

CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

April 1, 2016 to March 31, 2017

BREEDING CRISPHEAD AND LEAFY LETTUCE

Richard W. Michelmore

María José Truco

The Genome Center and
The Department of Plant Sciences

University of California, Davis

rwmichelmore@ucdavis.edu

mjtruco@ucdavis.edu

SUMMARY

The program continues to emphasize the identification and incorporation of genes for disease resistance, particularly to downy mildew, and *Verticillium* and *Fusarium* wilts into crisphead and the four leaf 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. New sources of resistance in our breeding program are more effective than the known resistance genes. 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 have identified at least one QTL for resistance to *Verticillium* race 2. Genetic analysis of several populations revealed multiple QTLs for resistance to *Fusarium*. Different QTLs were identified in different populations providing the possibility of combining them to provide high levels of resistance.

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

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

COOPERATING PERSONNEL: **Pauline Sanders**
Cayla Tsuchida
Huaqin Xu
German Sandoya
Aubrey Kenefick
Juliana Gil
The Genome Center and
The Department of Plant Sciences
University of California, Davis
Ivan Simko
Jim McCreight
UDSA-ARS, Salinas
Krishna Subbarao
Thomas Gordon
The Department of Plant Pathology
University of California, Davis
Steve Koike
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 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 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). 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	<i>Lactuca</i>	Type	Status	Category
PI491000	<i>L. saligna</i>	Green Leaf	BC ₇ S ₁	Advanced
05G1411	<i>L. serriola</i>	Green Leaf	BC ₇ S ₁	Advanced
ISR-380	<i>L. serriola</i>	Romaine	BC ₅	Advanced
CGN5309	<i>L. serriola</i>	Green Leaf	BC ₅	Advanced
CHEC-023	<i>L. serriola</i>	Crisphead	BC ₄	Advanced
CHEC-063	<i>L. serriola</i>	Crisphead	BC ₄	Advanced
CHEC-076	<i>L. serriola</i>	Crisphead	BC ₄	Advanced
CHEC-147	<i>L. saligna</i>	Romaine	BC ₃	Intermediate
CHEC-101	<i>L. saligna</i>	Romaine	BC ₂	Intermediate
CHEC-136	<i>L. saligna</i>	Red Leaf	BC ₃	Intermediate
RUSS-635	<i>L. serriola</i>	Red Leaf	BC ₃	Intermediate
KYRGY-237	<i>L. serriola</i>	Romaine	BC ₃	Intermediate
GEOR-301	<i>L. serriola</i>	Romaine	BC ₃	Intermediate
GEOR-289	<i>L. serriola</i>	Romaine	BC ₃	Intermediate
GEOR-297	<i>L. serriola</i>	Red Leaf	BC ₃	Intermediate
GEOR-292	<i>L. serriola</i>	Green Leaf	BC ₃	Intermediate
GEOR-299	<i>L. serriola</i>	Crisphead	BC ₃	Intermediate
CHEC-022	<i>L. serriola</i>	Crisphead	BC ₂	Intermediate
CHEC-075	<i>L. serriola</i>	Crisphead	BC ₂	Intermediate
CHEC-082	<i>L. serriola</i>	Crisphead	BC ₂	Intermediate
CHEC-083	<i>L. serriola</i>	Crisphead	BC ₂	Intermediate
CHEC-088	<i>L. serriola</i>	Crisphead	BC ₂	Intermediate
CHEC-089	<i>L. serriola</i>	Crisphead	BC ₂	Intermediate
05G1421	<i>L. serriola</i>	Butterhead	BC ₃	Intermediate
CHEC-158	<i>L. saligna</i>	Green Leaf	BC ₂	Intermediate
KYRGY-247	<i>L. serriola</i>	Butterhead	BC ₂	Intermediate
CHEC-108	<i>L. saligna</i>	Green Leaf	BC ₁	Early
CHEC-132	<i>L. saligna</i>	Green Leaf	BC ₁	Early
CGN13330	<i>L. saligna</i>	Romaine	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
CGN5157	<i>L. saligna</i>	Green Leaf	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
CGN5301	<i>L. saligna</i>	Romaine	F ₁	Early

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 (BC₆S₁) are then tested a diverse panel of contemporary virulent Californian isolates. In the past year, the two most advanced green leaf lines in Table 1 were tested for resistance against five different LDM isolates that represented much of the current genetic variation observed in *B. lactuca* (see Figs. 1 & 2 below). One line was resistant to all of the isolates tested; the other line was resistant to three of the five isolates assayed but susceptible to the other two. Analysis with additional isolates will be made in the coming year. These two lines will be intercrossed and existing segregating progeny will be analyzed to genetically characterize their resistance and provide molecular markers to select for lines with both sources of resistance. Release of lines with pyramids of resistance genes should increase the durability of the individual genes.

Downy Mildew Surveys

In order to ensure that we are breeding for resistance against virulent phenotypes of the pathogen *Bremia lactuca* currently present in California, we have continued to sample *B. lactuca* 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 1,750 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.

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. lactuca* in California. During this period the number of isolates characterized was considerably higher, averaging ~180 isolates per year. This provided a comprehensive understanding of variation in California. Reference isolates have been sequenced to provide information on variability at the DNA level. Since the end of this funding, we have had to return to opportunistic characterization of lower numbers of isolates.

We have 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. 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. lactuca* 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 now use the same core differential set of resistant cultivars so that data can be compared.

Nomenclature is being standardized so that denominated isolates will be designated Bl:#EU or US. The US Pathotypes I to VIII will become 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. 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 Race and used in cultivar resistance descriptions. There are currently eight official Pathotypes/Races; 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, 105 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 Pathotype CAV or CAVI; 5% were CAVII; 7% were CAVIII; and 1% were candidate Pathotype CAIX (see below). By far the majority of isolates (89%) had novel virulence phenotypes that differed from the Pathotypes (Fig. 1). Of all the isolates, only eight (8%) were able to overcome *Dm17*. *Avr36*, *Avr37* and *Avr38* were present in 25, 44, and 89% of the isolates, respectively. *Avr4* was detected at a frequency of 25%; *Avr6* had increased from 30% to 66% (Fig. 2). European cultivars Silvinas (n1), Murai (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 2009-2016.

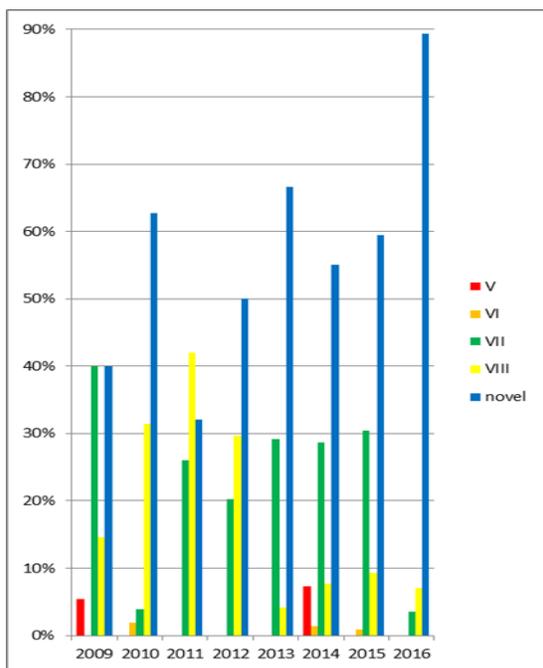
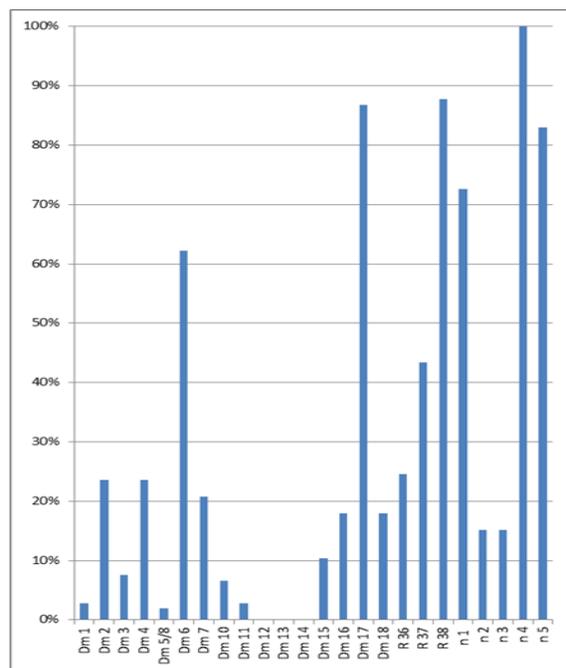


Figure 2: Frequency (%) of avirulence genes observed in California in 2016.



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*. This group of novel isolates was nominated 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. A candidate type isolate for Pathotype IX was distributed to seed companies for testing; however, its virulence phenotype was not stable. In the meantime, isolates with this virulence phenotype were rarely detected; therefore this group of isolates was not denominated as Pathotype IX.

At the October 2015 IBEB-US meeting, two more groups of isolates 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. Testing of candidate type isolates by the companies is ongoing.

Of 105 isolates characterized for mating type, 87 (83%) and 18 (17%) were B₂ and B₁ respectively. Isolates of B₁ mating type were distributed throughout California and all had novel avirulence phenotypes (Table 2). In 2015, 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 (CLGRB report 2015). 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/Races and sexual oospores surviving in the soil, which will alter the epidemiology of downy mildew, possibly causing earlier more widespread epidemics.

Table 2: Geographical distribution and virulence phenotypes of isolates with B₁ mating type collected in 2016.

Number of isolates	Location	Avirulence phenotype
2	Davis	1,3,5/8,10,15,16,17,18,36,37,n1,n2,n3,n4,n5,
1	Chualar	2,11,16,17,18,36,37,38,n3,n4,n5,
1	Chualar	2,3,7,16,17,18,36,37,38,n3,n4,n5,
1	Chualar	2,4,7,15,36,37,38,n4,n5,
1	Santa Maria	2,4,7,15,38,n4,r1,r2,
2	King City & Chualar	2,4,7,17,18,36,37,38,n4,
1	Santa Maria	2,4,7,Rsal-1,38,n4,r1,r2,
1	Chualar	2,6,7,11,16,17,18,36,37,38,n3,n4,n5,
3	Gonzales & San Lucas	2,7,16,17,18,36,37,38,n3,n4,n5,
3	Gonzales, Salinas & Chualar	2,7,16,17,18,36,37,38,n4,n5,
1	Chualar	2,7,17,18,36,37,38,n4,
1	Greenfield	4,6,15,17,n1,n4,n5,

Of the 104 isolates analyzed for metalaxyl sensitivity in 2016, 63 (61%), 4 (4%), 2 (2%), and 35 (34%) 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. However, of the 18 B₁ isolates phenotyped, only one was insensitive to metalaxyl.

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.

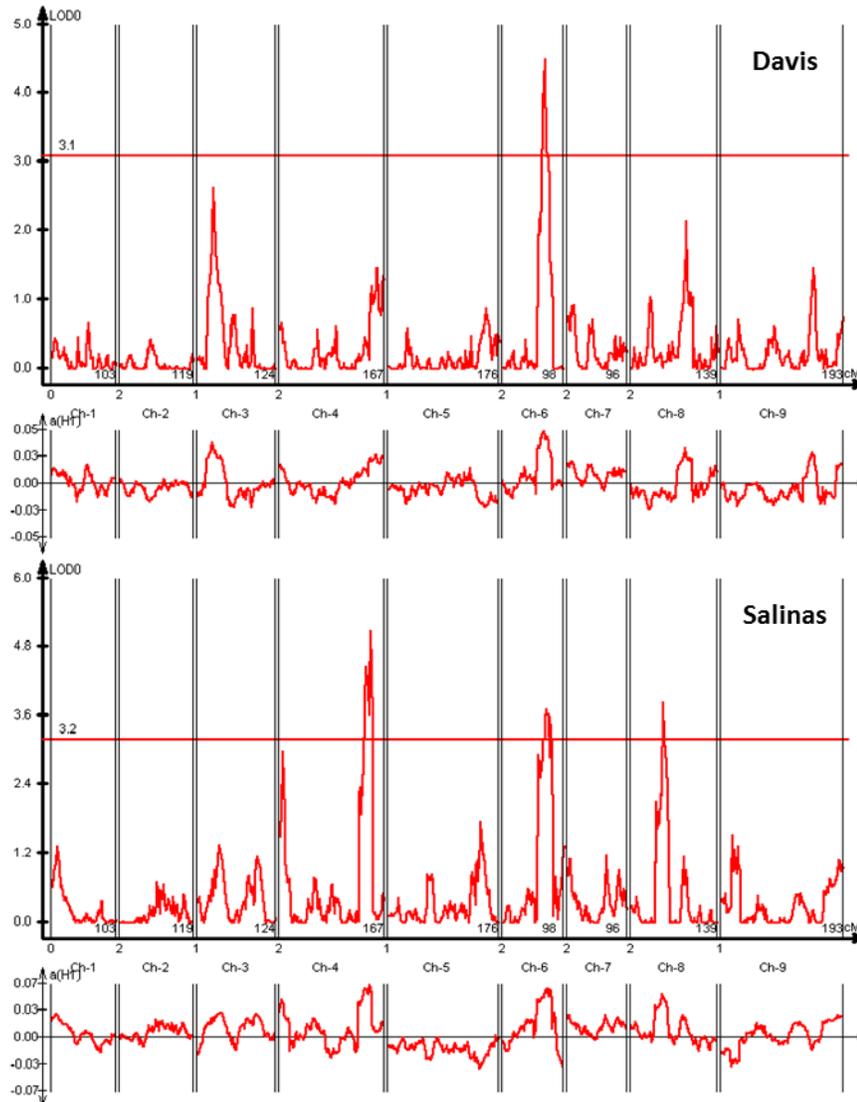
We have continued to screen germplasm for resistance to race 2. Thirty-five new *L. serriola* lines from Georgia, Kyrgyzstan and Uzbekistan were received via the USDA and a subset of them was evaluated for resistance in 2015. None of these lines was completely resistant but a few lines had individuals 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. In 2016 we received 67 new accessions of *L. serriola* from Azerbaijan that were seed increased; these will be screened for resistance in 2017.

The majority of accessions screened for resistance over the past five 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. These were intermated 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, this population 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 were evaluated in replicate for resistance to *Verticillium* race 2 in our greenhouse sick plots. The same population plus additional individuals (134 individuals in total) was evaluated for resistance in the greenhouse by German Sandoya at the USDA Salinas station. These RILs were genotyped by sequencing and QTL analysis was performed for data from both trials. A QTL in linkage group 6 was significant in both trials. Two additional QTLs were identified in the Salinas trial in linkage groups 4 and 8 but were not significant in the Davis trial (Fig. 3). This variation is consistent with environmental sensitivity of this resistance. In collaboration with German Sandoya (now at University of Florida) we are comparing these

results with QTLs for resistance observed in other populations. We are introgressing this QTL for resistance into lettuce cultivars.

Figure 3: QTLs for resistance to *Verticillium* race 2 in Davis and Salinas trials. The horizontal red line is the 5% LOD significance threshold as determined by 1,000 random permutations of the data.

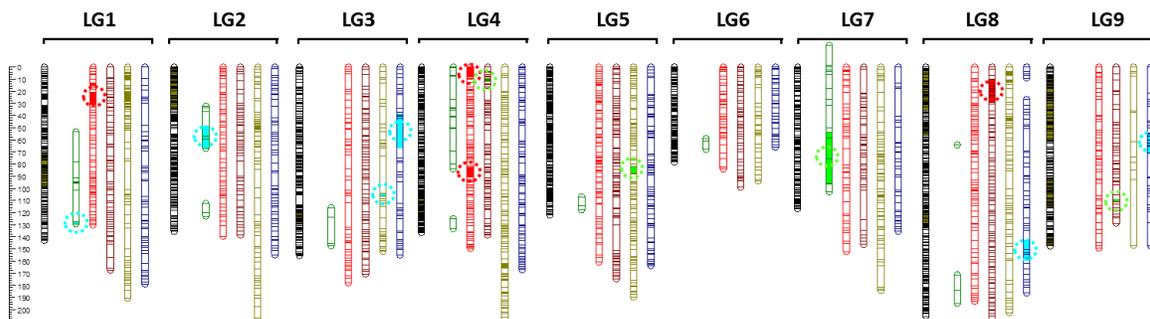


Resistance to *Fusarium* Wilt

We have continued our genetic analysis of resistance to *Fusarium oxysporum* race 1 in collaboration with Dr. Thomas Gordon (Dept. Plant Pathology, UC Davis). We previously developed four populations involving resistant (R), partially resistant (PR) and susceptible (S) cultivars: Salinas (PR) x Green Towers (R), Lolla Rosa (R) x Salinas (PR), Lolla Rosa (R) x Green Towers (R) and Lolla Rosa (R) x Red Tide (S) (R = resistant, PR = partially resistant, S = susceptible). Analysis in previous years of crosses between Lolla Rosa x Salinas and Salinas x Green Towers indicated one or two genes responsible for resistance. Also, QTL analysis of progeny from Lolla Rosa x Red Tide indicated the

presence of one major QTL in LG1 and three minor ones, two on LG4 and one on LG8. We had previously identified a QTL for resistance to *Fusarium* also in LG1 from Valmaine. Current data indicated that the resistance QTLs from Lolla Rosa and Valmaine are in different regions. However, precise integration of data across populations awaited more complete genetic maps for each population. We have now genotyped three of these populations (Lolla Rosa X Salinas, Lolla Rosa x Red Tide and Salinas x Green Towers) using Genotyping by Sequencing (GBS), have developed detailed genetic maps for each and conducted QTL analysis. In collaboration with Jim McCreight, we phenotyped an F₂ population generated from a cross between King Louie (R) x Autumn Gold (S) for *Fusarium* resistance. Different QTLs were identified for resistance to *Fusarium* in the five populations (Fig. 4). This provides the possibility of combining multiple sources of resistance in a single genetic background. Only the top of LG4 had a QTL identified in two populations. Fine mapping of the QTL regions is underway.

Figure 4: QTLs for resistance against *Fusarium* and *Verticillium* wilts. Linkage groups for each chromosome (LG1 to LG 9) from left to right correspond to: Salinas x *L. serriola* core population, Valmaine x Salinas, Red Tide x Lolla Rosa, Lolla Rosa x Salinas, Salinas x Green Towers and King Louie x Autumn Gold. Resistant QTLs are color coded depending on the parental conferring the *Fusarium* resistance as follows: In aqua from Valmaine, Green Towers and King Louie (romaines), in green from Salinas, and in red from Lolla Rosa.



In 2014 and 2015 we planted a subset of the best ~20 resistant lines based on previous field data from each of Valmaine x Salinas, Salinas x Green Towers, Lolla Rosa x Salinas and Red Tide x Lolla Rosa populations in order to select *Fusarium* resistant lines of different lettuce types. Resistant selections were evaluated for uniformity in non-infected field in Davis in 2016. We selected 11 lines from Valmaine x Salinas, 13 lines from Salinas x Green Towers, 20 lines from Lolla Rosa x Salinas and 8 lines from Red Tide x Lolla Rosa populations. Uniform lines were planted in the *Fusarium* sick plot in Davis in the summer of 2016 to confirm resistance. These lines are being characterized to identify markers associated with the QTLs for resistance prior to release to seed companies.

Supply of Isolates

We have continued to supply current California isolates of downy mildew and corky root to breeding companies and other research groups. In particular, we have supplied isolates of the nominated Pathotypes V to VIII (Bl:5US to Bl:8US) as well as candidate isolates for ring tests. We have trained personnel from the seed industry and others to handle lettuce downy mildew and corky root.