CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

April 1, 2018 to March 31, 2019

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

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CONTINUING 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 overall strategy used in the UC Lettuce Breeding Program continues to be 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 have screened 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 (Table 1). 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 genes for resistance. Backcrossing programs to introgress the next generation of genes for resistance to DM into crisphead and leafy types is a continual ongoing process.

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

Donor	*Lactuca*	Type	Status	Category
PI491000	*L. saligna*	Green Leaf	BC_7S_1 testing for Hm	Advanced
05G1411	*L.serriola*	Green Leaf	BC_7S_1 testing for Hm	Advanced
ISR-380	*L. serriola*	Romaine	BC_7S_1 testing for Hm	Advanced
CHEC-023	*L. serriola*	Iceberg	BC_7	Advanced
CHEC-063	*L. serriola*	Iceberg	BC_7	Advanced
CHEC-132	*L. saligna*	Green Leaf	BC_6	Advanced
RUSS-635	*L. serriola*	Red Leaf	BC_6	Advanced
KYRGY-237	*L. serriola*	Romaine	BC_6	Advanced
GEOR-289	*L. serriola*	Romaine	BC_6	Advanced
GEOR-297	*L. serriola*	Red Leaf	BC_6	Advanced
CGN5309	*L. serriola*	Green Leaf	BC_5	Advanced
CHEC-022	*L. serriola*	Iceberg	BC_5	Advanced
CHEC-075	*L. serriola*	Iceberg	BC_5	Advanced
CHEC-082	*L. serriola*	Iceberg	BC_5	Advanced
CHEC-088	*L. serriola*	Iceberg	BC_5	Early donor
CHEC-089	*L. serriola*	Iceberg	BC_5	Early donor
CHEC-147	*L. saligna*	Romaine	BC_5	Advanced
CHEC-076	*L. serriola*	Iceberg	BC_4	Intermediate
CHEC-101	*L. saligna*	Romaine	BC_3	Intermediate
CHEC-136	*L. saligna*	Red Leaf	BC_4	Intermediate
GEOR-301	*L. serriola*	Romaine	BC_4	Intermediate
GEOR-292	*L. serriola*	Green Leaf	BC_4	Intermediate
GEOR-299	*L. serriola*	Iceberg	BC_4	Intermediate
CHEC-083	*L. serriola*	Iceberg	BC_4	Intermediate
05G1421	*L.serriola*	Butterhead	BC_3	Intermediate
CHEC-108	*L. saligna*	Green Leaf	BC_3	Intermediate
CHEC-158	*L. saligna*	Green Leaf	BC_2	Early donor
CGN13330	*L. saligna*	Romaine	BC_1	Early donor
RUSS-653	*L.serriola*	Butterhead	BC_1	Early donor
GEOR-282	*L. serriola*	Butterhead	BC_1	Early donor
KYRGY-247	*L. serriola*	Butterhead	BC_2	Early donor
GEOR-284	*L. serriola*	Red Leaf	BC_1	Early donor
GEOR-288	*L. serriola*	Iceberg	BC_1	Early donor
AZER-805	*L.serriola*	Red Leaf	BC_1	Early donor
AZER-811	*L.serriola*	Romaine	BC_2	Early donor
AZER-822	*L.serriola*	Red Leaf	BC_2	Early donor
AZER-840	*L.serriola*	Romaine	BC_2	Early donor
AZER-843	*L.serriola*	crisphead	BC_2	Early donor
AZER-853	*L.serriola*	Green Leaf	BC_2	Early donor
AZER-854	*L.serriola*	Green Leaf	BC_2	Early donor
AZER-871	*L.serriola*	Butterhead	F_1	Early donor
CGN5157	*L. saligna*	Green Leaf	BC_1	Early donor
ARM09-158	*L. serriola*	crisphead	BC_1	Early donor
ARM09-169	*L. serriola*	crisphead	BC_1	Early donor
ARM09-172	*L. serriola*	crisphead	BC_1	Early donor
CGN5301	*L. saligna*	romaine	F_1	Early donor

Each line is tested in each generation against a current, highly virulent isolate and the resistant progeny used as the resistance donor parent for the next generation. Selfed generations of advanced lines (BC_6S_1) are then tested for resistance to a diverse panel of contemporary virulent Californian isolates. Susceptible lines are discarded. We are genetically characterizing the chromosomal position of all of our resistance genes. Once these genomic regions are identified, molecular markers will be used for MAS for selection of lines with multiple resistance genes that confer resistance to all Californian isolates. Release of lines with pyramids of several resistance genes should increase the durability of the individual genes and provide longer lasting resistance.

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. On average ~50 isolates have been characterized per year with a total of more than 2,070 isolates characterized since 1982. In most years the sampling was opportunistic; this provided a qualitative rather than a quantitative understanding of the phenotypes of isolates in the field. From September 2013 to June 2016, we were funded by the California Department of Food and Agriculture to conduct a detailed characterization of variation of *B. lactucae* in California. During this period the number of isolates characterized averaged ~180 isolates per year. This provided a comprehensive understanding of variation in California. While most sampling was opportunistic in 2018, we received additional funding from the International Lettuce Genomics Consortium in the fall and we are now characterizing ~20 isolates a month that have been more objectively sampled from throughout the state.

We maintain 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. Isolates are entered as their phenotypes are characterized so that collaborators, pest control advisors, and others can access the data as soon as it is available; however, this is still several (4 to 8) weeks after an isolate is collected due to the need to increase each isolate prior to inoculation and scoring on the differential series of resistance cultivars. We are currently developing PCR-based assays that will allow more rapid characterization and analysis of large numbers of lesions. This will allow us to triage isolates into groups and to focus our phenotyping efforts on potentially novel and problematic isolates.

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 nominates official Pathotypes for *B. lactucae* in the western US using a similar protocol to that used in Europe. In May 2015, it was decided to coordinate the activities in Europe and the Western US under a single IBEB-G(lobal) umbrella with two sub-groups IBEB-EU and IBEB–US responsible for activities in Europe and the US, respectively. Both groups now use the same core differential set of resistant cultivars so that data can be compared. Nomenclature has been standardized so that denominated isolates are designated Bl:#EU or

US. The US Pathotypes I to VIII are now designated as Races Bl:1US to Bl:8US. 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 by IBEB-US for designation as a new Race. Nominated isolates will be distributed to the companies to confirm their phenotype and stability; if confirmed, they will be designated as an official Race and used in cultivar resistance descriptions. There are currently nine official races in California; however, Races Bl: 1US to Bl: 6US have not been observed for many years; therefore, they are not of agricultural relevance and are not available for distribution and screening. Reference isolates for Bl: 7 and 8 are being or will be ring tested to ensure their stability. Reference isolate Bl: 9US is available for screening; this isolate has been derived by passaging a common but unstable isolate (referred to as Bl: 9US-progenitor) on FrSAL-1. Individuals interested participating in IBEB-US should contact Nicki Phillips (nicki.phillips@enzausa.com).

Over the past year, 57 isolates of *B. lactucae* mostly representing opportunistic samples provided by collaborators from several regions in California were characterized for virulence phenotype, mating type, and metalaxyl sensitivity. None of the isolates analyzed were Race Bl: 5US, 6US or 7US; 9% were 8US; and 12% were Race Bl: US9 progenitor (see below). By far the majority of isolates (74%) had novel virulence phenotypes that differed from the designated races (Fig. 1). Of all the isolates in 2018, only twenty-three (27%) were able to overcome *Dm17*. *Avr36* and *Avr38* were present in 25 and 62% of the isolates, respectively. *Avr4* was decreased slightly to a frequency of 13%; *Avr6* increased to 89% of isolates (Fig. 2). The new inclusions in the differential set RYZ2164 (n1), RYZ910457 (n2), Bedford (n3), Balesta (n4) and Bellissimo (n5) showed resistance; of these, Balesta (100% of isolates avirulent) and Bellissimo (88% of isolates avirulent) were the most effective against the isolates tested.

Figure 1: Frequency of downy mildew Pathotypes detected in CA 2011-2018

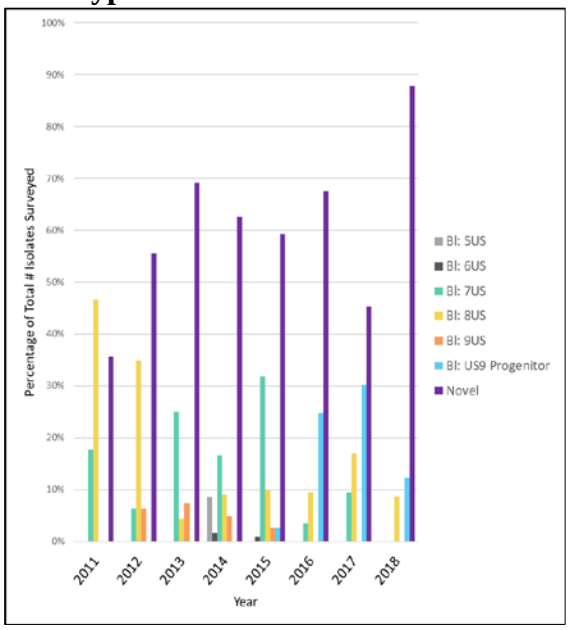
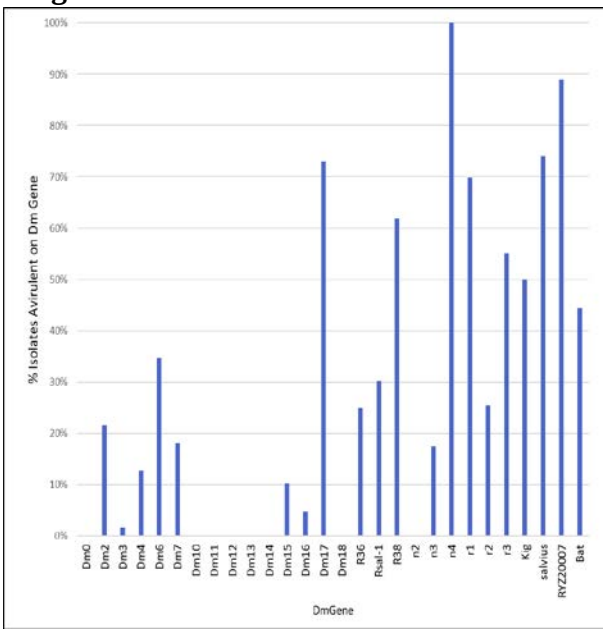


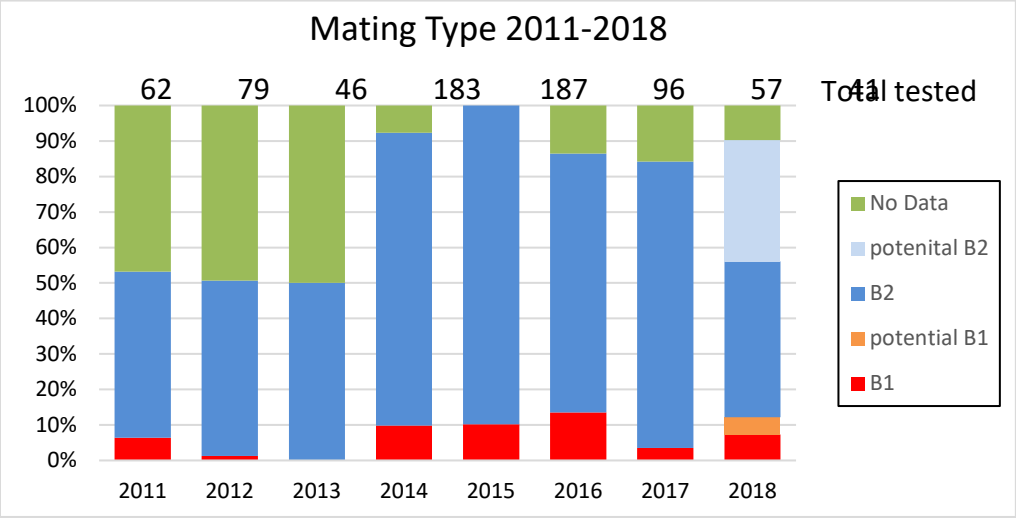
Figure 2: Frequency (%) of avirulence genes observed in California in 2018.



We have identified 528 isolates with novel virulence phenotypes between 2010 and 2018. Of these, 14 were avirulent on *Dm6*, *Dm17*, *n1*, n4, r1 and r3. This group of novel isolates was nominated by IBEB-US as a candidate for Race (Bl: 10US) because they have similar virulence phenotypes, had been detected in multiple locations and over multiple years, and overcome important *Dm* genes. A candidate type isolate for Race Bl: 10US was distributed to seed companies for ring testing; however, its virulence phenotype was not stable. Therefore, we are filtering a representative isolate on a differential cultivar in an attempt to produce a stable reference isolate.

Of 41 isolates characterized for mating type in 2018, 32 (78%) and 5 (7%) were B_2 and B_1 respectively. The frequency of isolates of B_1 mating type varied during the years (Fig. 3) with the highest frequencies in years 2014, 2015, 2016 and 2018.

Figure 3: Frequency of isolates with B_1 or B_2 mating types between 20011 and 2018.



Of the 56 isolates analyzed for metalaxyl sensitivity in 2018, 22 (58%), 6 (16%), 2 (5%), and 8 (21%) were insensitive, delayed 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.

Development of molecular markers to quickly characterize isolates of *B. lactucae*

We are developing molecular markers in order to quickly characterize field isolates. Current phenotyping is too labor intensive and slow for real-time monitoring of changes in *B. lactucae* populations. Genotyping isolates before phenotyping will allow increased sampling and a better understanding of population dynamics. We used the SF5 reference genome assembly and whole genome sequencing data from a diverse set of isolates to identify polymorphic regions in the genome of *B. lactucae*. We have developed 25 PCR-based, SSR markers distributed over 14 scaffolds (90% of the genome). These markers were able to uniquely identify isolates from a diversity panel. Over the next year, we will test these SSR markers on fresh lesions of field isolates. We are also developing PCR based markers for SNPs for mating type. These markers will be distributed so that populations of Bremia can be analyzed with a standard set of markers.

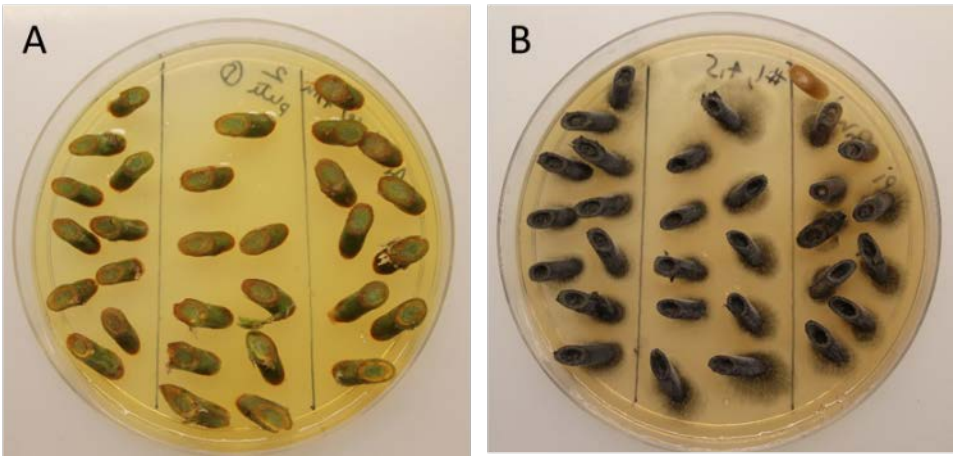
Resistance to *Verticillium* wilt

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 (see previous reports). We continue to screen 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.

The majority of accessions screened for resistance over the past six years have been highly susceptible to race 2, 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. 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. A QTL for resistance was identified in linkage group 6 that was in significant trials conducted in Davis and Salinas. The same QTL was significant in other populations (collaboration with German Sandoya; now at University of Florida). We are introgressing this QTL for resistance to *Verticillium* race 2 into cultivated lettuce.

In 2016, we received 67 new accessions of *L. serriola* from Azerbaijan; these were seed increased in 2017 and screened for resistance in the greenhouse in 2018. The experiment had random design without repetition. Up to six derived siblings were screened for each accession with six plants representing each sibling. Resistance was scored as absence of brown discoloration on the stem-root transition region. In additional, stems with no discoloration were plated on NP-10 medium to rule out the presence of *Verticillium* (Fig. 7). For no accession were all the siblings tested was resistant. For five accessions, one or two siblings were resistant; these accessions may be segregating for resistance. We have initiated crosses to different susceptible and resistant genotypes to confirm and study the genetic basis of this resistance. Original accessions and progeny of the crosses will be tested for resistance this year in the greenhouse.

Figure 7: NP-10 medium plates with stems of resistant (A) and susceptible plants (B).



Supply of Isolates

We have continued to supply current California isolates of downy mildew to breeding companies and other research groups. In particular, we have supplied isolates of the nominated Races as well as candidate isolates for ring tests. We have trained personnel from the seed industry and others to handle lettuce downy mildew.