

CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

April 1, 2014 to March 31, 2015

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. Of the characterized genes for resistance to downy mildew, *Dm17* remains the most effective known resistance gene but does not provide resistance against all California isolates. 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 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 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 for the UC Crisphead and Leafy Lettuce 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 or modified 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 crisp and leafy types is underway.

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

	Type	Donor Line	Donor Species	Status
Advanced breeding lines	green leaf	CGN491000	<i>L. saligna</i>	BC ₇
	green leaf	05G1411	<i>L. serriola</i>	BC ₇
	butterhead	05G1421	<i>L. serriola</i>	BC ₃
	romaine	TUR00-A	<i>L. saligna</i>	BC ₃
	red leaf	TUR00-C	<i>L. saligna</i>	BC ₆
Early breeding lines	crisphead	ARM09-158	<i>L. serriola</i>	BC ₂
	crisphead	ARM09-169	<i>L. serriola</i>	BC ₂
	crisphead	ARM09-172	<i>L. serriola</i>	BC ₂
	crisphead	CZE07-23	<i>L. serriola</i>	BC ₂
	crisphead	CZE07-63	<i>L. serriola</i>	BC ₂
	crisphead	CZE07-76	<i>L. serriola</i>	BC ₂
	romaine	CZE08-101	<i>L. saligna</i>	BC ₁
	greenleaf	CZE08-132	<i>L. saligna</i>	BC ₁
	romaine	CZE08-134	<i>L. saligna</i>	BC ₁
	red leaf	CZE08-147	<i>L. saligna</i>	BC ₁
	green leaf	CZE08-158	<i>L. saligna</i>	BC ₁
	red leaf	RUS10-1	<i>L. serriola</i>	BC ₁
	crisphead	GEO14-299	<i>L. serriola</i>	BC ₁
	crisphead	GEO14-288	<i>L. serriola</i>	BC ₁
	romaine	GEO14-301	<i>L. serriola</i>	BC ₁
	romaine	GEO14-289	<i>L. serriola</i>	BC ₁
	romaine	KYR14-237	<i>L. serriola</i>	BC ₁
	red leaf	GEO14-297	<i>L. serriola</i>	BC ₁
	butterhead	GEO14-282	<i>L. serriola</i>	BC ₁
	butterhead	KYR14-247	<i>L. serriola</i>	BC ₁
	red leaf	GEO14-297	<i>L. serriola</i>	BC ₁
	red leaf	GEO14-284	<i>L. serriola</i>	BC ₁
	green leaf	GEO14-292	<i>L. serriola</i>	BC ₁
	romaine	CGN13330	<i>L. saligna</i>	F ₁
	green leaf	CGN5157	<i>L. saligna</i>	F ₁
	butterhead	RUS10-19	<i>L. saligna</i>	F ₁

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 growers, the seed industry 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 samples were sampled opportunistically; this provided a qualitative rather than a quantitative picture 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. This is providing a comprehensive picture 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 and other phenotypes 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, PCAs and others can access the data as soon as it is generated; this is still several weeks after the isolate is collected due to the need to increase each isolate prior to inoculation 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. 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, 185 isolates of *B. lactucae* originating from several regions in California were characterized for virulence phenotype, mating type, and metalaxyl sensitivity. Thirteen % of the isolates analyzed in 2014 were Pathotype CAV; 24% were CAVII; only 2% of the isolates were each of Pathotypes VI, VIII and candidate Pathotype

CAIX (see below). Nearly half (46%) of the isolates had novel virulence phenotypes (Fig. 3). *Dm17* remains effective against most (95%) but not all isolates; it has not, however, been widely deployed and so has not been under extensive selection pressure. *Avr36*, *Avr37* and *Avr38* were present in 20, 30 and 75 % of the isolates respectively. *Avr4* was detected at a frequency of 60% (Fig. 4). Of the 86 novel isolates we identified, 57 (66%) were variants of previously characterized pathotypes and the rest had new combinations of avirulent genes. European cultivars Silvinas (a), Murai (b), Bedford (c), Balesta (d) and Bellissimo (e) also showed resistance. Of these, Balesta (99% of isolates avirulent) and Bellissimo (91% of isolates avirulent) remain most effective against *B. lactucae*.

From 2009 to 2013 we identified 36 isolates with novel virulence phenotypes. Of these 34 were avirulent on *Dm3*; 18 were avirulent on *Dm17*, *Dm36*, *Dm37* and *Dm38* and variable on *Dm6*. 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 potential type isolate 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 will be distributed for testing.

Figure 1: Frequency of downy mildew Pathotypes detected in CA 2008-2014.

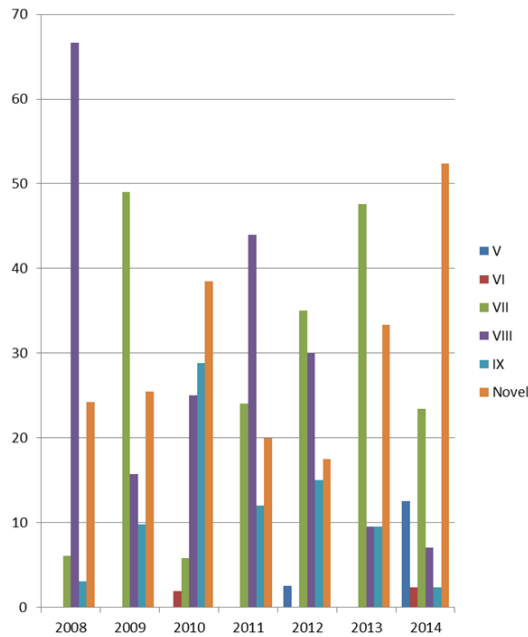
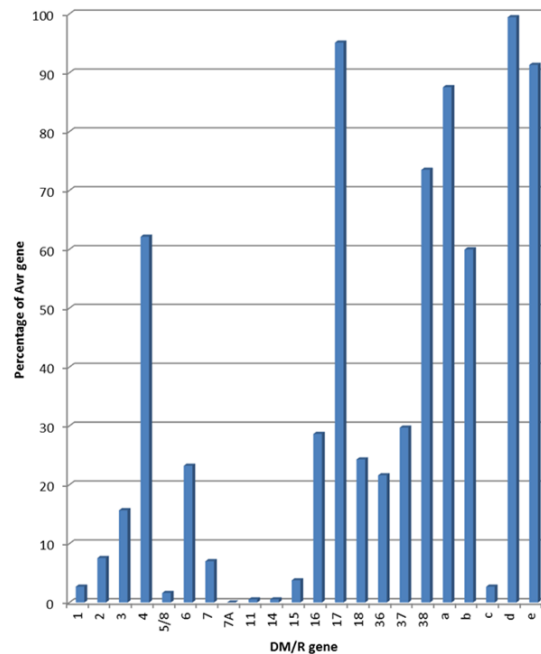


Figure 2: Frequency (%) of avirulence genes observed in California in 2014.



Of 170 isolates characterized for mating type, 151 (89%) and 18 (11%) were B₂ and B₁ respectively. Isolates with B₁ mating type were distributed throughout California and all had novel avirulence phenotypes (Table 2). 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 2014 with B₁ mating type.

Number of isolates	Location	Avirulence phenotype
1	Huron, CA	1,17,18,a,d,e
2	Hollister, CA	2,7,16,17,18,36,37,38,d
9	Monterey County	2,7,16,17,18,36,37,38,d,e
1	San Lucas, CA	2,3,7,16,17,18,36,37,38,c,d,e
2	Watsonville, CA and San Lucas, CA	2,3,7,16,17,18,36,37,38,d,e,
1	Salinas, CA	3,17,36,38,a,d,e,
1	Salinas, CA	4,6,16,17,38,a,b,d,e,
1	Salinas, CA	4,17,38,a,d,e

Table 3. Phenotypes of progeny from cross of isolates C14C1485 x C14C1486 that were collected from the same field.

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	+	+	+	+	+	+	(-)	+	+	+	+	+	(-)	+	+	+	+	+	
Captan	11*	(-)	+	(-)	(-)	+	+	(-)	+	(-)	+	(-)	+	(-)	+	+	+	+	+	
Hide II	12	+	+	+	+	+	+	+	+	+	+	+	(-)	+	+	+	+	+	+	
Penlake	13	+	+	+	+	+	+	+	+	+	+	(-)	+	+	+	+	+	+	+	
UCDM14	14	+	+	(-)	+	+	+	(-)	+	+	+	+	+	(-)	+	+	+	+	+	
Num DM15	15	+	+	+	+	+	+	(-)	+	+	+	+	+	(-)	+	+	+	+	+	
CGDM16	16*	-	-	-	(-)	(-)	(-)	(-)	-	(-)	-	(-)	-	(-)	(-)	(-)	-	-	(-)	
NunDm17	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	NT	B1	B2	B2	NT	B1	B1	B2	B2	NT	NT	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	insen	insen	

Of the 30 isolates analyzed for metalaxyl sensitivity in 2014, 5 (17%), 7 (23%), and 18 (60%) were insensitive, intermediate, and sensitive respectively. Insensitivity is denoted as the ability to sporulate by 15 dpi at 50 ppm or above. An intermediate reaction is denoted as sporulation by 15 dpi at 5 or 10 ppm but not at 50 ppm. Sensitivity is denoted as no sporulation by 15 dpi at 5 ppm or above. There was no clear correlation of metalaxyl sensitivity with virulence phenotype.

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 *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 are presently being evaluated for resistance (Fig. 3). 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 individuals of this population have been evaluated for resistance to *Verticillium* race 2. The same population is being re-evaluated for resistance by G. Sandoya at the USDA Salinas station. These RILs have been genotyped and QTL analysis will be performed shortly.

Multiple Disease Resistances

Crosses between advanced breeding lines, field selections, and released lines to generate lines with multiple disease resistances continues.

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.

Figure 3. New germplasm from Georgia, Kyrghystan and Uzbekistan being screened in containment bins for resistance to *Verticillium* race 2.

