CALIFORNIA LEAFY GREENS RESEARCH PROGRAM
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REPORT

Project Title: Lettuce Breeding and Genetics (USDA)

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Abstract:

The lettuce industry of California requires continued development of improved, adapted cultivars to meet new disease and insect problems, changes in the market, and changes in growing procedures. The lettuce breeding and genetics project aims to incorporate valuable traits into crisphead, mixed lettuce, and spring mix cultivars and breeding lines that are adapted to coastal California and low desert production conditions. In parallel, we develop information and tools to increase the utility of our germplasm. In 2017 and 2018, we emphasized developing resistance to Verticillium wilt, lettuce drop, Fusarium wilt, corky root, downy mildew, tospoviruses, bacterial leaf spot, leafminer, lettuce aphid, as well as bolting resistance, extending fresh-cut salad shelf-life, and development of molecular markers for marker-assisted selection. In all programs, horticultural traits, adaptation, and resistance to tipburn were considered essential.

Objectives:

Develop landmark cultivars, advanced breeding lines, information, and tools for use by other breeders, scientists, producers and growers. Address problems facing the lettuce industry using genetic approaches that are suited to coastal, desert, and interior valley locations, to the various types of lettuce, and to the demands of different production and marketing approaches.

Procedures and Results:

VERTICILLIUM WILT (with R. Hayes and K. Subbarao)

Verticillium wilt is a highly destructive soil borne disease caused by the fungus *Verticillium dahliae*. Two races (race 1 and race 2) of the pathogen are known. Resistance to race 1 is provided by the *Vr1* gene. We have developed iceberg breeding lines that combine resistance to race 1 isolates of *V. dahliae* with resistance to corky root. The three breeding lines are derived from crosses between breeding line carrying *Vr1* (RH12-1158) to the corky root resistant cultivar Telluride (Fig. 1). Horticultural traits were evaluated in three field experiments in Salinas. Data from multiple experiments show that RH14-1156, RH14-1157, and RH14-1158 are completely resistant to race 1 isolates of *V. dahliae* and have very high level of resistance to corky root (similar to resistant cv. Telluride). Head width and core height were within the range of performance of the control cultivars Glacier, Tiber and Salinas. RH14-1158 demonstrated somewhat longer core compared to other tested cultivars and breeding lines. The RH14-1156 to 1158 breeding lines have circular shape of head. RH14-1157 showed the best combination of head weight, core height, head shape, and tipburn resistance (Table 1). These breeding lines were released in 2018. Limited quantities of seeds are available for distribution. Seeds can be requested from Ivan.Simko@ars.usda.gov.

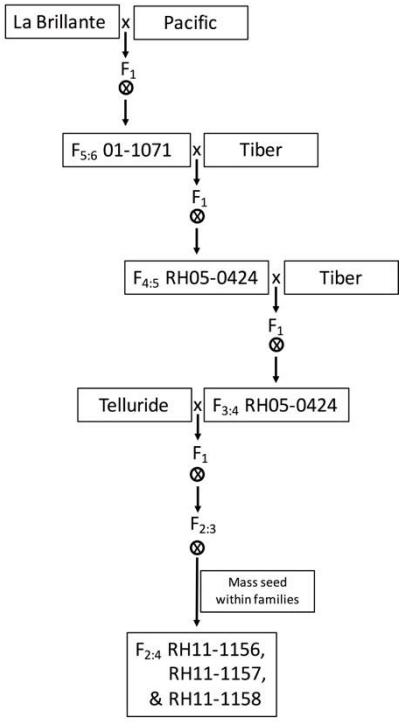

Figure 1. Pedigree of iceberg breeding lines RH11-1156, RH11-1157, and RH11-1158 with combined resistance to verticillium wilt and corky root disease. Seed from each generation of self-pollination were kept separate to develop $F_{5:6}$ 01-1071, $F_{4:5}$ RH05-0424, and $F_{3:4}$ RH09-0689. Seed from self-pollination of approximately 90 $F_{2:3}$ plants within three families derived from Telluride x RH09-0689 was massed to create three $F_{2:4}$ seed lots (Hayes et. al. 2018, HortScience).

Table 1. Performance of three iceberg breeding lines, parents, and check cultivars for disease and horticultural traits in three replicated field experiments in Salinas, California.

	Vert. wilt	Corky root	TB (%)	Weight (g)	Height (cm)	Diam. (cm)	Core (cm)
RH14-1156	R	5.9	26	621	14	16	7
RH14-1157	R	5.9	23	768	14	16	6
RH14-1158	R	6.0	39	712	15	17	11
RH12-3197	R	7.1	31	644	13	17	5
Telluride	S	5.9	39	653	15	16	8
Glacier	S	6.0	30	596	13	16	7
Salinas	S	7.8	27	613	14	16	7

Resistance to Verticillium wilt: R – resistant, S – susceptible.
Corky root disease severity rated as 0 = no disease to 9 = severe disease causing plant death.
Tipburn incidence in percent.
Head height, diameter, and core length at harvest maturity.

In addition, we tested over 6,000 plants from 203 cultivars, and breeding lines. Fifty-seven of the accessions had disease index below 10%, indicating presence of the *Vr1* gene. Forty-five new crosses were made to combine resistance to Verticillium wilt, corky root, bacterial leaf spot, and lettuce drop.

LETTUCE DROP (with B. Mamo, K. Subbarao, R. Hayes)

Lettuce drop is a near ubiquitous soil borne disease of lettuce caused by the fungal pathogens *Sclerotinia minor* and *S. sclerotiorum* resulting in decay of the crown tissue, wilting of leaves and total collapse of the entire plant before harvest. The use of genetic resistance as part of an integrated lettuce drop management strategy would provide a sustainable approach of reducing yield loss. We initiated a single seed descent breeding program to develop lettuce cultivars resistant to drop. Overall, this method is expected to increase selection efficiency and reduce the time it takes to develop desired cultivars compared to previous methods.

Lettuce drop resistance is often associated with traits related to plant development, primarily rapid bolting. Due to this reason, lettuce drop resistance identified in the past could not be used in breeding of modern lettuce cultivars. They do not fit to commercial interest. Recently, high level of resistance to *S. minor* and *S. sclerotiorum* independent of plant morphology and is genetically determined was identified in the slow-bolting red Latin type cultivar ‘Eruption’. To use this resistance in breeding, it is important to determine the inheritance of drop resistance and the number and location of resistance loci in ‘Eruption’. To this effect, a study population was

developed from a cross of ‘Eruption’ to the susceptible cultivar ‘Batavia Reine des Glaces’ (‘BRG’). Recombinant inbred lines (RIL) totaling 162 from ‘BRG’ × ‘Eruption’ cross were selfed to the F_8 generation. These RIL were used for genetic experiments to determine the inheritance of lettuce drop resistance in ‘Eruption’, map its genetic basis and characterize the relationship to those that underlie rapid bolting. The population was genotyped by sequencing (GBS) from which 840 single nucleotide polymorphism (SNP) markers were identified. The SNP markers mapped to and evenly distributed along the nine linkage groups of lettuce (Fig. 2).

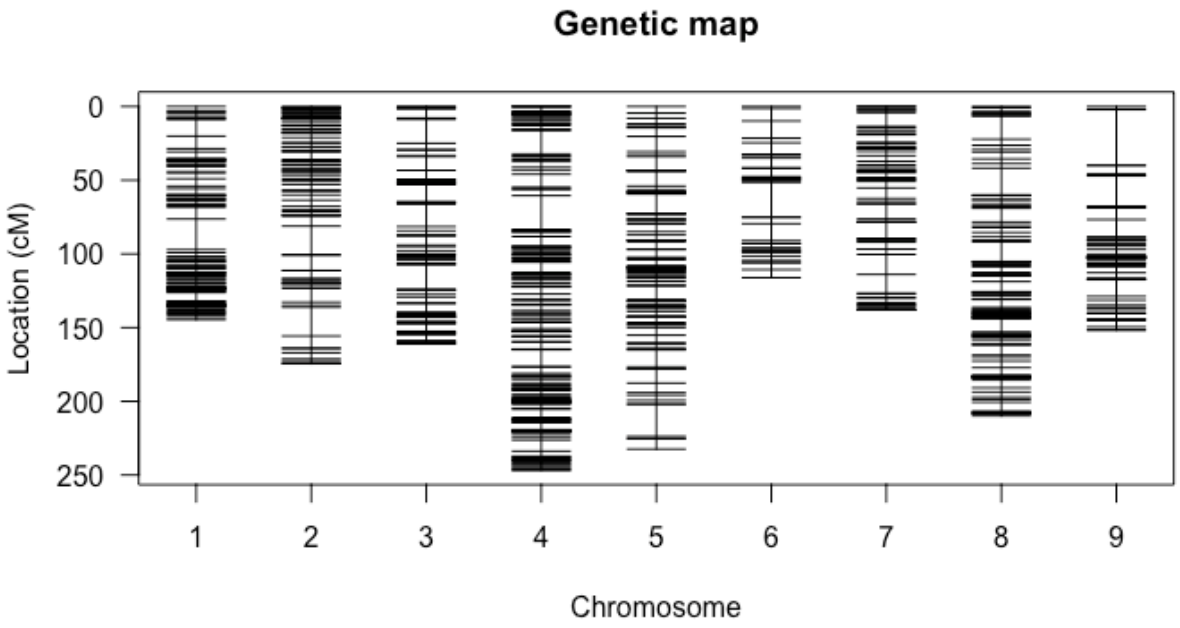

Figure 2. Illustration of 840 single nucleotide polymorphism markers (SNP) along the nine linkage groups of lettuce. Individual SNP markers are represented by a single horizontal line (marker not shown for brevity).

We evaluated the 162 RIL and their parents (‘BRG’ and ‘Eruption’) for lettuce drop incidence at the USDA-ARS, Salinas research station in a field infested with the sclerotia of *S. minor* as replicated alpha design with three replications for the third time season in spring 2017. In agreement with the results from the first two evaluations (spring and fall 2016), the RIL population showed a continuous phenotypic variation in response to lettuce drop (Fig. 3). The population had ‘disease rating’ scores that ranged from 0.53 to 1.45, with a mean of 1.07 in spring 2016 (Fig. 3A). In fall 2016, the RIL had ‘disease rating’ ranging from 0.29 to 1.30 with a mean of 0.86 (Fig. 3B). Whereas, the ‘rating’ scores ranged from 0.45 to 1.21, with a mean of 0.85 in spring 2017 (Fig. 3C). These phenotypic distributions in the mapping population indicates that lettuce drop resistance in ‘Eruption’ has quantitative inheritance; i.e., more than one ‘gene’ controls drop resistance. Consistent with the initial observation, the two parents exhibited distinct levels of lettuce drop. The lettuce drop datasets from the three experiments had significant positive correlation (Table 2). In other words, RIL with low disease incidence in one experiment had similar low disease levels during the other two experiments and vice versa. On the other hand, as expected, lettuce drop and rate of bolting were negatively associated (Table 2), indicating that fast bolting lines had relatively lower disease levels and vice versa.

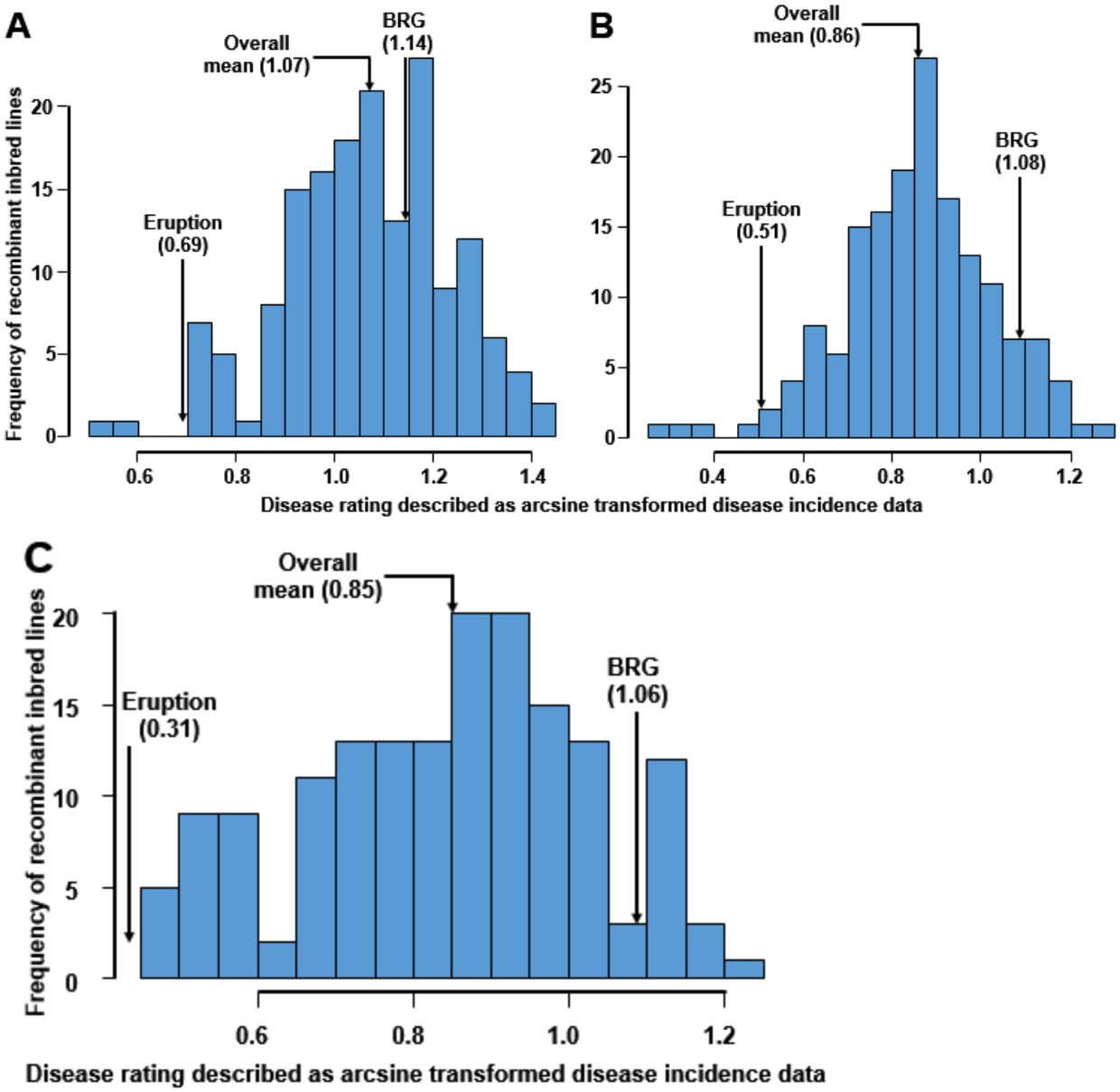

Figure 3. Distribution of 'disease rating' of Batavia Reine des Glaces x 'Eruption' recombinant inbred lines and parents in *Sclerotinia minor*-infested field experiment (A) spring 2016, (B) fall 2016, and (C) spring 2017

Table 2. Correlation coefficients for lettuce drop disease incidence and rate of bolting in the 'BRG' x 'Eruption' RIL evaluated in three experiments (LD16.1, LD16.2 and LD17.1) in Salinas, CA, in 2016 and 2017

Phenotype	Drop LD16.1	Drop LD16.2	Drop LD17.1	Bolting LD16.1	Bolting LD16.2	Bolting LD17.1
Drop incidence (LD16.1)	1					
Drop incidence (LD16.2)	0.589	1				
Drop incidence (LD17.1)	0.589	0.650	1			
Rate of bolting (LD16.1)	-0.298	-0.256	-0.325	1		
Rate of bolting (LD16.2)	-0.294	-0.246	-0.260	0.668	1	
Rate of bolting (LD17.1)	-0.243	-0.286	-0.269	0.697	0.634	1

Correlation coefficients >0.255 (in absolute values) are significant at p <0.001; coefficients 0.209 to 0.255 (in absolute values) are significant at p <0.01.

The genotype and phenotype data were associated through quantitative trait loci (QTL) analysis to map the location of 'genes' responsible for lettuce drop resistance in 'Eruption'. The QTL analysis identified some consistent QTL in all three seasons. The consistent quantitative resistance loci mapped on lettuce linkage groups 1 and 5, explaining 9-12% and 11-25% of the variation in lettuce drop, respectively (30-41% total). The lettuce drop resistance at these genomic locations is independent of rate of bolting.

In addition to lettuce drop, 'Eruption' has complete (100%) resistance to the major soil-borne disease of lettuce known as Verticillium wilt. Verticillium wilt is caused by more than one races of the fungal pathogen *Verticillium dahlia*. Of the two recognized races, 'Eruption' is resistant to race 1. This is significant as it is unusual to find lettuce genotypes resistant against two soil-borne pathogens (along with lettuce drop caused by *S. minor*). Like for lettuce drop, identifying the gene(s) and liked molecular markers is important to develop breeding lines that are resistant to Verticillium wilt and transfer the resistance to commercial cultivars. To this effect, a replica of the 162 RIL was also evaluated in a field infested with the pathogen during the spring-summer 2017 experimental season. Verticillium wilt incidence data was recorded at harvest maturity. Analysis of the disease incidence data, after conversion to the more appropriate dataset of 'disease rating', indicated the existence of phenotypic variation in the RIL population (Fig. 3). 'Eruption' had a 'disease incidence' or 'rating' of 0. Whereas, 'BRG', the susceptible parent, exhibited a 'rating' score of 0.72. For the RIL, the disease 'rating' scores ranged from 0 to 1.15 with a mean of 0.41. The distribution of 'disease incidence' or 'rating' data of the RIL indicated that 'Eruption' likely has one major gene for resistance against race 1 of *V. dahlia*. QTL mapping identified one genomic region on linkage group 9, explaining 47–51% of the variation in Verticillium wilt. This genomic region of linkage group 9 was previously identified to have the *Vr1* (*Verticillium resistance 1*) gene, and the gene in 'Eruption' appears to be the same.

FUSARIUM WILT (with M. Matheron, S. Koike, and T. Turini)

No progress was made in the lower desert. We planned a field test to confirm reactions of best F_4 and F_5 families (from the cross of susceptible iceberg cultivar Autumn Gold with the resistant romaine cultivar King Louie), but the field site was not ready until after the planting period for maximum disease pressure.

No significant progress was made in California coastal districts, though a commercial field trial was planted at the end of March.

CORKY ROOT

Corky root disease of lettuce is caused by a soil bacterium, Sphingomonas suberifaciens, formerly Rhizomonas suberifaciens. It may reduce yield 30-70% from reduced head size (van Bruggen, 1997). One resistant gene, cor, has been identified (Brown and Michelmore, 1988) and used in commercial cultivars.

We have previously screened more than 1,000 PI lines and cultivars for new sources of resistance to corky root, and four L. serriola lines (PI 273597c, PI 491096, PI 491110, and PI 491239) were found highly resistant to the disease. In growth chamber tests, PI 491239 and PI 273597c had lower corky root severity than cultivars with cor resistant gene in soil from Watsonville that has cor-resistance breaking strains. The resistance from these lines is being incorporated into cultivated lettuces through crosses and selections.

We continued to make crosses to transfer resistant gene cor from ‘Glacier’ to green leaf, red leaf, romaine, and butterhead lettuce types, and to combine corky root resistance with resistances to leafminers, downy mildew, and tipburn. F2 to F6 plants from these crosses were selected in the field for horticultural traits and resistances to other diseases and insects using a pedigree method. Backcrosses were used as necessary to restore horticultural types.

Eighteen F7 to F11 breeding lines of crisphead, leaf, butterhead, and romaine lettuces were tested in a replicated field trial at the USDA Spence Farm in Salinas from June to September 2017, and disease resistance and horticultural traits were recorded. The corky root disease rating of the breeding lines was significantly lower than the cultivars but was similar to the resistant controls ‘Glacier’, ‘Clemente’, and ‘Heart’s Delight’ (Tables 3-6). The plant weight, core length, and tipburn of the breeding lines were comparable or better than the cultivars. A leaf lettuce breeding line, MU11-332, had a low downy mildew disease rating, similar to the resistant control ‘Grand Rapids’ (Table 5).

Table 3. Mean values of corky root severity and head characteristics of crisphead lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2017.

Genotype	Corky root[z]	Plant Wt. (g)	Core length (cm)	Tipburn leaves[y]
Cardinale	8.0 A	698.8 C	26.2 A	0.9 C
Salinas	8.0 A	961.5 B	7.9 C	0.0 C
Glacier	6.2 B	1,017.7 B	10.7 C	5.1 A
MU16-455-1	6.0 BC	1,271.2 A	15.4 B	1.3 C
MU14-360	5.9 C	1,205.4 A	15.0 B	3.1 B

[z] On Brown and Michelmore's scale of 0-9 (0, no disease; 9, plant is dead from the disease). Means in the same column followed by different letters indicate significant differences at $P < 0.05$. [y] Number of leaves with tipburn in a head.

Table 4. Mean values of corky root and head characteristics of butterhead lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2017.

Genotype	Corky root[z]	Plant Wt. (g)	Core length (cm)	Tipburn leaves[y]
Cobham Green	8.0 A	450.3 E	6.4 DE	2.5 B
Margarita	8.0 A	443.4 E	5.3 E	0.4 BC
Bibb	7.9 A	473.5 E	6.6 DE	0.0 C
Dark Green Boston	7.9 A	424.0 E	8.0 CD	5.0 A
MU16-447	6.3 B	725.5 BC	6.8 DE	1.3 BC
MU16-448	6.2 BC	717.5 BCD	6.5 DE	0.3 C
Glacier	6.2 BC	1,017.7 A	10.7 A	5.1 A
MU16-450	6.0 BC	502.0 E	8.8 BC	0.0 C
MU16-451	6.0 BC	741.4 B	9.0 ABC	0.0 C
MU16-446	5.9 CD	633.5 D	8.0 CD	0.1 C
MU16-445	5.5 D	649.9 CD	10.2 AB	1.2 BC
MU16-442	5.4 D	701.0 BCD	9.2 ABC	0.6 BC

[z] On Brown and Michelmore's scale of 0-9 (0, no disease; 9, plant is dead from the disease). Means in the same column followed by different letters indicate significant differences at $P < 0.05$. [y] Number of leaves with tipburn in a head.

Table 5. Mean values of corky root and head characteristics of leaf lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2017.

Genotype	Corky root[z]	Plant Wt. (g)	Core length (cm)	Downy mildew[y]
Big Red	8.0 A	410.7 DE	8.9 BCD	3.5 B
Grand Rapids	8.0 A	401.5 E	8.3 CD	1.0 F
Lolla Rossa	8.0 A	248.2 F	8.3 CD	2.0 D
Prizehead	8.0 A	529.3 D	6.5 DE	3.5 B
Red Fox	8.0 A	658.5 C	10.1 BC	3.5 B
Merlot	7.5 B	291.5 EF	11.1 B	2.0 D
Redina	7.3 B	327.3 EF	15.0 A	2.0 D
MU11-332	6.5 C	396.2 E	5.5 E	1.0 F
Glacier	6.2 D	1,017.7 A	10.7 BC	4.0 A
MU14-357	6.1 D	1,055.9 A	14.4 A	3.0 C
MU14-368	6.0 D	394.0 E	5.2 E	1.5 E
MU14-391	6.0 D	791.0 B	9.8 BC	3.0 C

[z] On Brown and Michelmore's scale of 0-9 (0, no disease; 9, plant is dead from the disease). Means in the same column followed by different letters indicate significant differences at $P < 0.05$. [y] On a scale of 0 – 5 (0, no lesion; 5, large lesions covering nearly 100% of the exposed leaf surface).

Table 6. Mean values of corky root severity and head characteristics of romaine lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2017.

Genotype	Corky root[z]	Plant Wt. (g)	Core length (cm)	Tipburn leaves[y]
Flashy Troutback	8.0 A	924.7 CDE	23.2 A	12.5 A
Green Forest	8.0 A	1,145.3 ABC	11.2 BCD	1.7 DE
Green Towers	8.0 A	940.7 BCDE	9.2 CDE	2.8 DE
Parris Island Cos	8.0 A	884.5 DE	9.1 CDE	4.5 CD
Red Hot	8.0 A	877.7 DE	7.5 E	3.1 DE
Valmaine	8.0 A	904.8 DE	8.1 E	3.8 DE
Clemente	6.0 B	1,275.7 A	12.4 B	10.1 AB
Heart's Delight	6.0 B	1,070.2 ABCD	11.1 BCD	8.1 BC
MU14-382-1	6.0 B	994.7 BCD	9.0 CDE	2.7 DE
MU16-538	5.9 B	1,150.8 AB	11.1 BCD	2.2 DE
MU16-553-1	5.9 B	481.3 F	8.4 DE	0.0 E
MU16-555	5.9 B	496.5 F	11.2 BC	0.3 DE
MU14-333	5.8 C	737.7 E	8.4 CDE	10.3 AB

[z] On Brown and Michelmore's scale of 0-9 (0, no disease; 9, plant is dead from the disease). Means in the same column followed by different letters indicate significant differences at $P < 0.05$. [y] Number of leaves with tipburn in a head.

DOWNY MILDEW

Downy mildew ("DM", caused by *Bremia lactucae*) is a widespread lettuce disease, frequently found in California fields. This disease primarily affects the foliar tissue, reducing yield and decreasing quality of the marketable portion of the crop. Management of DM is achieved by the combined use of fungicide applications and agronomic practices that avoid excess irrigation, but the use of resistant varieties is the most effective method for controlling this disease. However, the use of fungicides is constrained by high costs and the development of fungicide resistant strains of the pathogen, while the use of resistant varieties is complicated by high pathogen variability, which leads to the defeat of cultivars containing major single gene resistance by new variants of the pathogen.

Plants were evaluated for DM severity in five field experiments in Salinas, Marina, and Gonzales, CA. All accessions were planted during spring using RCBD with three replicates. The phenotyping of DM severity was carried out in weekly intervals, after the most susceptible cultivars showed first symptoms of disease:

0: No DM lesions on any of the plants in the plot (consisting in 10 to 20 plants).
1: Few small lesions on some of the plants in the plot.
2: Few lesions on most of the plants in the plot.
3: Several lesions on most of the plants in the plot.
4: Multiple lesions on most of the plants in the plot.
5: Multiple lesions on most of the plants, with presence of dead tissue.

In total, we have evaluated over 35,000 plants from 512 accessions (Fig. 4). Two of the accessions of *Lactuca indica* showed non-host type of resistance. Other 11 accessions had a disease score between 0.5 to 1.5, indicating possible polygenic resistance to DM. These accessions will be retested in multiple experiments to confirm observed results.

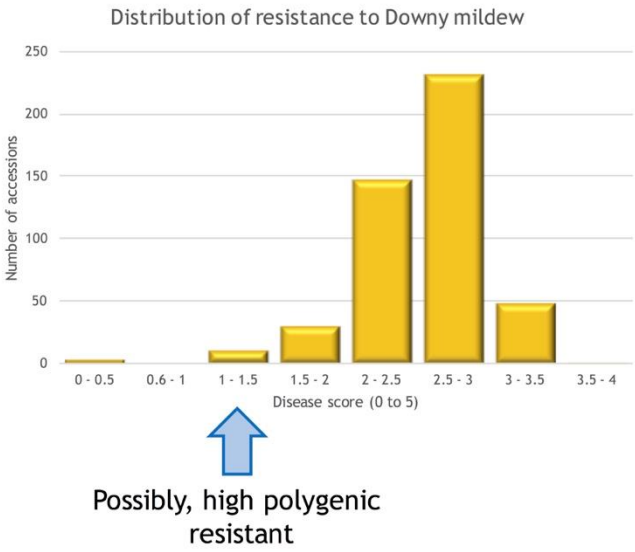

Figure 4. Distribution of resistance to downy mildew in 512 accessions of lettuce.

Forty-two breeding lines of F_2 to F_5 generation originating from crosses with cultivars with high polygenic resistance (Balady Banha, Iceberg, Grand Rapids, Holborn's Standard, La Brillante, Merlot, and Primus) were also tested for DM resistance using RCBD with three replications. The trials were used to select plants with a minimum number of lesions that were not bolting at the time of evaluation and otherwise appeared healthy and robust. The selected material will be used for further evaluations of resistance to downy mildew, bolting, tipburn, and horticultural characteristics.

TOSPOVIRUSES (with W. Wintermantel and C. Richardson)

Tospoviruses (Tomato spotted wilt virus - TSWV and Impatiens necrotic spot virus - INSV), reduce lettuce yield and quality (Fig. 5). Unreliable and unpredictable field conditions directed research to a more controlled approach in testing tospovirus resistance. Experiments were conducted in greenhouses to determine the efficacy and availability of resistance within lettuce (*Lactuca sativa*) to INSV. This project focuses on identifying and characterizing lettuce accessions with resistance to TSWV, INSV, develop methods for testing TSWV and INSV resistance, and determine the inheritance of resistance.

These experiments involve propagation of virus in lettuce and a California native weed species in the Asteraceae family, *Emilia sonchifolia*. Initial characterization focused on INSV. Titers of this virus in several hosts were compared by enzyme-linked immunosorbent assay (ELISA) and related to efficiency of manual (rub inoculation of virus) and viruliferous-thrip transmission to lettuce. Results showed a wide range of virus titers among the different host plants. Lettuce seedlings are grown to the four leaf-stage (two large leaves and two smaller emerging leaves) before being inoculated and exposed to viruliferous thrip populations. Source plants are confirmed to be singly infected with the appropriate virus using immunostrips. Virus-infected leaf samples from *E. sonchifolia,* is ground in inoculum buffer (sodium phosphate, pH 7.0 with sodium sulfite) and immediately used to mechanically inoculate seedling test plants. 2-3 days after the inoculated plants have recovered from inoculation they are moved to the greenhouse and exposed to viruliferous thrip populations. Viruses need to be able to multiply in both plants and thrips vectors during propagation to maintain infectivity in lettuce. Propagation hosts include *E. sonchifolia* and lettuce. Test plants are maintained for five weeks and evaluated weekly for symptoms. Visual symptoms of Tospoviruses include necrotic lesions, stunting, chlorotic lesions, necrotic apex, or dieback of apex, and die-off. At the conclusion of the trial plants are evaluated by ELISA to determine infection. All subsequent transmissions for maintenance of source plants is exclusively by thrips transmission and reared in mesh cages within dedicated greenhouses. Varieties of interest (potential resistance or mild symptoms) are evaluated for virus titer as well. Full scale studies have begun using these methods to evaluate diverse germplasm for resistance to INSV. We have tested 142 *Lactuca sativa* cultivars and accessions of *L. serriola, L. saligna, L. aculeata, L. indica, and L. virosa* for Tospovirus resistance. Viruliferous thrip populations have been reared and maintained in a greenhouse environment and have proven to be successful vectors in transmitting Tospovirus to host, and test plants. More than 40 successful ELISA screens have been completed on different varieties of lettuce. Greenhouse and growth chambers are also utilized for cultivating plants in between inoculation and exposure to viruliferous thrips, as well as acclimating plants once they have been transplanted from growth chambers.

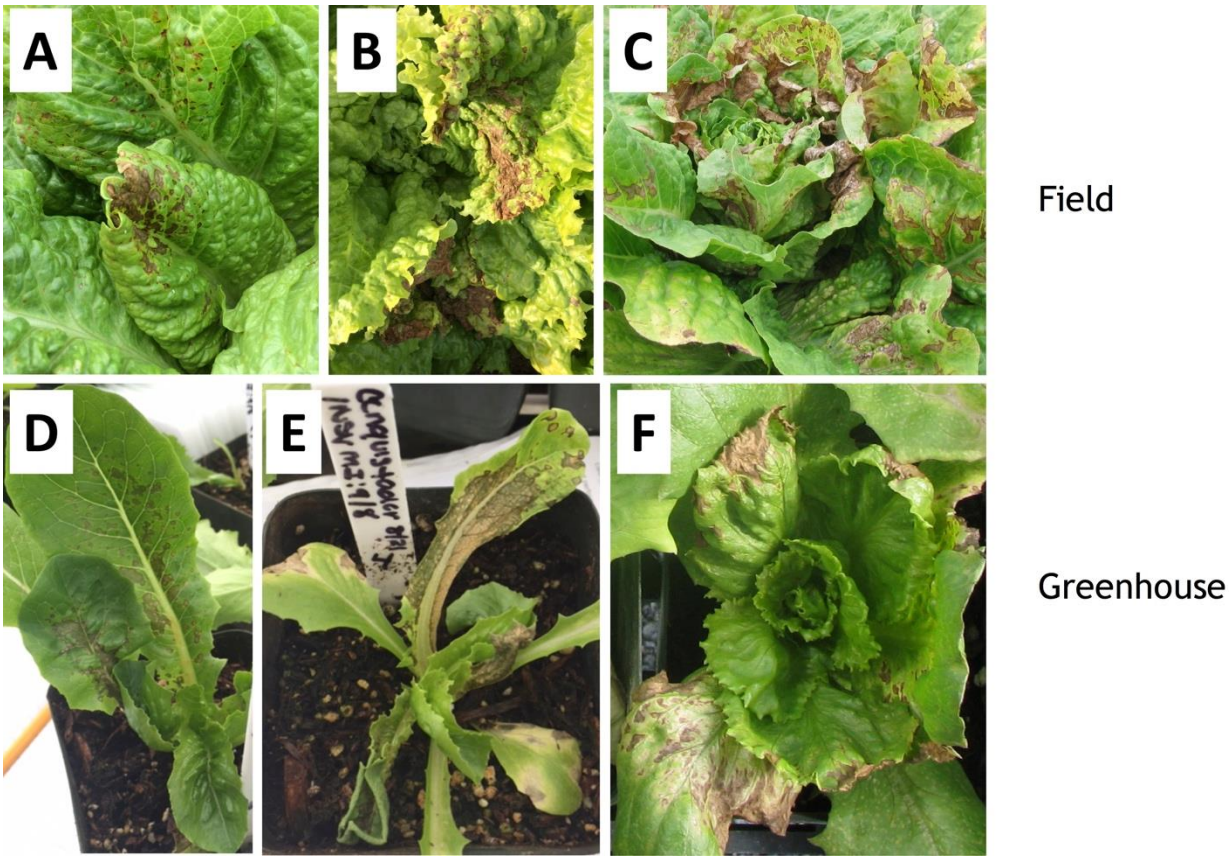

Figure 5. Typical symptoms of INSV infection on lettuce plants grown in field or a greenhouse (Simko et al. 2018, Plant Disease).

No *Lactuca* species was determined to be completely resistant to INSV, thus they are potential reservoirs from which the virus can be acquired by thrips and transmitted to lettuce and other susceptible crops. A partial resistance was identified in ten cultivars: Amazona, Ancora, Antigua, Commodore, Eruption, Iceberg, La Brillante, Merlot, Telluride, and Tinto. These cultivars were used for crosses to develop breeding lines with improved resistance to INSV and to study inheritance of the resistance.

BACTERIAL LEAF SPOT (with C. Bull and R. Hayes)

The foliar disease bacterial leaf spot is caused by *Xanthomonas campestris* pv. *vitians* (*Xcv*). We have identified a new gene, *Xar1*, that confers resistance to California strains of the bacteria (Hayes et al. 2014, Horticulture Research). *Xar1* can be detected by infiltrating the backside of a leaf with *Xcv*. Lettuce with *Xar1* rapidly develops a dry tan spot at the infiltration site, rather than the black watery lesions typical of bacterial leaf spot. We are breeding BLS resistance into cultivars suitable for spring mix production. 163 recombinant inbred lines from the Batavia Reine des Glaces x Eruption population were tested with three diverse strains of the pathogens. Three resistance QTLs were identified, explaining between 9% to 22% of the total phenotypic variation of the trait. Additional ~15,000 plants from 87 accessions of cultivated lettuce and

related wild species were tested in replicated trials. Our breeding effort continues to develop resistant breeding lines from a cross between RH08-0111 and cv. Merlot. Thirty-four new crosses were made to incorporate resistance loci from primitive cultivars and wild species into modern type of lettuce.

LEAFMINER

The predominant species of leafminers in central California is *Liriomyza langei.* They have a wide host range including broccoli, cauliflower, celery, lettuce, melons, spinach, tomato, and many weeds. Chemical control is not long lasting, and it is well documented that leafminers can develop a high degree of resistance to insecticides.

We made crosses to transfer leafminer resistance from wild species into iceberg and mixed lettuce types. BC_1F_2 to BC_1F_6 plants from these crosses were selected in the field for horticultural traits and resistance to leafminer, and were backcrossed if necessary to restore horticultural types. We also continued to make crosses to combine leafminer resistance with resistances to other diseases and insects for multiple-resistance. Crosses were also made among resistant sources in an effort to elevate the level of resistance to leafminers.

F_2 to F_6 progeny plants from crosses between leafminer resistant source (PI 169513, Red Grenoble, Merlot, Lolla Rossa, Bibb, and Tom Thumb) and good horticultural types (Salinas, Salinas 88, Tiber, Prizehead, and Lobjoits) were selected in the field for leafminer and multiple resistances, and some of them were backcrossed to restore horticultural traits. Ten promising F_7 to F_{11} breeding lines of green leaf, red leaf, and romaine lettuces were trialed at Spence Farm in Salinas from June to September 2017 with four replications, along with commercial cultivars and resistant controls. Many breeding lines had significantly lower leafminer sting density than commercial cultivars and resistant controls, and the plant weight, core length, and tipburn of the breeding lines were generally similar to or better than commercial cultivars (Tables 7-9). Some of these breeding lines also have the *cor* resistant gene, so they were resistant to corky root disease as well (Tables 7 and 9). Some green leaf lettuce breeding lines showed moderate resistance to downy mildew (Table 7). These breeding lines will be evaluated in the field again in 2019 to confirm the results.

Table 7. Mean values of leafminer sting density and head characteristics of green leaf lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2017.

Genotype[z]	Stings/cm^{2}[,y]	Plant Wt. (g)	Core length (cm)	Downy mildew[y]
Waldmann's Green	2.8 A	578.0 A	13.4 A	3.0 B
Two Star	2.8 A	680.3 A	7.3 B	4.0 A
Grand Rapids	2.7 A	401.5 B	8.3 B	1.0 D
Shining Star	2.5 A	575.1 A	8.9 B	4.0 A
MU16-310 (*cor*)	0.5 B	677.2 A	8.0 B	2.0 C
MU16-307 (*cor*)	0.4 B	658.5 A	7.4 B	2.0 C
MU16-318 (*cor*)	0.4 B	588.2 A	7.4 B	2.0 C
MU16-322 (*cor*)	0.4 B	710.4 A	7.8 B	2.0 C

[z] Some breeding lines have the *cor* gene and are resistant to corky root. [y] Means in the same column followed by different letters indicate significant differences at $P < 0.05$. [y] On a scale of 0 – 5 (0, no lesion; 5, large lesions covering nearly 100% of the exposed leaf surface).

Table 8. Mean values of leafminer sting density and head characteristics of red leaf lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2017.

Genotype	Stings/cm^{2}[,z]	Plant Wt. (g)	Core length (cm)	Tipburn[y]
Prizehead	5.4 A	529.3 AB	6.5 B	2.8 A
Big Red	4.8 A	410.7 BC	8.9 AB	0.3 B
Red Fox	3.1 B	658.5 A	10.1 A	0.0 B
Lolla Rossa	2.6 B	248.2 D	8.3 AB	0.0 B
Merlot	2.1 BC	291.5 CD	11.1 A	0.0 B
MU16-321	1.1 C	511.7 AB	9.8 A	0.0 B
MU16-314	1.0 C	485.3 B	8.9 AB	0.0 B

[z] Means in the same column followed by different letters are significantly different at $P < 0.05$.
[y] Number of leaves with tipburn in a head.

Table 9. Mean values of leafminer sting density and head characteristics of romaine lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2017.

Genotype[z]	Stings/cm^{2}[,y]	Plant Wt. (g)	Core length (cm)	Tipburn[x]
Green Forest	5.7 A	1,145.3 ABC	11.2 AB	1.7 CD
Clemente	5.1 AB	1,275.7 A	12.4 A	10.1 A
Red Hot	4.4 BC	877.7 D	7.5 D	3.1 CD
Valmaine	4.1 BC	904.8 CD	8.1 CD	3.8 CD
Green Towers	4.1 BC	940.7 BCD	9.2 CD	2.8 CD
Heart's Delight	4.0 BC	1,070.2 ABCD	11.1 AB	8.1 AB
Parris Island Cos	3.8 C	884.5 D	9.1 CD	4.5 BC
MU14-489 (*cor*)	1.2 D	856.7 D	8.0 D	3.2 CD
MU16-329-1 (*cor*)	0.8 D	1,032.2 ABCD	12.2 A	9.4 A
MU16-331 (*cor*)	0.5 D	1,015.0 BCD	10.0 BC	8.5 A
MU14-481-1	0.3 D	1,153.5 AB	10.0 BC	0.3 D

[z] Some breeding lines have the *cor* gene and are resistant to corky root. [y] Means in the same column followed by different letters indicate significant differences at $P < 0.05$. [x] Number of leaves with tipburn in a head.

LETTUCE APHID (with Y.B. Liu)

No real progress as lettuce aphid colonies were plagued by thrips.

BOLTING RESISTANCE (with L. Rosental, R. Hayes, and D. Still)

Research on bolting resistance for fall plantings is being conducted collaboratively with Dr. David Still from California State Polytechnic University, Pomona. Funding for the collaborative research comes from the Agricultural Research Initiative with matching funds from the California Leafy Greens Research Program.

The objectives of this project are:
(1) Breeding commercially acceptable romaine cultivars with resistance to pre-mature bolting.
(2) Mapping QTL controlling environmentally dependent early bolting, and identification of genes underlying environmentally-dependent QTL.
(3) Screening wild *Lactuca* for winter annual or biennial flowering habit and identifying genes involved in or influencing the biennial flowering habit.

We have previously reported the evaluation of recombinant inbred line populations from PI 251246 x Salinas, Pavane × Parade, Western Red Leaf × Cool Guard and Diplomat × Margarita for plant development in various environments. Development was recorded on a scale of 1 = rosette; 2 = bolting; 3 = visual buds; 5 = flowering; 7 = open involucres. In total, these

populations have been evaluated for plant development in two fall plantings in Yuma, AZ, two spring plantings in the Salinas Valley and two spring plantings in Pomona, CA. Genetic maps using genotyping by sequencing for the PI 251246 × Salinas, Western Red Leaf × Cool Guard and Diplomat × Margarita populations have been constructed. The Pavane × Parade population has been genotyped using the Lettuce GeneChip.

In addition, a recombinant inbred line populations from 11-G99 (*Lactuca serriola*) × PI 251246, received from the R. Michelmore lab, was evaluated for development in a fall planting in Yuma, AZ. Another two RIL populations, Iceberg x PI491224 and Grand Rapids x Salinas, were evaluated in a late summer planting in Salinas. A diversity panel of mostly romaine cultivars for GWAS study was evaluated in a summer field experiment in California's Central Valley.

Preliminary analysis of QTLs from the PI 251246 x Salinas, the Pavane × Parade and the Iceberg x PI491224 populations revealed three large-effect QTLs with significant genetic by environment interactions in chromosomes 2, 6 and 7. Additional small-effect QTLs were also identified. For the Pavane × Parade population three QTL for bolting were found under certain environments (on chromosome 2, 8 and 7), and four for flowering time (two on chromosome 2, on 6 and 7). The QTL in chromosome 7 for bolting and flowering co-locates in these two populations. The 11-G99 × PI 251246 population has five significant QTLs for time to bolting (on chromosome 1, 3, two on 4 and 7), two QTL in additional locations for budding time (on chromosome 4 and 9) and another one on chr 9 for flowering time.

A sub population of recombinant inbred lines from the PI 251246 × Salinas population was developed to enable fine mapping of QTLs identified in the analysis of data from the field experiments. Additional DNA markers in the QTL regions were developed using high resolution melting technology with the LightScanner instrument. By fine mapping we identified a region of approximately 1.15Mbp as the QTL region in chromosome 7. Known homologs of flowering time genes and candidate genes which co-locate with the three major QTL were targeted in a gene expression study. The study includes samples form plant grown in growth chambers under various day length and temperature conditions. Candidate flowering genes' expression responded more to temperature than to changes in day length. A lettuce homolog of SOC1 gene displayed the greatest increase in expression during development, and in response to elevated temperatures.

SHELF-LIFE (with J. Sthapit, H. Peng)

To study the genotypic and phenotypic variation in shelf life, 493 lettuce accessions were selected. The accessions were phenotyped for shelf life in four experiments in 2016 and 2017 that were planted in three locations (Marina, Gonzales, Salinas, CA) in different planting seasons. When the field plots were ready, lettuce heads were harvested and processed into three salad bags (340 g each) per plot, and stored at 4°C for evaluation. Salad bags were evaluated on weekly intervals for at least six weeks using a 0 (no deterioration) to 10 (100% deterioration) scale. Least square means (LS means) of the deterioration scores were calculated for each accession for evaluation every week, which was then used to calculate area under deterioration progress stairs (*AUDePS*) for all the accessions for each experiment. As all evaluations were performed at regular intervals and at every time unit (weekly), *AUDePS* was calculated as

follows: *AUDePS* = $\bar{y}$ x *n*, where $\bar{y}$ is the arithmetic mean of all observations and *n* is the total number of observations (Fig. 6).

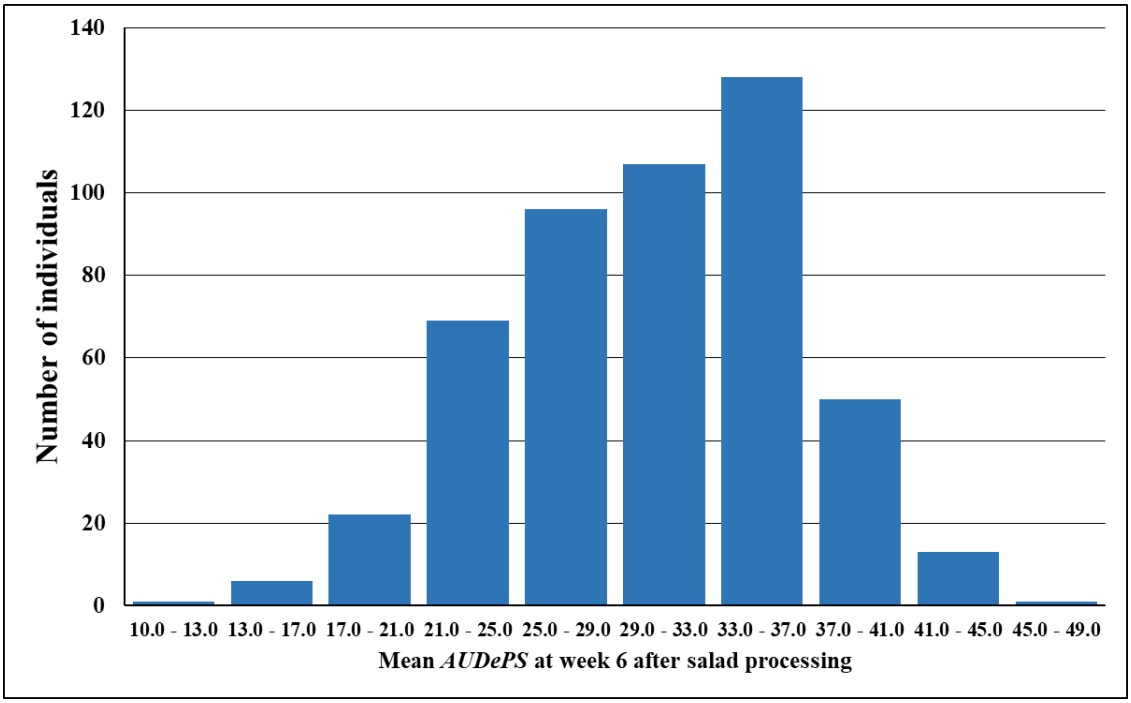

Figure 6. Distribution of mean *AUDePS* (from 4 experiments) of 493 lettuce accessions at week 6 after salad processing.

Along with shelf life data, yield data (weight in kg per head) was also collected for the 493 lettuce accessions. Shelf-life stability and yield stability of the accessions were assessed by calculating static and dynamic stability coefficients for each accession and statistical analyses were performed.

Genotypic data for 493 lettuce accessions was generated by Data2Bio company using tunable genotyping-by-sequencing (tGBS) technique. A total of 4,615 high-quality polymorphic SNP (single-nucleotide polymorphism) markers were used to perform genome-wide association study (GWAS). Shelf-life data (*AUDePS*), shelf-life stability, yield, and yield stability were used as phenotypic data for GWAS. Significant marker-trait associations were detected indicating genetic region associated with variation in shelf life and yield and their stability.

Among all the lettuce accessions, fifty were selected for nutrient analysis based on previously known shelf life variation. Nutrient analyses (vitamin C, vitamin A, sugar profile) were performed in two experiments at different times of storage to assess original nutrient content and relatedness of shelf life and nutrient retention post processing. Salad samples were shipped to Medallion Labs (Minneapolis, MN) on first and third week of processing for vitamin A (beta carotene) and sugar analyses. Vitamin C analysis was performed during the first, second, and third week of processing. Statistical analysis indicated that UC96US23, Taiwan, and PI 491086 has good nutrient quality with high vitamin C and beta carotene and low amount of sugars, whereas Salinas 88, Clemente, and PI 665200 are low in vitamin C and beta carotene, but have high sugars.

Deterioration rate of fresh-cut lettuce are affected by many factors such as atmosphere, temperature, and hormone level. Information of those factors highly associated with decay development can give some clues to identify the critical pathway in which *qSL4* (the gene conferring the rate of decay) might function and thus could help narrow down the candidate pool.

Atmosphere around samples in the sealed bag may play a role on deterioration rate and we thus first investigated the atmospheric change in salad bags. In two preliminary experiments launched on Aug 3rd and Aug 24th, we observed that 1) ethylene level reached 0.1-0.3 ppm in both cultivar salad bags after being stored for eight days at 4 ºC in the dark, 2) O_2 level quickly dropped off and approached to zero unlimitedly, 3) CO_2 content went up in sample bags of both cultivars, and 4) CO_2 level in the bags carrying La Brillante samples (fast decay) increased faster than that in the bags carrying Salinas 88 samples (slow decay). To verify the change of CO_2 and O_2 level, we further repeated the test with romaine and iceberg lettuce (unknown variety) from market on Feb 26th, 2018. As shown in Fig. 7, O_2 level in the empty bag went up constantly, indicating the material of bags allowed O_2 to be transmitted into bags as expected. Both romaine and iceberg samples consumed O_2 but the later used O_2 slightly less than the former, indicating the respiration rate of iceberg salad might be lower than that of romaine salad. CO_2 level in the empty bags kept as low as in the air, suggesting CO_2 didn't accumulated in the bag. Thus, increase of CO_2 in both sample bags were the consequence of respiration. Notably, CO_2 content in romaine sample bags was significantly higher than that in iceberg sample bags at both early and middle stages, indicating the faster respiration rate of romaine lettuce. In view of the faster decay of romaine lettuce, it can be concluded that respiration rate is highly associated with deterioration rate. Considering that stomata status or movement can directly affect respiration, we planned to compare stomata distribution and activity on alive or processed leaves of both parent plants. Lettuce have been planted in Spence in April 3rd, 2018.

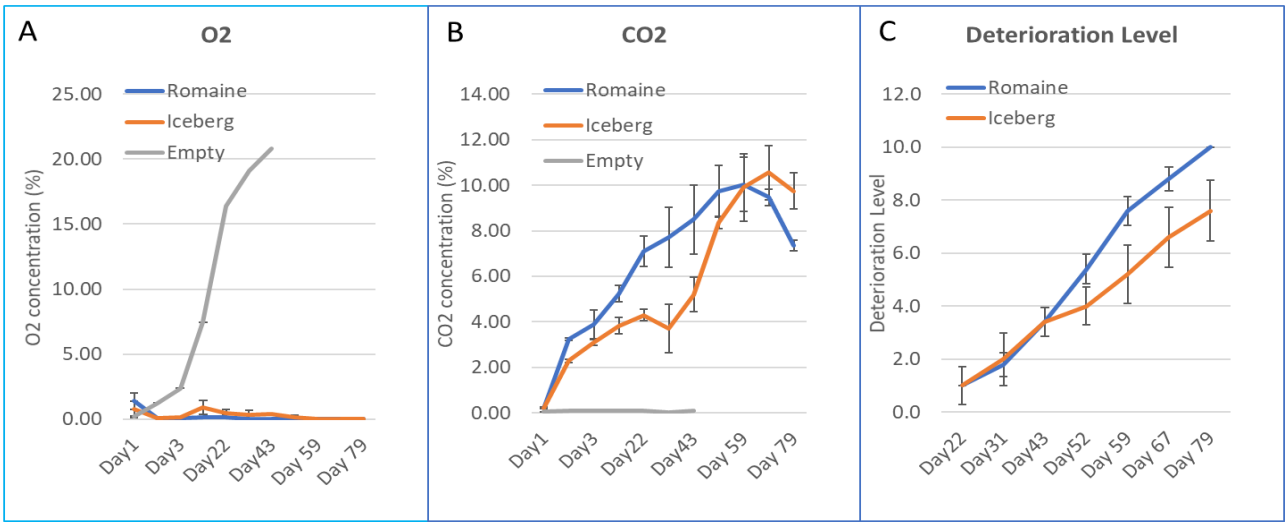

Figure 7. Deterioration rate of fresh-cut market romaine and iceberg lettuce and atmosphere change in the bags. A, O_2 content (%) in bags carrying cut lettuce (romaine or iceberg) or no lettuce (empty); B, CO_2 content (%) in bags carrying cut lettuce (romaine or iceberg) or no lettuce (empty); A, Deterioration rate of cut lettuce (romaine or iceberg) during storage.

Based on observation above, it is worth to investigate the effect of ethylene and CO_2 on shelf-life of cut lettuce. In the experiment, cut lettuce (250g) were enclosed in the bag with a stop cock. N_2 was flushed into the bag in triplicate. To stabilize the samples, all bags were set in the cold room (4°C) under dark for 3 hours before treatments. There were five treatments with N_2 as base, i.e. N_2+Ethylene (10 ppm), N_2+Ethylene absorbent (potassium permanganate, Power Pellets; 10 g/bag), N_2, N_2+ CO_2 absorbent (SodaSorb; 10 g/bag), N_2+ CO_2 absorbent+Ethylene absorbent. Each treatment had 5 replicates of Salinas 88 and La Brillante. All samples were kept at 4°C under dark condition. The ethylene and CO_2 content were measured in 10th day by GASTEC Ethylene and Bacharach's CO_2 Analyzer 2820, respectively. Also, the Ethylene treated bags were injected with ethylene to 10 ppm. Appearance of samples was evaluated by eyes twice per week. Total of three rounds of ethylene and CO_2 sensitivity tests were performed on June 9th, Aug 3rd, and Aug 24th, 2017. Results from the latest experiment were displayed in Fig. 8. Both addition and removal of ethylene had no obvious effect of decay rate for both cultivars, indicating decay rate is not majorly regulated by ethylene pathway. Removal of CO_2 didn't alleviate the deterioration rate of La Brillante salad but slightly reduced that of Salinas 88 salad, indicating toxic effect of CO_2 might be cultivar dependent. Interestingly, shelf-life of Salinas 88 salad could be significantly improved when removing both ethylene and CO_2, indicating that both ethylene and CO_2 have slight impact to shelf-life or the interaction of ethylene and CO_2 plays a role during decay development. One more scaled up experiment is ongoing. Both La Brillante and Salinas 88 plants have been grown in Spence since April 3rd, 2018.

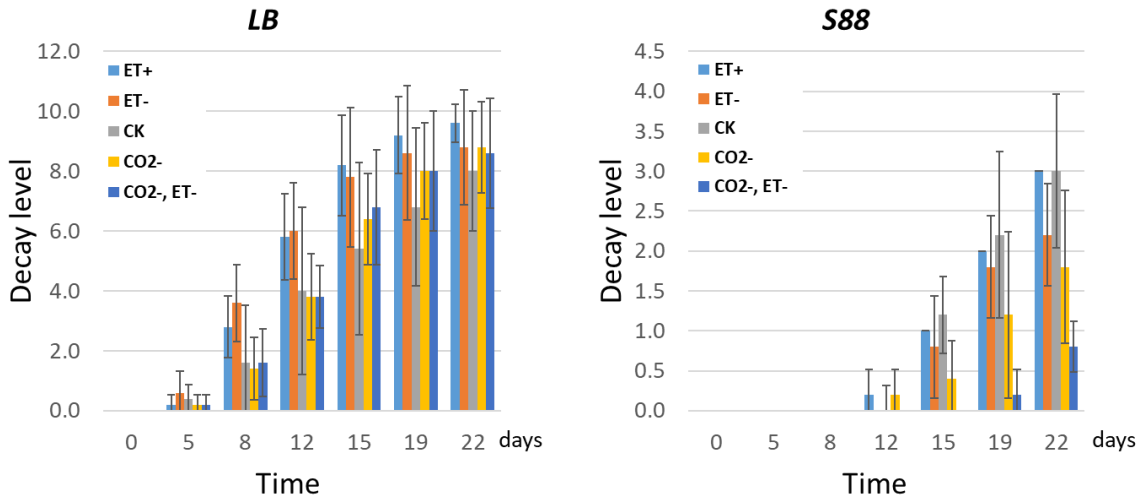

Figure 8. Shelf life of fresh-cut tissues of La Brillante (LB)and Salinas 88 (S88) in the Modified Atmosphere Packaging (MAP) with ethylene (ET+), ethylene absorbent (ET-), or carbon dioxide absorbent (CO_2-).

In addition, effect of other potential factor including leaf age, melatonin, ABA, MeJA, SA, and ethanol on shelf-life of cut lettuce will be tested on June 2018. Materials (La Brillante) were planted in Spence on April 3rd, 2018.

MOLECULAR MARKER DEVELOPMENT

Verticillium wilt (with P. Inderbitzin, K. Subbarao, and R. Michelmore)

Verticillium wilt is a highly destructive soil borne disease of lettuce caused by the fungus *Verticillium dahliae*. Resistance to race 1 is conferred by a single dominant gene (*Verticillium resistance 1*, *Vr1*) located on linkage group 9 (Hayes et al. 2011, Theoretical and Applied Genetics). The resistance allele has been first described in Batavia-type cultivar La Brillante, but multiple copies of the resistance allele are present in the *Vr1* genomic region. To determine the number of *Vr1* gene copies, and to identify the functional allele, we sequenced and performed genome assembly on 61 lettuce accessions. Additional 90 accessions were studied using PCR assays designed to amplify specific alleles. Phenotyping of all accessions was performed in at the USDA-ARS station in Salinas, at the field infected with the race 1 of the pathogen. Results of this study are being analyzed. The PCR assay resulting from this study will be published and can be used for accurate identification of genotypes with resistance alleles against Verticillium wilt, race 1.

Post-harvest deterioration of fresh-cut lettuce (with R. Michelmore, M. Truco, and R. Hayes)

Lettuce is widely used as the main ingredient of packaged leafy vegetable salads. Salad lettuce can have short shelf-life, decaying as early as eight days after harvest and reducing the nutritional quality. Identification of lettuce cultivars with extended shelf-life is important for the industry, but phenotyping is exceptionally labor intensive and slow process. Genetic studies showed that the deterioration rate is a heritable trait (broad spectrum heritability, H^2 of 0.56–0.87). The major genetic determinant of the deterioration rate is the quantitative trait locus (QTL), *qSL4*, that we have located on linkage group 4. This QTL explained 40– 74% of the total phenotypic variation of the trait in the two bi-parental populations (Salinas 88 x La Brillante and Pavane x Parade). Saturating the *qSL4* region with single-nucleotide (SNP) markers allowed detection of six haplotypes in a set of 16 lettuce accessions with different rates of deterioration. Three of the haplotypes were always associated with very rapid rates of deterioration, while the other three haplotypes were associated with slow rates of deterioration. Two SNPs located 53 bp apart were sufficient to separate the 16 accessions into two groups with different rates of deterioration. The accuracy of markers-trait association was subsequently tested on 350 plants from seven F_2 families that originated from crossing parents with different rates of deterioration (Fig. 9). The H^2 of deterioration rate in these seven families ranged from 0.64 to 0.90. The SNP-based analysis accurately identified individuals with rapid, intermediate, and slow rates of deterioration in each family. Intermediate rate of deterioration was found in individuals having heterozygous alleles at *qSL4*, indicating an additive effect of the alleles. The assay can be used for fast, accurate, and reliable identification of deterioration rate after processing for salad. A combination of results obtained with these two molecular SNP markers with information about phenotypes of parents can be used to accurately select slow deteriorating genotypes in breeding populations, thus contributing to the development of lettuce cultivars with superior shelf-life.

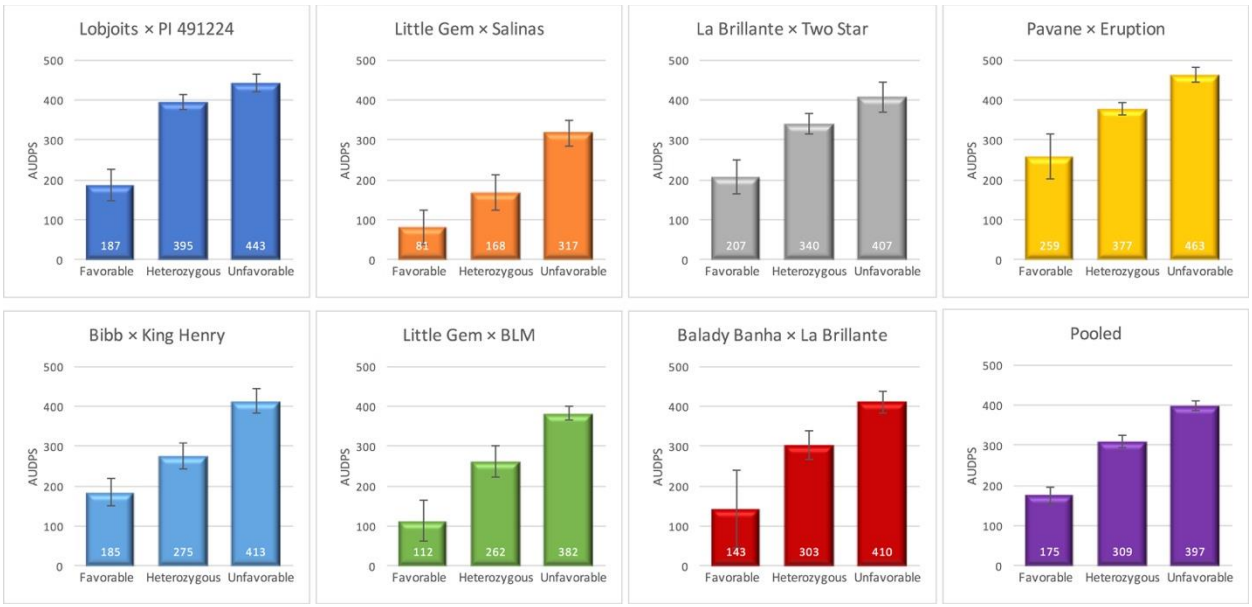

Figure 9. Deterioration scores in seven F_2 families (and pooled data from all families) after separating plants in each family into three genotypes using the newly developed PCR-based assay. Larger values of AUDPS (Areas Under the Deterioration Progress Steps) indicate more rapid deterioration. Within each family, the group of plants with favorable alleles for shelf-live deteriorated slower than heterozygous individuals. The fastest deterioration was observed on the group of plants with unfavorable alleles. More details about the marker assay, sequence data, and phenotypic screening can be found in Simko et al. 2018, Horticulture Research.

RECENT (2017 - 2018) PUBLICATIONS RELEVANT TO THIS PROJECT

Hayes, RJ, Sandoya, G, Mou, B, Simko, I, Subbarao, KV (2018) Release of three iceberg lettuce populations with combined resistance to two soilborne diseases. HortScience 53:247-250.

Lafta, A., T. Turini, G. Sandoya, and B. Mou. 2017. Field evaluation of green and red leaf lettuce genotypes in the Imperial, San Joaquin, and Salinas Valleys of California for heat tolerance and extension of the growing seasons. HortScience 52: 40-48.

Simko,I, Hayes, RJ (2018) Accuracy, reliability, and timing of visual evaluations of decay in fresh-cut lettuce. PLoS One https://doi.org/10.1371/journal.pone.0194635

Simko, I, Hayes, RJ, Truco, MJ, Michelmore, RW, Antonise, R, Massoudi, M (2018) Molecular markers reliably predicts post-harvest deterioration of fresh-cut lettuce in modified atmosphere packaging. Hort. Res. 5:21 DOI 10.1038/s41438-018-0022-5

Simko, I, Richardson, CE, Wintermantel, WM (2018) Variation within Lactuca spp. for resistance to Impatiens necrotic spot virus. Plant Dis. 102:341-348.

Xu, C. and B. Mou. 2017. Drench application of fish-derived protein hydrolysates affects lettuce growth, chlorophyll content, and gas exchange. HortTechnology 27: 539-543.