

CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

April 1, 2015 to March 31, 2016

BREEDING CRISPHEAD AND LEAFY LETTUCE

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SUMMARY

The program continues to emphasize the identification and incorporation of genes for disease resistance, particularly to downy mildew, *Verticillium* and *Fusarium* wilts, and corky root into crisphead and the four leaf horticultural types suitable for California. Resistance for downy mildew is being introduced from multiple new sources and combined with resistance to *Verticillium* wilt race 1 and corky root. We have continued to monitor variation in the ability of the downy mildew pathogen to overcome resistance genes and are conducting a large scale comprehensive survey. New but as yet uncharacterized sources of resistance in our breeding program are more effective than the known resistance genes. The International *Bremia* Evaluation Board – US (IBEB-US; previously the American *Bremia* Evaluation Board) has been constituted to formalize the nomination of new Pathotypes. Deployment of multiple new sources will minimize the chances that changes in the pathogen will render all cultivars susceptible simultaneously. We continue to screen for high levels of resistance to *Verticillium* race 2. Genetic studies are continuing 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**

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OBJECTIVES:

- 1) To identify new genes for disease resistance in wild germplasm and incorporate multiple genes from diverse sources into advanced crisphead and leafy breeding lines to maximize the likelihood of durable resistance.
- 2) To monitor variation in pathogen populations, particularly downy mildew, to facilitate the deployment of effective resistance genes.
- 3) To utilize the genetics of agriculturally important traits, particularly disease resistance.
- 4) To release advanced crisphead and leafy 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 long-term strategy used in the UC Lettuce Breeding Program is to initiate crosses and grow early generations at Davis; later generations are trialed and selected at several different lettuce-growing areas in collaboration with USDA, Cooperative Extension in Salinas, and California growers. Backcross and/or single-seed descent strategies are employed for most of the early generations. We select 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 are intercrossed to create lines with multiple disease resistances for lettuce downy mildew (LDM), corky root (CR), anthracnose (ANT) lettuce mosaic virus (LMV), and *Verticillium* race1 (*Ve1*). Additionally we are screening germplasm to identify genetic resistances for *Verticillium* race 2 and *Fusarium* wilts, which are being 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 currently using Salinas, Green Towers, Tropicana, Red Fox, Red Tide, Bibb and Buttercrunch 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 as they advance.

Introgression of Resistance to Downy Mildew

We are continuing to develop crisphead and leafy lettuce lines with resistance genes from diverse sources to provide protection against downy mildew in California. We are focused on generating advanced breeding lines with new resistance genes identified through germplasm screens in previous years (Table 1). Mapping and characterization of the new sources are in progress to provide a pipeline of new sources of resistance. Backcrossing programs to introgress the next generation of genes for resistance to DM into crisphead and leafy types is underway.

Table 1: Status of introgression of new sources of DM resistance into crisphead and leafy types.

Donor	<i>Lactuca</i>	Type	Status	Category
PI491000	<i>L. saligna</i>	Green Leaf	BC7S ₁	Advanced
05G1411	<i>L. serriola</i>	Green Leaf	BC7S ₁	Advanced

ISR-380	<i>L. serriola</i>	Romaine	BC ₄	Advanced
CHEC-023	<i>L. serriola</i>	Iceberg	BC ₃	Intermediate
CHEC-063	<i>L. serriola</i>	Iceberg	BC ₃	Intermediate
CHEC-076	<i>L. serriola</i>	Iceberg	BC ₃	Intermediate
CHEC-101	<i>L. saligna</i>	Romaine	BC ₂	Early
RUSS-635	<i>L. serriola</i>	Red Leaf	BC ₂	Early
KYRGY-237	<i>L. serriola</i>	Romaine	BC ₂	Early
GEOR-301	<i>L. serriola</i>	Romaine	BC ₂	Early
GEOR-289	<i>L. serriola</i>	Romaine	BC ₂	Early
GEOR-297	<i>L. serriola</i>	Red Leaf	BC ₂	Early
KYRGY-247	<i>L. serriola</i>	Butterhead	BC ₂	Early
GEOR-292	<i>L. serriola</i>	Green Leaf	BC ₂	Early
GEOR-299	<i>L. serriola</i>	Iceberg	BC ₂	Early
CHEC-147	<i>L. saligna</i>	Romaine	BC ₁	Early
CHEC-136	<i>L. saligna</i>	Red Leaf	BC ₁	Early
CHEC-132	<i>L. saligna</i>	Green Leaf	BC ₁	Early
RUSS-653	<i>L. serriola</i>	Butterhead	BC ₁	Early
GEOR-282	<i>L. serriola</i>	Butterhead	BC ₁	Early
GEOR-284	<i>L. serriola</i>	Red Leaf	BC ₁	Early
GEOR-288	<i>L. serriola</i>	Iceberg	BC ₁	Early
CHEC-022	<i>L. serriola</i>	Iceberg	BC ₁	Early
CHEC-075	<i>L. serriola</i>	Iceberg	BC ₁	Early
CHEC-082	<i>L. serriola</i>	Iceberg	BC ₁	Early
CHEC-083	<i>L. serriola</i>	Iceberg	BC ₁	Early
CHEC-088	<i>L. serriola</i>	Iceberg	BC ₁	Early
CHEC-089	<i>L. serriola</i>	Iceberg	BC ₁	Early
ARM09-158	<i>L. serriola</i>	crisphead	BC ₁	Early
ARM09-169	<i>L. serriola</i>	crisphead	BC ₁	Early
ARM09-172	<i>L. serriola</i>	crisphead	BC ₁	Early
05G1421	<i>L. serriola</i>	Butterhead	BC ₁ and F ₁	Early
CGN13330	<i>L. saligna</i>	Romaine	F ₁	Early
CGN5157	<i>L. saligna</i>	Green Leaf	F ₁	Early
CGN5301	<i>L. saligna</i>	Romaine	F ₁	Early

Downy Mildew Surveys

In order to ensure that we are breeding for resistance against virulent phenotypes of the pathogen *Bremia lactucae* currently present in California, we have continued to sample *B. lactucae* in collaboration with agricultural and seed companies, growers, and extension personnel, particularly Steve Koike. We have analyzed isolates of downy mildew since 1982. On average ~50 isolates have been characterized per year with a total of more than 1,700 isolates characterized to date. In most years the sampling was opportunistic; this provided a qualitative rather than a quantitative understanding of the phenotypes of isolates in the field. The last comprehensive survey of ~400 isolates was in 1995 to assess the distribution of Ridomil insensitivity in field isolates.

Beginning in September 2013, we have been funded for 32 months by the California Department of Food and Agriculture to conduct a detailed characterization of variation of *B. lactucae* in California. Therefore, the number of isolates characterized has increased considerably, averaging ~180 isolates per year. This is providing a comprehensive understanding of variation in California. Reference isolates are being sequenced to provide information on variability at the DNA level. As part of this project, we developed the *Bremia* database to display the virulence of California isolates characterized by us and others (http://bremia.ucdavis.edu/bremia_database.php). This database has information on the origin, virulence phenotype, mating type and fungicide sensitivity of isolates dating back to 2001. Current isolates are entered as the phenotypes are characterized so that collaborators, pest control advisors, and others can access the data as soon as it is generated; however, this is still several weeks after the isolate is collected due to the need to increase each isolate prior to inoculation and scoring on the differential series of resistance cultivars.

In 2014 the American *Bremia* Evaluation Board (ABEB) was initiated by interested breeding companies and UC Davis. This is a breeding company coordinated group that will nominate official Pathotypes for *B. lactucae* in the western US using a similar protocol to that used in Europe by the International *Bremia* Evaluation Board (IBEB). In May 2015, there was a joint meeting of ABEB and IBEB and it was decided to coordinate the activities of both groups under a single IBEB umbrella with two sub-groups IBEB-EU and IBEB-US responsible for activities in Europe and the US, respectively. Both groups will use the same core differential set of resistant cultivars so that data can be compared. Discussions are on-going to revise and standardize the primary set of differential cultivars used to characterize isolates in both California and Europe. When isolates of the same virulence phenotype are observed in multiple years and locations in California that overcome important *Dm* genes, they will be nominated for designation as a new Pathotype. A reference isolate will be distributed to the companies to confirm the phenotype and its stability; if confirmed it will be designated as an official Pathotype and used in cultivar resistance descriptions. There are currently eight official Pathotypes; however, Pathotypes I to IV have not been observed for many years and therefore are not of agricultural relevance and are not available for distribution and screening. Individuals interested in IBEB-US should contact Nicki Phillips (nicki.phillips@enzausa.com).

Over the past year, 187 isolates of *B. lactucae* originating from several regions in California were characterized for virulence phenotype, mating type, and metalaxyl sensitivity. None of the isolates analyzed were Pathotype CAV; 0.5% were CAVI; 32%

were CAVII; 8.5% were CAVIII; and 7% were candidate Pathotype CAIX (see below). Nearly half (52%) of the isolates had novel virulence phenotypes (Fig. 3). More isolates (80%) are able to overcome *Dm17*. *Avr36*, *Avr37* and *Avr38* were present in 26, 48, and 76% of the isolates, respectively. *Avr4* was detected at a frequency of 51%; *Avr6* has increased from 24% to 30% (Fig. 4). Of the 97 novel isolates we identified, 62 (64%) were variants of previously characterized Pathotypes and the rest had new combinations of avirulence genes. European cultivars Silvinas (n1), Murai (n2), Bedford (n3), Balesta (n4) and Bellissimo (n5) also showed resistance. Of these, Balesta (100% of isolates avirulent) and Bellissimo (86% of isolates avirulent) were the most effective against the isolates tested.

Figure 1: Frequency of downy mildew Pathotypes detected in CA 2009-2015.

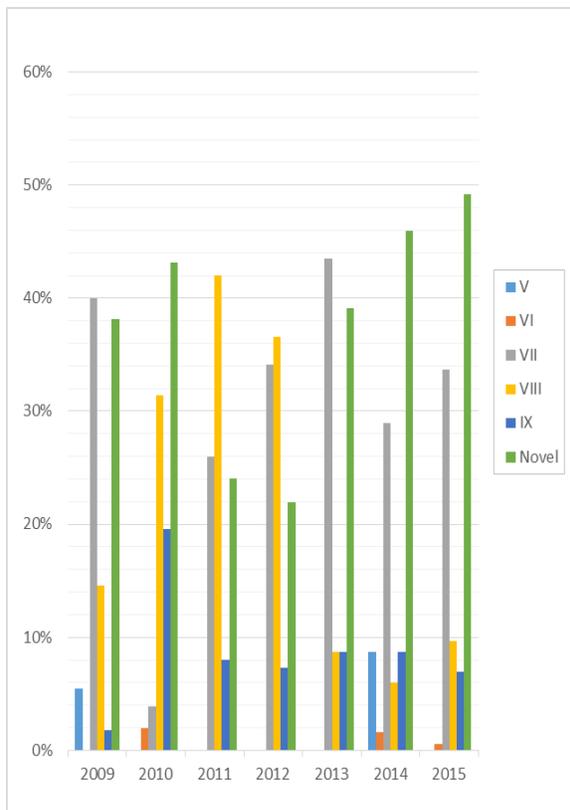
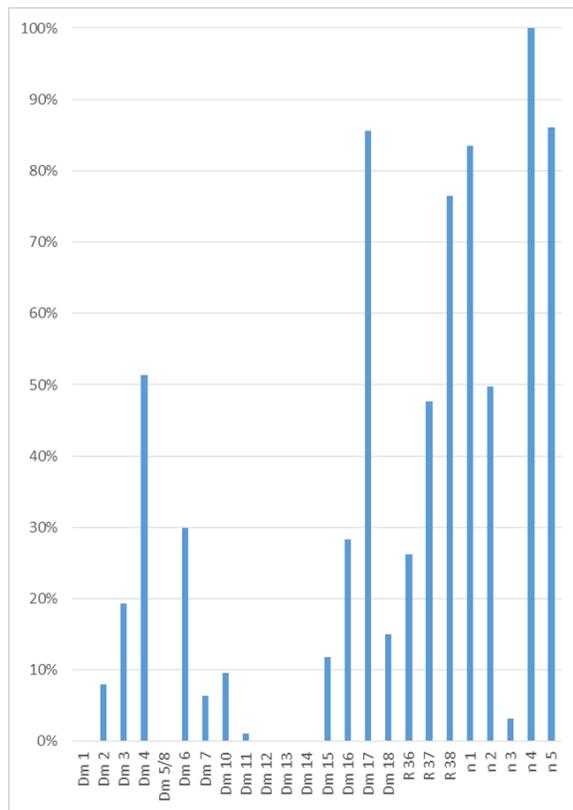


Figure 2: Frequency (%) of avirulence genes observed in California in 2015.



From 2009 to 2015 we identified 298 isolates with novel virulence phenotypes. Of these 49 were avirulent on *Dm3*, *Dm6*, *Dm17*, *Dm36*, and variable on *Dm37* and *Dm38*. We nominated this group of novel isolates as a candidate for Pathotype CAIX because they have similar virulence phenotypes, have been detected in multiple locations and over multiple years, and overcome important *Dm* genes. In 2014, a candidate type isolate for Pathotype IX was distributed to seed companies for testing but its virulence phenotype was not stable and therefore Pathotype IX was not ratified as an official Pathotype at this time. Another candidate Pathotype IX type isolate has been distributed for testing.

At the October 2015 IBEB-US meeting, two more groups were nominated for Pathotype designation due to their ability to overcome *Dm17* and *R37* resistance respectively. From 2009 to 2015, 38 isolates were able to overcome *Dm17*. Of these, 13 collected in 2015 from multiple locations were avirulent on *Dm6*, *Dm10*, *Dm15*, *R37*, n2, and n4. From 2009 to 2015, 280 isolates were virulent on *R37*. Of these, 75 isolates collected from multiple years and locations were avirulent on *Dm4*, *Dm17*, *R38*, n1, n2, n4, and n5. Two candidate type isolates for each group have been distributed to breeding companies for testing.

Of 187 isolates characterized for mating type, 167 (89%) and 19 (10%) were B₂ and B₁ respectively. Isolates of B₁ mating type were distributed throughout California and all had novel avirulence phenotypes (Table 2). One isolate was self-fertile, capable of forming sexual spores when inoculated alone; this isolate is sensitive to metalaxyl and has the same virulence phenotype as the candidate Pathotype able to overcome *R37*. On one occasion, isolates of both mating types were collected from the same field. When these were crossed in the laboratory, a wide range of virulence phenotypes were recovered (Table 3). Therefore, although B₁ isolates have been extremely rare in the past, the California population of *B. lactucae* seems to be transitioning to a sexual, more variable population. This will result in fewer stable Pathotypes and sexual oospores surviving in the soil, which will alter the epidemiology of downy mildew, possibly causing earlier epidemics.

Table 2: Geographical distribution and virulence phenotype of isolates collected in 2015 with B₁ mating type.

Number of isolates	Location	Avirulence phenotype
1	King City, CA	2,3,7,11,16,17,18,36,37,38,n3,n4,n5,
1	King City, CA	2,3,7,11,16,17,18,36,37,38,n4,n5,
3	Greenfield, King City, and San Lucas, CA	2,3,7,16,17,18,36,37,38,n3,n4,n5,
6	Salinas, Gonzales, Soledad, Greenfield, and King City, CA	2,3,7,16,17,18,36,37,38,n4,n5,
1	Hollister	2,7,16,17,18,36,37,38,n4,n5,
1	Chualar	3,4,16,17,38,n1,n2,n4,n5,
1	Salinas	3,4,17,18,36,37,38,n1,n3,n4,
2	Watsonville and Salinas, CA	4,17,38,n1,n2,n4,n5,
1	Salinas	4,6,17,37,38,n1,n4,
1	Chualar	4,6,17,38,n1,n4,n5,
1	King City	6,17,37,n1,n2,n4,n5,

Table 3. Example phenotypes of progeny from cross of isolates C14C1485 x C14C1486 that were collected from the same field illustrating the diversity generated through sexual crosses.

Differential lettuce cultivar	Dm	Parental isolates		Progeny isolates															
		C14C1485	C14C1486	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Green Towers	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lednický	1	+	+	+	+	+	+	+	+	+	+	(-)	+	+	+	+	+	+	
UCDM2	2	-	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	
Dandle	3	-	+	(-)	(-)	+	(-)	-	+	+	+	-	+	(-)	+	(-)	+	(-)	
R4T57D	4	+	+	+	+	-	-	+	+	-	-	+	+	-	-	+	-	-	
Valmaine	5/8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Sabine	6	+	-	+	+	+	+	(-)	+	+	+	+	+	-	+	+	+	+	
LSE 57/14	7	-	+	(-)	+	-	-	-	-	-	-	+	-	-	-	+	-	-	
UCDM10	10	+	+	+	+	+	+	(-)	+	+	+	+	+	(-)	+	+	+	+	
Capitan	11*	(-)	+	(-)	(-)	+	+	(-)	+	(-)	+	(-)	+	(-)	+	+	+	+	
Hilde II	12	+	+	+	+	+	+	+	+	+	+	+	(-)	+	+	+	+	+	
Perlake	13	+	+	+	+	+	+	+	+	+	+	(-)	+	+	+	+	+	+	
UCDM14	14	+	+	(-)	+	+	+	(-)	+	+	+	+	+	(-)	+	+	+	+	
Num DM15	15	+	+	+	+	+	+	(-)	+	+	+	+	+	(-)	+	+	+	+	
CGDm16	16*	-	-	-	(-)	(-)	(-)	(-)	-	(-)	-	(-)	-	(-)	(-)	(-)	-	(-)	
NunDn17	17	-	-	+	-	-	-	-	-	-	-	(-)	-	+	-	-	-	-	
Colorado	18	-	+	-	-	(-)	+	-	+	-	-	+	-	-	-	-	-	+	
Ninja	36	-	+	-	-	-	-	-	-	(-)	-	-	-	-	-	-	-	-	
Discovery	37	-	-	-	(-)	-	-	-	-	-	-	-	-	-	-	-	-	-	
Argeles	38	-	(-)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
RYZ-2164	A [#]	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
RYZ-910457	B [#]	+	+	(-)	+	+	-	-	-	(-)	-	-	-	-	+	-	-	-	
Bedford	A [#]	(-)	+	-	+	+	(-)	(-)	+	+	+	+	-	+	+	+	(-)	+	
Balesta	D [#]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Bellisimo	E [#]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
* may have a partial resistance phenotype	# provisional	-	No sporulation.			+	Profuse sporulation.			(-)	Delayed, sparse sporulation. Those with multiple (-) scores may have low fitness.								
	Mating type	B1	B2	B2	B1	B2	B2	B2	B2	B1	B1	B2	B2	B2	B2	B2	B2	B2	
	Metalaxyl sensitivity	sen	insen	sen	sen	sen	sen	intermed	intermed	intermed	intermed	intermed	insen	insen	insen	insen	insen	insen	

Of the 171 isolates analyzed for metalaxyl sensitivity in 2015, 101 (59%), 13 (8%), and 57 (33%) were insensitive, intermediate, and sensitive respectively. Insensitivity is considered as the ability to sporulate by 15 dpi at 50 ppm or above. An intermediate reaction is considered as sporulation by 15 dpi at 5 or 10 ppm but not at 50 ppm. Sensitivity is considered as no sporulation by 15 dpi at 5 ppm or above. There was no obvious correlation of metalaxyl sensitivity with virulence phenotype. However, of the 19 B₁ isolates phenotyped, only one was insensitive to metalaxyl.

Screening for resistance to *Verticillium* wilt

Resistance to *Verticillium* wilt is a high priority for our program. We previously developed an efficient, reliable, and contained method for screening for the reaction of lettuce to *V. 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 resistance to race 1.

We have continued to screen germplasm for resistance to race 2. Forty seven new accessions that had been received from Israel and Russia in 2012 and increased in 2013 were screened for resistance in 2014. No lines had good resistance. Thirty-five new *L. serriola* lines from Georgia, Kyrgyzstan and Uzbekistan were received via the USDA and a subset of them was evaluated for resistance in 2015. None of the lines screened was all resistant but few lines had individuals that were asymptomatic and stem cuttings plated on NP-10 medium were free of *Verticillium*. Selfed progeny of these individuals will be re-

evaluated to confirm resistance this year. We have received 67 new accessions of *L. serriola* from Azerbaijan that are being seed increased prior to screening.

The majority of accessions screened over the past five years have been highly susceptible, although differences in symptoms have been observed. A few accessions of *L. serriola* showed delayed development of symptoms or were asymptomatic. In particular, seven lines from Armenia showed no symptoms when initially screened against VdLs17 (race 2) and exhibited no seed transmission. These were inter-mated as well as crossed to *L. sativa* genotypes. QTL analysis of an F₂ population from a cross between *L. serriola* acc. Arm09-170-1-5 and a *L. sativa* breeding line analysis failed to detect significant QTL. However, it segregated for big differences in plant development that may have obstructed assessment of resistance because evaluations of resistance to *Verticillium* are confounded by differences in plant development and maturity.

Therefore we developed a RIL population from a cross between *L. sativa* PI251246 that is highly susceptible to *Verticillium* race 2, and *L. serriola* acc. Arm09-170-1-5 that was asymptomatic. These lines have similar vegetative development patterns and flowering times. Ninety six RILs of this population have been evaluated in replicate for resistance to *Verticillium* race 2 in our greenhouse sick plots. The same population was evaluated for resistance in the greenhouse by German Sandoya at the USDA Salinas station. These RILs have been genotyped and QTL analysis was performed for both trials. Results were not conclusive. QTLs were observed in the Davis trial but these were not significant in the Salinas trial. When the most extreme lines for resistance and susceptibility in both trials were compared they were not coincident; lines that were resistant in the Davis trial showed intermediate responses in the Salinas trial and the most resistant lines in the Salinas trial had intermediate responses in the Davis trial. These results may be indicative of major environmental sensitivity to this resistance. These RILs will be phenotyped further.

Supply of Isolates

We have continued to supply current 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 and corky root.