CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

April 1, 2019, to March 31, 2020

GENETIC VARIATION IN LETTUCE

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SUMMARY:

We continue to develop and apply novel methods for detecting, analyzing, and manipulating genetic variation in lettuce. This project aims to ensure that lettuce benefits from the application of genomic and biotechnological techniques and has four components: (i) Transgene expression and genome editing in lettuce. (ii) Cloning and characterization of disease resistance genes. (iii) Genetic mapping with the goal of locating and developing markers for most of the disease resistance genes known in lettuce. (iv) Comparative genomics to identify candidate genes controlling horticultural traits. We continue to make extensive use of high-throughput sequencing, marker technologies, and genome editing for gene identification. Genome sequences have been and are being mined for candidate genes for traits such as disease resistance, development, and horticulturally important traits such as tipburn resistance. Ten new genes for resistance to downy mildew have been mapped to the lettuce genome and candidate genes identified using k-mer analysis. Lines for resistance to *Fusarium* were successfully trailed in Yuma and are ready for release. New sources of resistance to *Fusarium* have been identified in a diversity panel trialed in an infested field at Davis. We have initiated a program to study salinity tolerance in lettuce. The v8 version of the reference genome assembly of lettuce cv. Salinas is publically accessible and is being widely used. The v10 version with nine chromosomal scaffolds is being polished that has much improved contiguity and sequence accuracy. We continue to curate several databases that include genetic, molecular marker, cultivar, phenotypic and sequence data for lettuce.

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PROJECT TITLE: **GENETIC VARIATION IN LETTUCE**

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OBJECTIVES:

To develop and apply new methods for detecting, analyzing and manipulating variation in lettuce. We continue to pursue these objectives in four sub-projects:

1) Analysis of transgenes in lettuce and genome editing.

2) Molecular cloning of genes for disease resistance and other horticultural traits.

3) Development of a detailed genome assembly and identification of reliable, readily assayed markers linked to disease resistance genes.

4) Utilization of comparative genomics to identify candidate genes controlling horticultural traits and development of robust molecular markers for them.

In the first three projects we are mostly emphasizing either novel forms of disease resistance or increasing the efficiency of selection for disease resistant genotypes. The fourth objective includes a wide range of horticultural traits. Some of these studies have been funded

from Federal grants and support from seed companies. All projects were initiated with CLGRP funds and application of the results to lettuce improvement is supported by CLGRP funds. All projects impact improvement of both crisphead and leafy types.

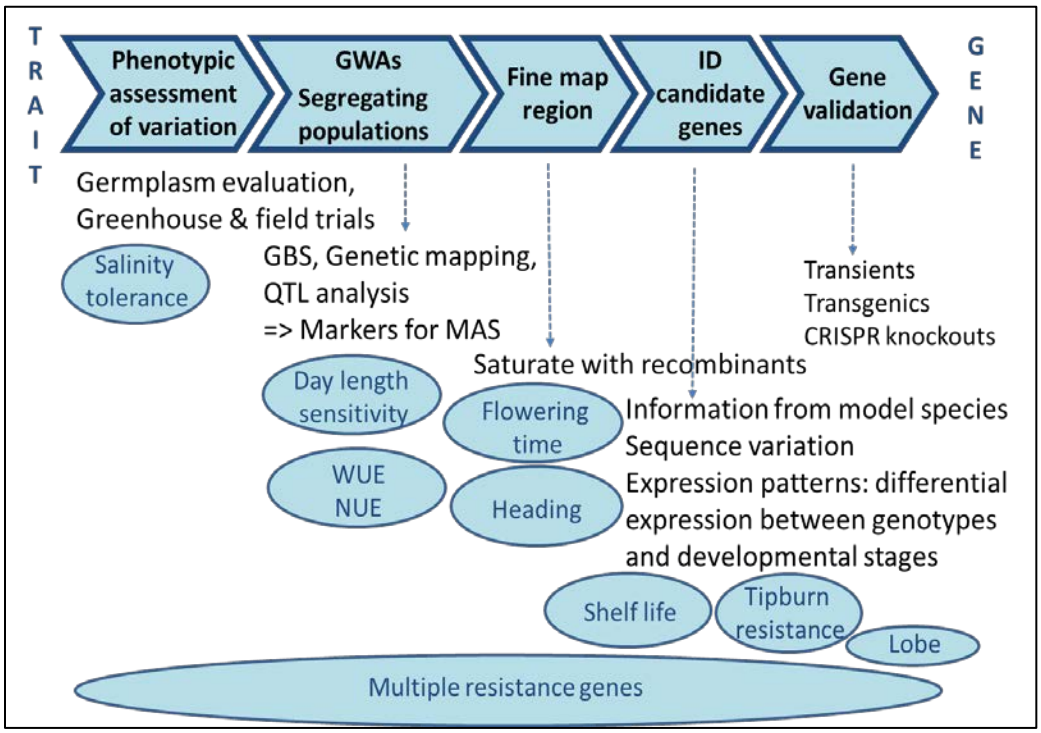
PROCEDURES AND RESULTS:

Transgene Expression and Genome Editing in Lettuce

Our studies on transgene expression remain a low priority, in part because there is not a major breeding objective that can only be addressed by transgenic lettuce and therefore commercial deployment of transgenes in lettuce is not a near-term need. Introduction of genes into lettuce using *A. tumefaciens* is routine. We continue to generate transgenics as components of other projects (see below); these provide additional data on transgene expression and stability.

Since 2012, technology for precise genome editing based on the CRISPR/Cas9 system has been developed for use in plants and animals. Gene knock-outs are currently much easier than sequence replacements or additions but the enabling technology is advancing rapidly. We continue to develop and apply genome editing technology for lettuce as part of our gene identification strategy (Fig. 1).

Figure 1. Workflow for identifying genes causing useful phenotypes.



In addition to validating potential candidate genes for disease resistance, we are targeting a subset of the many genes that have been previously functionally characterized in other plant species, particularly Arabidopsis. We are using CRISPR-mediated gene knockouts to deduce whether the homologous genes in lettuce maintain a similar function. We are initially investigating genes potentially controlling traits such as nutrient content, leaf, flower, and root development, and flavor profile.

In the longer term, once the technology has been developed adequately, we will use genome editing of lettuce to create stacks of resistance genes containing several resistance and other genes at single chromosomal positions so that they will be inherited as single Mendelian loci in breeding programs. Such stacks could contain multiple resistance genes effective against all known pathotypes of downy mildew and multiple viral, bacterial, fungal pathogens as well as insect pests and nematodes. This will enhance the durability of resistance by increasing the evolutionary hurdle that pathogens will have to overcome. Gene stacks could be expanded as more resistance genes become available and genes replaced when *Dm* genes are overcome by changes in the pathogens. A herbicide resistant gene (e.g. *ALS*) could be used as selectable marker for the gene stack. Gene stacking will greatly simplify breeding for disease resistance so that breeders can focus on more complex traits such as water and nitrogen use efficiencies and nutritional quality. However, in order for this to happen technology for inserting genes, preferably without tissue culture, need to be developed and genes for resistance to each disease need to be identified at the molecular level.

Resistance Gene Identification

We continue to map loci for resistance to downy mildew (DM), corky root, *Fusarium* and *Verticillium* wilts, onto the consensus genetic map and place them on the genome sequence. We are also continuing to develop molecular markers to assist the selection of resistance genes. Of the over 50 phenotypic resistance genes mapped in lettuce, most co-localize to one five major resistance clusters on chromosomes 1, 2, 3, 4, and 8 (MRCs 1, 2, 3, 4, and 8 respectively). The majority of these resistance phenotypes are linked to NB-LRR-encoding (NLR) genes as described in previous CLGRB reports that provide markers for selecting for these resistances.

Resistance to Downy Mildew

Numerous genes for resistance to DM have been introduced into cultivated lettuce from wild species (*L. serriola, L. saligna* and *L. virosa*) by repeated backcrossing by public and commercial breeders. This has resulted in near-isogenic lines (NILs) that only differ for small chromosome regions that are potentially associated with resistance. Twelve advanced breeding lines previously released from the UC program show resistance to many, although not all, isolates of *B. lactucae* in California, including recent highly virulent isolates.

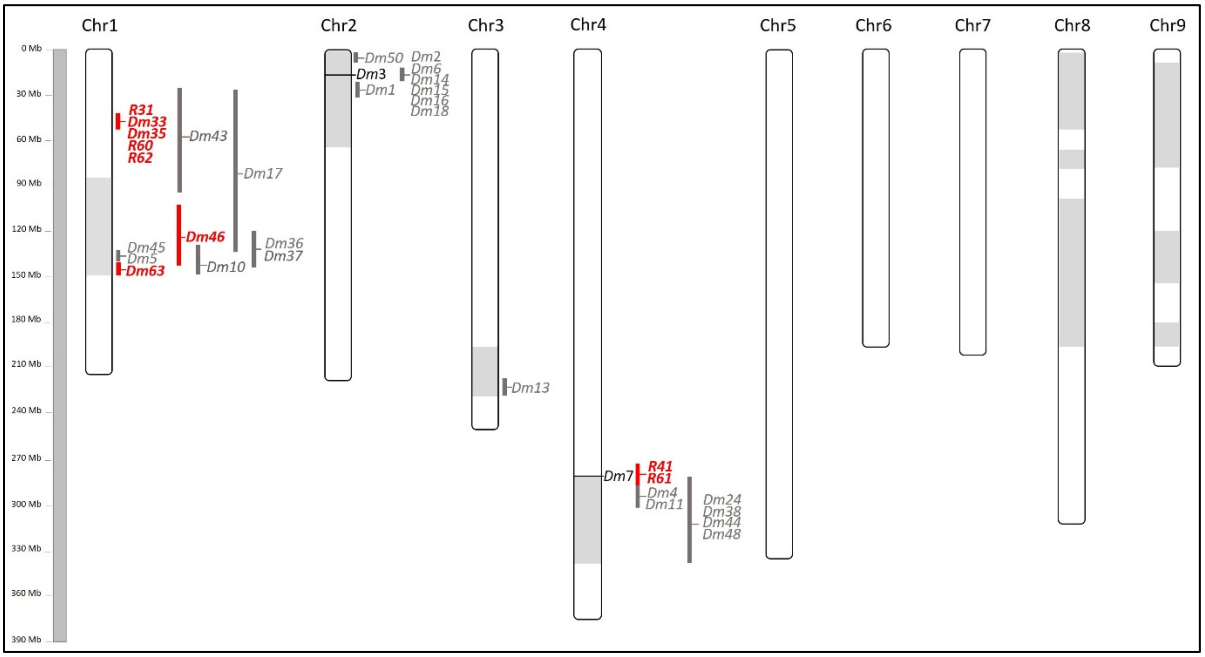
We have generated F_2 populations from crosses between these NILs and cv. Salinas in order to map the resistance to DM in the reference genome. F_2 progeny from these crosses were genotyped by sequencing and the resistance genes were mapped into the reference genome (Table 2, Fig. 1). New resistance genes from lines UC02202, UC02204, and UC07107 were named as numbered *Dm* genes consistent with their prior resistance factor denominations described in Parra *et al*. (2016, *Euphytica* **210**:309. https://doi.org/10.1007/s10681-016-1687-1). Resistances from UC02206, UC07105, UC07106, and UC07108 will remain as R factors until we can prove uniqueness of their resistance specificity. New resistance genes from lines UC12100, UC12101, UC12102 and UC12103 have been submitted to the International Bremia Evaluation Board (IBEB) for denomination.

Table 1. Advanced breeding lines with resistant donor accessions, *Dm* gene denominations and chromosomal locations.

ID	Donor	Dm-gene	Location	Isolate
UC02202	*L. saligna* LJ85314	*Dm33*	Chr1	C15L1742
UC02204	*L. virosa* LJ85289	*Dm35*	Chr1	C15L1777, C17L1926
UC02206	*L. serriola* W66331A	*R31*	Chr1	C17L1926
UC07105	*L. sativa* PI491226	*R41*	MRC4	C15L1777
UC07106	*L. serriola* PI491108	*R42*	n.d	C11O1326
UC07107	*L. saligna* PI491206	*Dm46*	MRC1	C11O1326
UC07108	*L. saligna* PI491208	*R47*	MRC2, MRC4	C15L1777
UC12100	*L. saligna* CGN9311	*R60*	Chr1	C15L1742, C17L1926
UC12101	*L. saligna* CGN5318	*R64*	MRC1, MRC2	C15L1691
UC12102	*L. saligna* CGN5282	*R61*	MRC4	C15L1777
UC12103	*L. saligna* CGN5147	*R62*	Chr1	C17L1926
UC12103	*L. saligna* CGN5147	*R63*	MRC1	C15L1691

n.d. Not determined

Figure 2: The genomic locations of new resistance genes (in red) on lettuce chromosomes.



In order to identify the resistance genes present in the advanced breeding lines we have used Resistance gene enrichment sequencing (RenSeq) and long fragment sequencing (PacBio) for the characterization of the resistance gene repertoires present in each breeding line and from 48 lettuce genotypes, carrying known *Dm* genes. This data in combination with phenotypic data from these lines was used for k-mer association analysis, which allowed the identification of several candidate genes for known and new resistances against *B. lactucae* (Table 2). Resistance genes will be validated using CRISPR-mediated knock-outs of the candidate gene.

Table 2: Candidate genes for resistance to downy mildew obtained using k-mer association mapping.

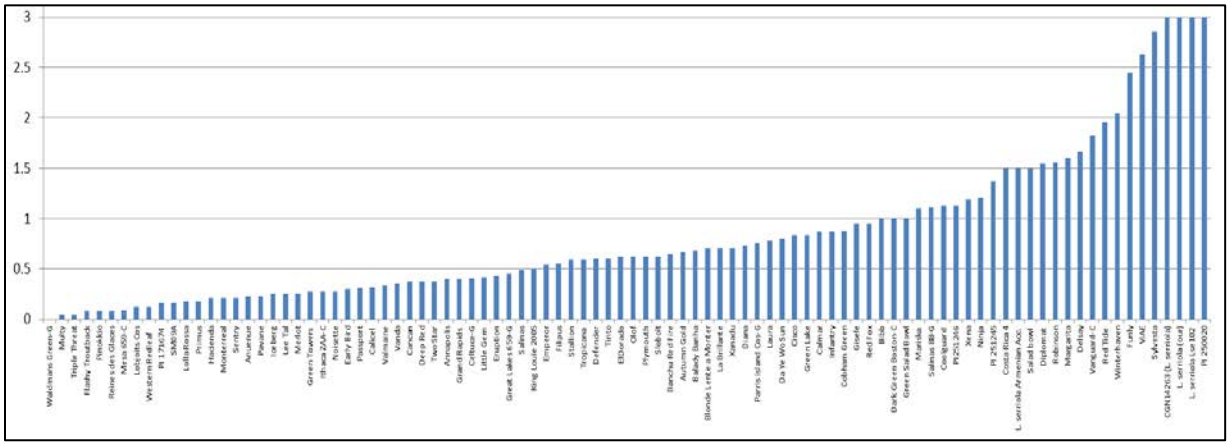
R-gene	cultivar/line	N candidate genes	(best candidate) type of gene
Dm33	UC02202	6	RLK
Dm35	UC02204	2	RLK
R31	UC02206	9	RLK
R41	UC07105	4	NLR
R42	UC07106	42	NLR
Dm46	UC07107	5	NLR
R61	UC12102	3	NLR
R62	UC12103	3	RLK
R63	UC12103	7	NLR

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). In the past, we tested several populations that were segregating for *Fusarium* resistance in an infested field at Davis. QTL analyses of these populations allowed us to identify regions on the genome associated with resistance in different chromosomes (see previous CLGB reports). Selections from these populations were trialed for resistance in Yuma in 2019 (see below).

In the summer of 2019, we trialed a diversity panel at Davis comprising 120 accessions mostly of *L. sativa* with few *L. serriola* in order to search for additional sources of resistance. We have genomic sequence information for all these lines. These lines showed a broad range of susceptibility to *Fusarium*; some lines showed high levels of resistance (Fig. 3). We are presently analyzing the resistance and genomic data to identify regions on the genome associated with resistance.

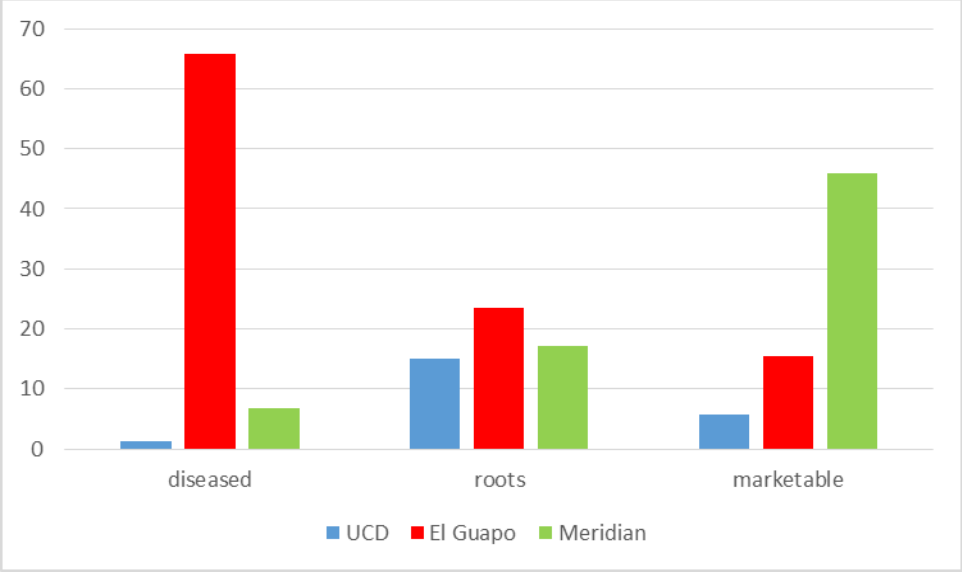
Figure 3. Disease distribution of 120 accessions in the summer 2019 trial at UC Davis.
Rating: 0 = no disease, 3 = all dead.



Yuma trials for resistance to *Fusarium*

In collaboration with Stephanie Slinski at the Yuma Center for Excellence for Desert Agriculture (University of Arizona), we trialed a set of lines that had been selected for resistance to *Fusarium* in Davis. Two trials were conducted in Wellton and Yuma, AZ. The trial in Wellton had weather and waterlogging problems that impacted seed emergence. The field in Yuma performed better, had good disease pressure, and provided informative data on resistance to *Fusarium*. UC Davis lines were compared to cultivars El Guapo and Meridian. Disease resistance was evaluated on the lettuce heads at harvesting time. In addition, the roots of four heads per plot were evaluated for the presence of brown discoloration indicative of infection. The UC Davis lines were highly resistant with lower percentages of diseased heads than cvs. El Guapo and Meridian (Fig. 4). The percentage of roots with brown discoloration was also smaller on the UCD lines. UC Davis lines still have residual heterozygosity that is reflected on a small percentage of marketable heads in comparison to cvs. El Guapo and Meridian. These lines are being released.

Figure 4. Percentage of diseased heads, presence of brown root discoloration and marketable heads in selected UC Davis lines and cvs. El Guapo and Meridian.



Remote Sensing

In collaboration with Dr. Duke Pauli (University of Arizona), we conducted a field trial at the Maricopa Agricultural Center, AZ to study water use efficiency in lettuce. The Maricopa Agricultural Center hosts the largest field analytical robot in the world. The high-throughput phenotyping field-scanning robot has a gantry that moves in rails while imaging the crops growing below with a diverse array of cameras and sensors including scanners for hyperspectral and thermal imaging, 3D laser and chlorophyll fluorescence (Fig. 5). The lettuce trial was conducted with three levels of water deficit stress. We trialed a RIL population from a cross between cvs. Grand Rapids x Iceberg that have been previously studied in Salinas for water use efficiency (WUE) and a diversity panel of 150 cultivars (iceberg, Batavia, butterhead, romaine, and green and red leaf types). There were three replications per irrigation level resulting in ~2,600 plots. The objectives were to understand the genetic base controlling agronomic and stress-adaptive traits, to monitor growth and development, quantifying water deficit stress and the impacts on photosynthetic efficiency in addition to develop analytical tools for remote sensing.

Figure 5. Lemnatec Field Scanalyzer at the University of Arizona Maricopa Agricultural Center scanning lettuce.

We harvested one head of lettuce for each plot that was measured fresh and dry weight and the stem length. In addition, we took pictures of the cut lettuce heads. These images are being analyzed for height and width of the head and the stem, and shape and area of the head. These measurements will be used to train algorithms for machine learning, so in future trials growth measurements can be performed with automatic sensors.

Genetics of Salt Tolerance in wild *Lactuca* species

We have initiated an investigation of the genetic basis of salt tolerance in order to breed lettuce cultivars with increased salt tolerance. In the past year, we have screened wild *Lactuca* germplasm for salinity tolerance. Phenotypic variation in fresh biomass reduction was observed among the *Lactuca* germplasm when treated with moderate salinity (100 mM NaCl; Table 2). Accessions with contrasting sensitivity have been selected based on fresh biomass as parents for RIL populations and are being crossed for mapping populations to identify QTLs and candidate genes for salinity tolerance. Greenhouse salinity trials using the selected parental accessions are underway to characterize the salinity stress response of *Lactuca* species as well as determining key traits to evaluate on the mapping populations.

Table 2. Average % reduction of fresh shoot weight in select accessions of *Lactuca* germplasm irrigated with 100 mM NaCl for 3 weeks (two replications, n = 8)

Tolerant Accessions	% Reduction of Fresh Shoot Weight in 100 mM NaCl	Susceptible Accessions	% Reduction of Fresh Shoot Weight in 100 mM NaCl
L. aculeata	-7.4	'Salinas'	-40.4
UC96US23	-8.9	PI 667821	-32.7
W6 37147	-13.1	PI 667815	-30.7

Genome and Transcriptome Sequencing

The v8 of the reference genome of lettuce is publically available at https://lgr.genomecenter.ucdavis.edu/ as well as several public databases such as GenBank, CoGe, and Phytozome and is being widely used. In the past year, analysis of the more contiguous v9.0 genome assembly revealed problems with the sequence and therefore v9 will not be released publically. We have generated the v10 genome assembly *de novo* from long reads generated with Oxford Nanopore and corrected using PacBio HiFi and Illumina reads resulting in chromosome-scale genomic scaffolds. This is currently being polished using Bionano data. This has greatly improved contiguity and sequence accuracy compared to v8 and v9 assemblies.

In collaboration with academic groups working on different aspects of lettuce biology, we are continuing to conduct RNAseq profiling experiments to provide an atlas of genes expressed in lettuce at different developmental stages, under different abiotic stresses, and during resistance and susceptibility to diverse diseases. We now have over 800 tracks of RNAseq data from multiple diverse experiments. These data are being made available through our website.

Databases

We continue to curate several publicly accessible databases for lettuce accessible through http://michelmorelab.ucdavis.edu. The G2G site (http://scri.ucdavis.edu/) provides access to information generated as part of the Next-Generation Lettuce Breeding: Genes to Growers (G2G) and CLGRP-funded projects. Our GBrowse genome viewer (http://gviewer.gc.ucdavis.edu/cgi-bin/gbrowse/lettucePublic/) provides access to the ultra-dense map as genetic chromosomal pseudomolecules. These databases continue to be revised to facilitate access to marker information for breeding purposes from disease-centric, breeder-oriented perspectives. The Bremia Database displays virulence phenotypes, mating type and fungicide sensitivity for Californian isolates of *B. lactucae* characterized from 2001 to the present (http://bremia.ucdavis.edu/bremia_database.php).