crisphead and the four leaf lettuce plant types. As far as possible we use different sources of resistance for each plant type. This will diversify the selection pressure on the pathogen. The use of multiple sources of resistance will tend to increase the longevity of each resistance gene and decrease the chances that a single change in the pathogen will render multiple lettuce types susceptible.

When resistant advanced lines of the desired plant type have been generated for individual resistances, they will be intercrossed to create lines with multiple disease resistances for lettuce downy mildew (LDM), corky root (CR), anthracnose (ANT) and lettuce mosaic virus (LMV). Additionally we are screening germplasm to identify genetic resistances for *Verticillium* and *Fusarium* wilts, which will be incorporated into the breeding program.

Cultivars representing each type were selected to be the recurrent parents in backcross programs to introgress resistance genes based on their horticultural type and performance in California, their status in the public domain, and the presence of additional disease resistance genes. We are now using Salinas, Green Towers, Tropicana, Red Fox, Red Tide, and Margarita for the crisphead, romaine, green leaf, red leaf, and butterhead programs, respectively (Tables 1 and 3). We welcome suggestions and further input on the field performance of these and other potential recurrent parents. The recurrent parents used in the final generations of backcrossing will be adjusted to reflect the industry standards at the time.

Four crisphead advanced breeding lines with novel resistance to LDM are being released (Table 2 and Appendix). A romaine advanced breeding line is also being prepared for release (Table 3).

Sources of Downy Mildew Resistance

We are continuing to develop crisphead and leafy coastal lettuce lines with resistance genes from diverse sources to provide protection against downy mildew in California. This year seventeen additional resistant accessions were identified from Israel and Armenia. We are focused on generating advanced breeding lines with new resistance genes identified through germplasm screens in previous years (Tables 1 and 3). Mapping and characterization of the new sources are in progress. Two new resistance genes from *L. serriola* (R48, R49) have been identified from germplasm provided by Ales Lebeda (Department of Botany, Faculty of Natural Sciences, Palacky University, Czech Republic). *L. saligna* acc. UC04US2509 has been characterized genetically as containing a new resistance gene, *Dm45*. Backcrossing programs to introgress the next generation of genes for resistance to LDM into crisp and leafy types have been initiated (Tables 1 and 3).

Table 1: Status of introgression of novel sources of LDM resistance into crisphead lettuce types.

	Source		Donor Species	Status	R gene
Early	UC04US2509	UC	<i>L. saligna</i>	To be released	45
	UC04UK2507	UC	<i>L. virosa</i>	Trial 2011	
New	UC07CS100	LEB-	<i>L. serriola</i>	BC4	48
	UC07CS101	LEB-	<i>L. serriola</i>	BC4	49
	UC07CS102	LEB-	<i>L. serriola</i>	BC4	
	UC07CS103	LEB-	<i>L. serriola</i>	BC4	
	UC07CS104	LEB-	<i>L. serriola</i>	BC3	
	UC07CS105	LEB-	<i>L. saligna</i>	BC3	
	UC07CS106	LEB-	<i>L. saligna</i>	BC3	
	UC07CS107	LEB-	<i>L. saligna</i>	BC3	
	UC07CS108	LEB-	<i>L. saligna</i>	BC3	
	UC07CS109	LEB-	<i>L. saligna</i>	BC3	
New	8 accessions	Israel	<i>L. saligna</i>	F1	
	4 accessions	Israel	<i>L. acuelata</i>	F1	
	5 accessions	Armeni	<i>L. serriola</i>	BC1	

Table 2: Releases of four crisphead BC₇S₂ advanced breeding lines.

	Source		Donor Species	Status	R gene
UC12100	CGN9311	CGN	<i>L. saligna</i>	Release	?
UC12101	CGN5318	CGN	<i>L. saligna</i>	Release	?
UC12102	CGN5282	CGN	<i>L. saligna</i>	Release	?
UC12103	CGN5147	CGN	<i>L. saligna</i>	Release	?

? Characterization and marker development in progress.

Downy mildew virulence surveys

In order to ensure that we are breeding for resistance against virulence phenotypes of the pathogen currently present in California, we have continued to sample the downy mildew pathogen on an opportunistic basis with the collaboration of growers, the seed industry and extension personnel, particularly Steve Koike. Over the past year, 50 isolates of *B. lactucae* originating from several regions in California (Table 4) were characterized for virulence phenotype and a subset of them were characterized for fungicide sensitivity and mating type (Table 5). This opportunistic sampling gives an indication of the diversity of *B. lactucae* isolates in the field but does not provide a quantitative measure of LDM variation.

Forty-two % of the samples analyzed in 2011 were CAVIII isolates; 32% were novel types and 26% were pathotype CAVII. California pathotypes V and VI were not detected in 2011 (Figure 1).

Table 3: Status of introgression of novel sources of LDM resistance into leafy lettuce types.

	Type	Donor line	Donor specie	Status
Advanced breeding lines	romaine	00G950	<i>L. saligna</i>	BC ₇ S ₁ to be released
	romaine	CGN5322	<i>L. saligna</i>	BC ₄
	red leaf	05G1411	<i>L. serriola</i>	BC ₅
	red leaf	PI509523	<i>L. saligna</i>	BC ₃
	green leaf	PI49100	<i>L. saligna</i>	BC ₇ S ₁ to be field trialed
	butterhead	05G1421	<i>L. serriola</i>	BC ₃
	butterhead	CGN13330	<i>L. saligna</i>	BC ₂
Early breeding lines	romaine	09G1077	<i>L. saligna</i>	F ₂ & BC ₂
	romaine	09G1080	<i>L. saligna</i>	BC ₃
	red leaf	09G1082	<i>L. saligna</i>	BC ₃
	green leaf	LB line	<i>L. saligna</i>	F ₂
	romaine	11G1058	<i>L. aculeata</i>	BC ₁
	red leaf	11G1057	<i>L. aculeata</i>	BC ₁
New donors	Israel	5 lines	<i>L. saligna</i>	to be crossed
	Israel	5 lines	<i>L. aculeata</i>	to be crossed
	Armenia	5 lines	<i>L. serriola</i>	to be crossed

Avr17 increased again to a frequency of 100 % of isolates in 2011. With the exception of 2010, *Avr17* has been detected in all isolates characterized over the last seven years, indicating that *Dm17* remains highly effective; it has not, however, been widely deployed and so has not been under extensive selection pressure. *Avr36*, *Avr37* and *Avr38* were present in 70, 75 and 90 % of the isolates respectively. The frequency of *Avr18* was less than 15%. *Avr4* was detected at a frequency less than 30%. Among the novel isolates, *Avr2*, *Avr3* and *Avr6* were all detected at frequencies of below 30 % (Figure 2). This is interesting because until recently avirulence to *Dm2*, *Dm3* or *Dm6* was very rarely detected in California. The presence of *Avr2*, *Avr3* and *Avr6* may indicate that the recently identified novel isolates have originated from different source(s) than isolates characterized in previous years.

Among the 2011 isolates analyzed for mating type, four were mating type B₁. Two of these isolates had novel virulence phenotypes, another was pathotype CAVII and the fourth was pathotype CAVIII. The presence of isolates of the B₁ mating type opens up the possibility of greater variation in LDM due to sexual reproduction as well as survival in the soil as oospores.

Of the 38 isolates tested for sensitivity to fungicides this year, 19 isolates were insensitive to Alliette and 26 were insensitive to Ridomil. One of these 19 had the B₁ mating type; this isolate was insensitive to Ridomil but sensitive to Alliette, indicating chemical control of this isolate is possible.

Table 4. Origin of *B. lactucae* isolates characterized in 2011.

Isolate ID	Collection	Location	Collector	On:
2011	Date			
C11O1311	1/12/11	UC Davis, Screenhouse	OOchoa	L.serriola
C11O1312	1/12/11	UC Davis, Screenhouse	OOchoa	L.serriola
C11O1313	1/12/11	UC Davis, Screenhouse	OOchoa	L.serriola
C11O1314	1/12/11	UC Davis, Screenhouse	OOchoa	L.serriola
C11O1315	1/12/11	UC Davis, Screenhouse	OOchoa	L.serriola
C11O1316-A	3/30/11	San Luis Obispo, Ca	?, Sent to Anna L Fabrilus	?
C11O1316-B	3/30/11	San Luis Obispo, Ca	?, Sent to Anna L Fabrilus	?
C11O1317	3/30/11	Genefresh?	S. Koike	Gunslinger
C11O1318	3/30/11	Genefresh?	S. Koike	Rifleman
C11O1319	3/30/11	Genefresh?	S. Koike	W124402
C11O1320	4/5/11	Salinas, Ca	J. Heintzberger	Breeding Material
C11O1321-A	5/3/11	King City	George Manos, Shamrock seeds	red romaine, Showdown
C11O1321-B	5/3/11	King City	George Manos, Shamrock seeds	red romaine, Showdown
C11O1322-A	5/4/11	King City	George Manos, Shamrock seeds	Lolarose type, Zion
C11O1322-B	5/4/11	King City	George Manos, Shamrock seeds	Lolarose type, Zion
C11O1323	5/26/11	?	S. Koike	San Lucas Lolla rose
C11O1324	5/26/11	?	S. Koike	?
C11O1325	5/26/11	?	S. Koike	?
C11O1326	5/26/11	?	S. Koike	?
C11O1327	6/13/11	?	S. Koike	Gunslinger
C11O1328	6/13/11	?	S. Koike	Gunslinger
C11O1329	6/22/11	?	S. Koike	?
C11O1330	6/22/11	?	S. Koike	?
C11O1331	6/22/11	?	S. Koike	?
C11O1332	8/3/11	Davis	OOchoa	KB RILs
C11O1333	8/3/11	Davis	OOchoa	KB RILs
C11O1334	8/3/11	Davis	OOchoa	KB RILs
C11O1335	8/3/11	Davis	OOchoa	KB RILs
C11O1336	9/6/11	USDA, Salinas	I. Simko	IS-S11-1
C11O1337	9/6/11	USDA, Salinas	I. Simko	IS-S11-2
C11O1338	9/6/11	USDA, Salinas	I. Simko	IS-S11-3
C11O1339	9/6/11	USDA, Salinas	I. Simko	IS-S11-4
C11O1340	8/8/11	Castroville,	J. Heintzberger	Steamboat
C11O1341	10/3/11	Rogers Ranch, Moss Landing, Ca ?	P. Serrial	?
C11O1342	10/3/11	Massa Ranch, Moss Landing, Ca ?	P. Serrial	?
C11O1343	10/11/11	San Ardo, Ca	J. Heintzberger	Regency
C11O1344	10/11/11	San Ardo, Ca	J. Heintzberger	Black Bell
C11O1345	10/13/11	?	S. Koike/ Genefresh	Sample 1?, Dm17 ?
C11O1346	10/13/11	?	S. Koike/ Genefresh	Sample 2?
C11O1347	10/13/11	?	S. Koike/ Genefresh	Sample 3?
C11O1348	10/13/11	?	S. Koike/ Genefresh	Sample 4?
C11O1349	10/13/11	?	S. Koike/ Genefresh	Sample 5?
C11O1350	10/13/11	?	S. Koike/ Genefresh	Sample 6?
C11O1351	10/13/11	?	S. Koike/ Genefresh	Sample 7?, Dm17 ?
C11O1352	11/4/11	?	M. Hughes, Syngenta	Cerini, spring Mix
C11O1353	11/4/11	?	M. Hughes, Syngenta	Paulita, Spring Mix
C11O1354	11/9/11	Spence(?), USDA	I. Simko	Iceberg
C11O1355	11/9/11	Spence(?), USDA	I. Simko	La Brillante
C11O1356	11/9/11	Spence(?), USDA	I. Simko	Balady Banha
C11O1357	11/9/11	Spence(?), USDA	I. Simko	RH08-0464
C11O1358	11/9/11	Spence(?), USDA	I. Simko	S1035-01

Figure 1: Frequency of California downy mildew pathotypes observed in 2006-2011.

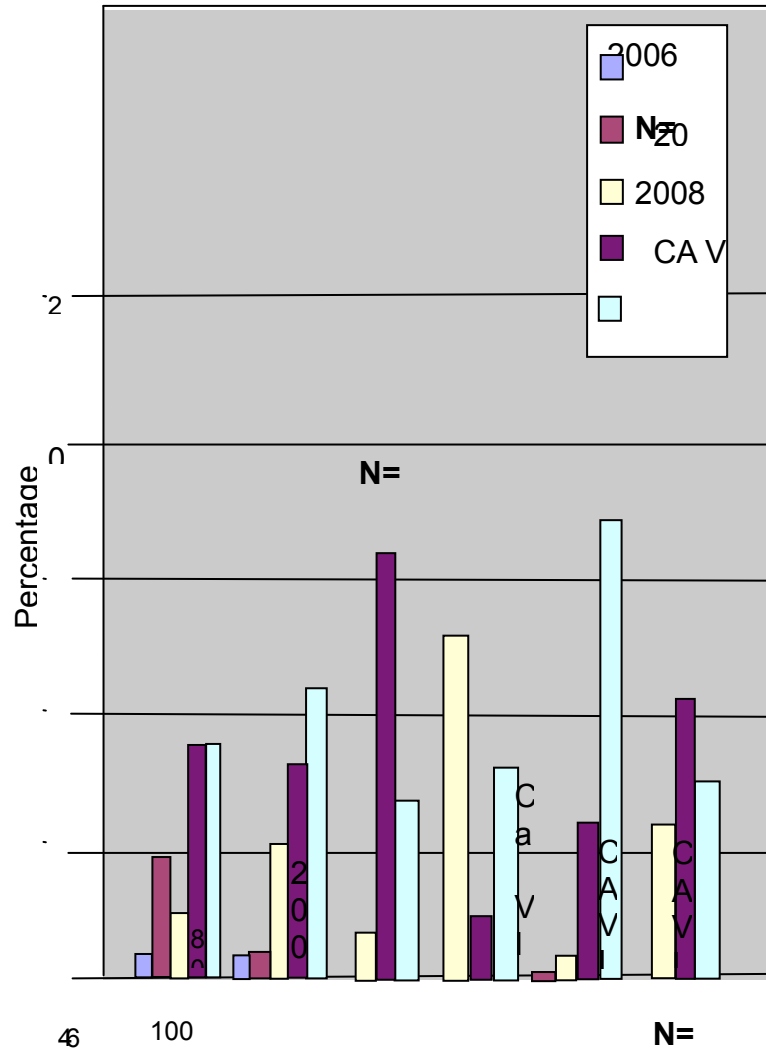
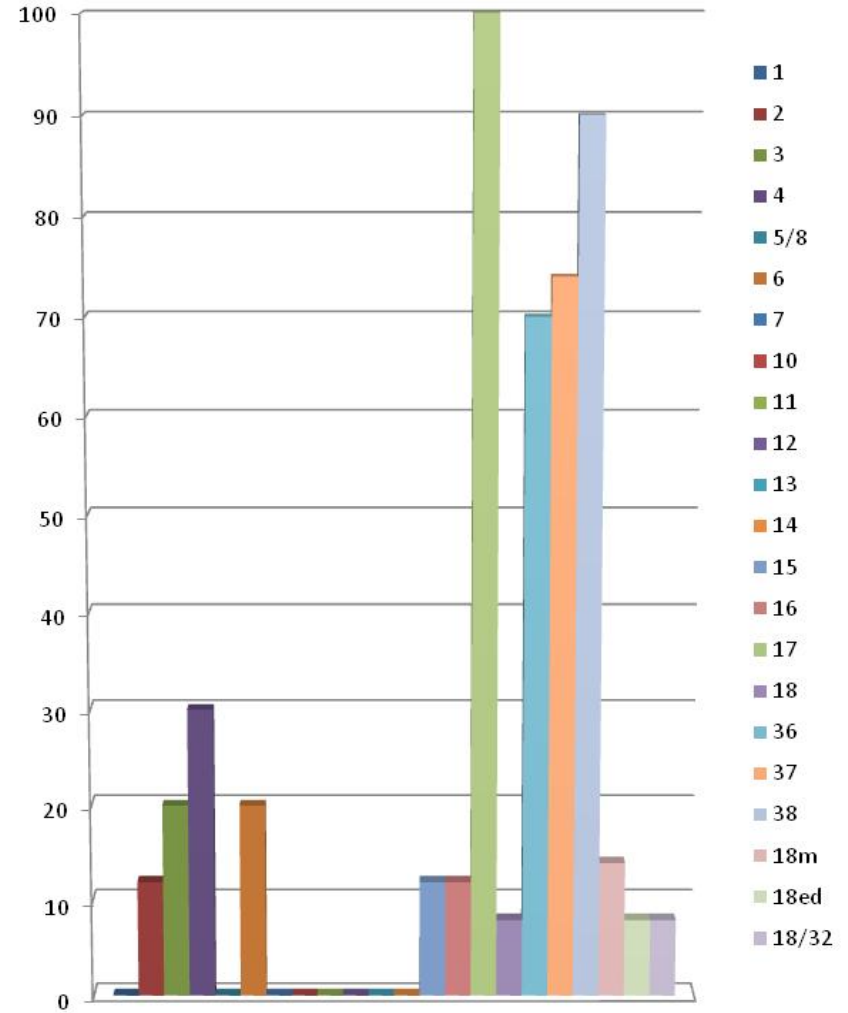


Figure 2: Frequency (%) of the avirulence genes detected in 2011.

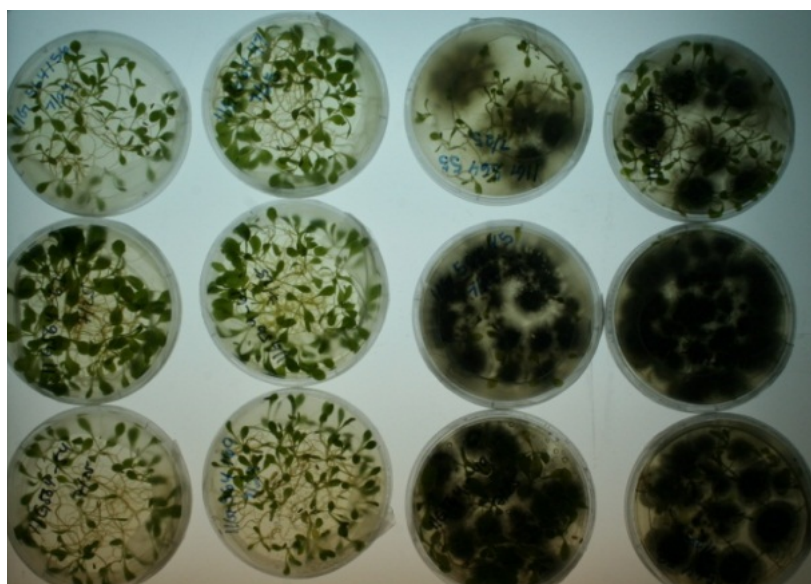


Screening for resistance to *Verticillium* wilt

Resistance to *Verticillium* wilt is a top priority for our program. We previously developed an efficient, reliable, and contained method for screening for the reaction of lettuce to *Verticillium dahliae* in the greenhouse. We utilize microplots within the greenhouse with restricted access to minimize the opportunity for spread of the pathogen. We are screening for resistance to *V. dahliae* strain VdLs17 (race 2) provided by Dr. Krishna Subbarao. We include cv. Salinas as the susceptible control genotype with cv. La Brillante representing a genotype that has shown less disease in the field.

We have continued to screen germplasm for resistance to race 2. New accessions recently received from Israel and Russia are being increased prior to screening for resistance. The majority of accessions screened over the past four years have been highly susceptible although differences in symptoms have been observed and a few accessions of *L. serriola* have been identified that show delayed development of symptoms. Seven lines from Armenia showed no symptoms when initially screened against Vdl 17(race2) and exhibited no seed transmission. These were inter-mated as well as crossed to cv. La Brillante and cv. Salinas; 196 F₂ plants from the cv. La Brillante x *L. serriola* acc. Arm09-170-1-5 cross were challenged with race 2. Disease phenotypes and the frequency of seedborne *Verticillium* seemed to segregate and show transgressive segregation. Some lines showed no symptoms and had no seed transmission. However, progeny testing of F₃ families derived from asymptomatic plants showed significant susceptibility. No families were detected that did not segregate for resistance. Genetic analysis failed to detect any significant QTLs. The same potentially resistant families were analyzed by USDA personnel who also observed delayed symptoms but all lines showed some level of susceptibility. We are reviewing our protocols to determine if there is an environmental explanation for these observations. In the meantime, we are continuing our germplasm screens to search for accessions with clear resistance to race 2.

Figure 3. Differences in seed transmission of *V. dahliae* race 2 observed in F₃ seed from cv. La Brillante x *L. serriola* acc. Arm09-170-1-5.



Multiple Disease Resistances

Crosses between advanced breeding lines, field selections, and released lines have been made to generate lines with multiple disease resistances. Screening for multiple diseases, including lettuce downy mildew, corky root, anthracnose and lettuce mosaic virus, continues.

Supply of Isolates

We have continued to supply California isolates of downy mildew and corky root to breeding companies and other research groups. We have trained personnel from the seed industry and others to handle lettuce downy mildew, corky root, anthracnose and other diseases.

Appendix:

Announcement of Releases of Advanced Breeding Lines of Crisphead Lettuce

Advanced breeding lines of crisphead lettuce are available for use by plant scientists and breeders in public and private institutions. These lines were developed by Richard Michelmore and Oswaldo Ochoa at University of California, Davis. When this germplasm contributes to a new cultivar, appropriate recognition should be given as to its origin.

These lines have been developed to provide new sources of disease resistance to downy mildew in a Salinas horticultural type by backcrossing resistant genotypes with cv. Salinas as the recurrent parent.

UC12100 is a BC₇S₂ line with resistance derived from *L. saligna* acc. CGN9311.
UC12101 is a BC₇S₂ line with resistance derived from *L. saligna* acc. CGN5318.
UC12102 is a BC₇S₂ line with resistance derived from *L. saligna* acc. CGN5282.
UC12103 is a BC₇S₂ line with resistance derived from *L. saligna* acc. CGN5147.

Resistant individuals were selected in each generation. These lines are now homozygous for resistance to California pathotypes CAVI, CAVII and CAVIII and several novel California isolates. We have not observed susceptibility to any California isolate; however, given the variability of *Bremia lactucae*, this does not preclude the existence of virulent isolates currently or in the future. We would be interested in receiving isolates in any cases where one of these lines appears to be susceptible.

To the extent possible, the Salinas plant type was selected in each generation. Field evaluations of the final two selfed generations were made in Salinas, California. These lines are close to horticultural types used in the coastal production areas of California. However, residual variation remains in these lines and further selections may be required to fix plant type. Trials and selections should be made to determine specific areas and seasons to which these lines are best adapted.

Requests for seed should be made to Richard Michelmore at rwmichelmore@ucdavis.edu with copies to Oswaldo Ochoa at oeochoa@ucdavis.edu.