PROJECT REPORT
CALIFORNIA LETTUCE RESEARCH BOARD

For the period
April 1, 2013 - March 31, 2014

PROJECT TITLE: Lettuce Breeding, USDA-ARS

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ABSTRACT:

In the 2013-2014 period, major efforts targeted resistance to lettuce big vein disease, lettuce drop caused by *Sclerotinia* species, Verticillium wilt, Fusarium wilt, lettuce powdery mildew, bacterial leaf spot, corky root, downy mildew, leafminer, lettuce aphid, tipburn, shelf-life of salad-cut lettuce, and multiple disease resistance. In all programs, horticultural traits, adaptation, and resistance to tipburn are essential.

OBJECTIVES:

Development of new landmark lettuce cultivars and breeding lines with improved disease resistance, insect resistance, tolerance to heat and cold stress, uniform growth and maturity, horticultural quality, postharvest quality, and adaptation to specific lettuce districts and seasons.

PROCEDURES AND RESULTS:

A. Cultivar and advanced breeding line development

1. Disease resistances

a. Lettuce big-vein disease (with W. Wintermantel)

A high level of partial resistance to big-vein is available in the butterhead cultivar Margarita; we are introgressing this resistance into iceberg breeding lines. In a January planted field experiment in Salinas, CA, we evaluated 8 BCF_2, 21 $BCF_{3:4}$, 6 $BCF_{3:5}$, and 3 F_7 iceberg type lines derived from crosses between Margarita and iceberg cultivars, along with the check cultivar Pacific. Nine plants were selected from $BCF_{3:4}$ iceberg families while 17 BCF_2 were selected. Seed from self-pollination of selected plants will be used for further evaluation and selection.

b. Lettuce drop (with K. Subbarao)

We initiated a single seed descent breeding program for lettuce drop resistance. Overall, this method is expected to increase selection efficiency and have a shorter breeding cycle time compared to previous approaches. In 2013 we inbred 89 iceberg lines from Hallmark × (Salinas 88 × (75-501-1 × Holborn Standard)) to the F_6 generation and 157 lines from Reine des Glaces × Eruption to the F_5 generation. We evaluated 129 F_6 romaine lines from Darkland × RH07-0731-4-3 for resistance in an unreplicated spring planted field experiment, followed by evaluation of 12 selected lines for resistance in a summer planted replicated experiment. From these experiments, three romaine breeding lines were selected for possessing better resistance than Darkland. Seed of these three lines will be increased for use in future experiments and these lines will be crossed to cultivars and advanced breeding lines to create new populations.

The red Latin type cultivar Eruption has demonstrated resistance to sclerotial infection by *Sclerotinia minor* and *S. sclerotiorum* and to ascospores from *S. sclerotiorum*. The resistance appears to be independent of plant morphology, which means that this resistance can be introgressed into diverse market types. We are currently introgressing resistance from the cultivar Eruption into romaine breeding lines. Several F_6 romaine breeding lines from Hearts Delight × Eruption have routinely demonstrated significantly lower mortality than commercial romaine cultivars. However, these breeding lines were variable for leaf margin ruffling and in 2012 over 60 plants with non-ruffled margins were selected and inbred. In 2013, we identified 48 of the resulting $F_{6:7}$ lines as uniform for non-ruffled margins. These lines were also evaluated in an unreplicated field experiment located in a commercial field site with high disease incidence. The Hearts Delight × Eruption breeding lines had lower disease incidence with head weights similar to or slightly lower than commercial cultivars (Figure 1**)**. The combination of high resistance and acceptable head weight resulted in 37 breeding lines with significantly greater plot yields (kg of lettuce from a 15 foot plot) than Green Towers and Hearts Delight. The Hearts Delight × Eruption breeding lines RH09-0488 and RH09-0519 were used in crosses to develop new populations for breeding. Nineteen, 39, and 14 F_3 families from the crosses RH09-0488 × RH09-0519, RH09-0488 × Green Towers, and RH09-0488 × PI 226641, respectively, were developed by making single plant selections for improved horticultural characteristics from F_2

populations grown in a non-infested field experiment. PI 226641 is a partially resistant romaine accession.

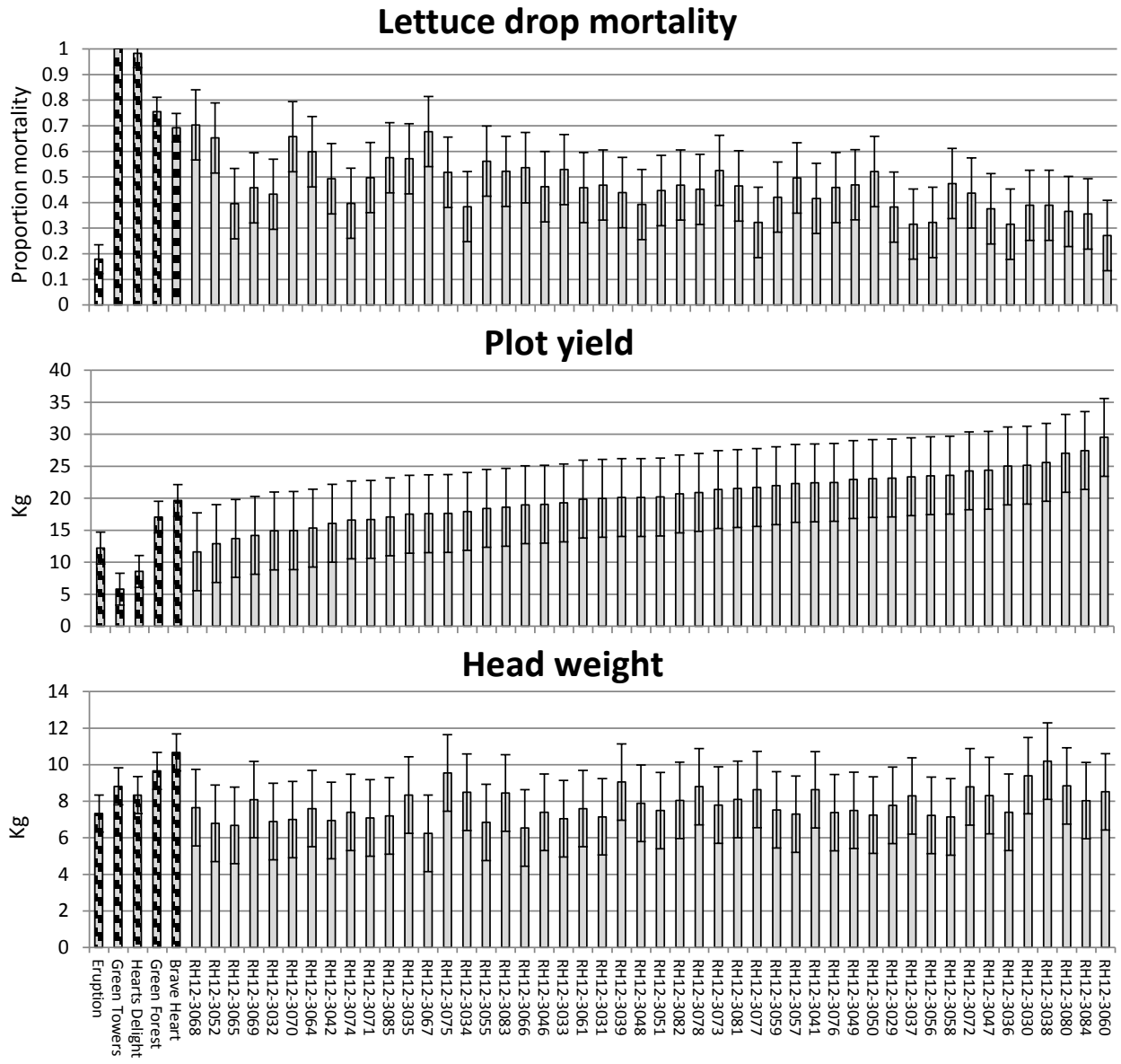

Figure 1. Lettuce drop mortality (proportion mortality), plot yield (kg of lettuce from a 15 foot plot), and head weight (kg per lettuce head) for 48 $F_{6:7}$ romaine breeding lines from Hearts Delight × Eruption (white bars) and the cultivars Green Towers, Hearts Delight, Eruption, Green Forest, and Braveheart (striped bars) in a field experiment located in a Salinas, CA commercial field site. Error bars are 95% confidence intervals.

Diverse sources of resistance are being used in a modified pedigree breeding approach to develop iceberg and leaf germplasm with resistance to lettuce drop. In this breeding scheme, spring and summer planted infested field experiments are conducted to identify families and breeding lines with improved resistance. A non-infested field experiment is conducted

concurrently; single plants with improved horticultural characters are taken from only the resistant families. Single plant selections are allowed to self-pollinate to develop seed of the next generation for use in subsequent experiments. Using this method, we identified 11 F_3 iceberg families from Hallmark × RH07-0388 and developed 41 $F_{3:4}$ families through single plant selection. Breeding line RH07-0388 is the source of resistance in this cross. Two leaf type F_3 families from Two Star × Eruption and three leaf type F_3 families from PI 178924 × Red Fox (PI 178924 has partial resistance to lettuce drop) were identified as having better resistance than Two Star and Red Fox. Single plant selections with improved horticultural characters were taken from these families resulting in 39 $F_{3:4}$ families. Twelve plants from these families were intercrossed to develop new populations for lettuce drop resistance breeding.

c. Verticillium wilt resistance breeding (with G. Sandoya and K. Subbarao)

We are evaluating a population of eight inbred iceberg breeding lines from Tiber × ((Pacific × La Brillante) × Tiber) in race 1 *V. dahliae*-infested and non-infested field experiments. Three experiments in Verticillium wilt-infested field sites demonstrated significantly lower disease incidence in the breeding lines compared to Salinas (Table 1). Experiments to collect data on yield, shelf-life, and horticultural quality from infested and non-infested fields were initiated in 2012 and continued in 2013. The breeding lines are generally similar to Salinas and Tiber for head weight, head diameter, and percent harvestable heads. The heads of the breeding lines are flatter than Salinas and Tiber. In one of the field experiments some breeding lines had a greater incidence of tipburn than Tiber and Salinas, although the difference was not statistically significant. Shelf-life of whole heads and minimally processed lettuce was evaluated in a single field experiment and appears to be similar to Salinas and Tiber. The breeding lines are susceptible to bacterial leaf spot and appear to be more susceptible to lettuce drop than Salinas. We will continue to characterize these breeding lines in field experiments in 2014.

Table 1. The percentage of plants with root discoloration typical of Verticillium wilt in two replicated experiments located in a race 1 *Verticillium dahliae* infested field site and a single replicated experiment located in a grower field site with a history of Verticillium wilt. Both field sites are in Salinas, CA.

Cultivar or breeding line	Number of plants evaluated	Percent plants with root discoloration
RH12-3194	70	3
RH12-3195	70	6
RH12-3196	70	3
RH12-3197	70	10
RH12-3198	70	1
RH12-3199	70	3
RH12-3200	70	4
RH12-3201	70	1
Salinas	100	74

We made progress on developing iceberg breeding lines that combine resistance to race 1 isolates of *V. dahliae* with resistances to other diseases. The breeding line RH08-0111 was

released in 2013 for combining resistance to race 1 of Verticillium wilt with resistances to downy mildew and bacterial leaf spot. Section D. (Release of breeding lines) of this report has more information on this release. In 2013, we evaluated F_2 plants from crosses between race 1 resistant breeding lines to the cultivars Telluride and Durango for horticultural characters in a non-infested field experiment. Telluride and Durango carry the *cor* gene for corky root resistance. Single plant selections with improved horticultural characteristics were taken from these families and allowed to self-pollinate resulting in 45 F_3 families. These families will be evaluated for resistance to corky root and race 1 Verticillium wilt in 2014.

We are working to develop race 2-resistant breeding lines using the currently available sources of resistance. Five F_3 families from PI 171674 × PI 204707 were identified with high level resistance (<15% disease incidence) in two greenhouse experiments. The disease incidence of Salinas in these experiments was 70%. The families will be crossed to adapted iceberg cultivars to develop new breeding populations.

d. Fusarium wilt

Three races of Fusarium wilt are known in Japan; to date only race 1 is known in California and Arizona. Two field tests of F_4 families from a cross of resistant 'King Louie' × susceptible 'Autumn Gold' were planted in Huron and Yuma. There was essentially no disease expressed in Huron in contrast to Yuma (Table 2). The mean rating of 'Costa Rica No. 4' and 12 F_4 families at Yuma was 1.0. Mean disease ratings for 'King Louie', 'Salinas' and 'Autumn Gold' were 1.2, 1.9 and 4.0, respectively, at Yuma. Mean disease ratings of 'Cost Rica No. 4' and 12 F_4 families were 1.0 at Yuma. Mean ratings for 'King Louie', 'Salinas', 'Autumn Gold', and 'Patriot' were 1.2, 1.9, 3.7, and 4.0, respectively at Yuma. Approximately 40 Fusarium wilt-resistant F_4 plants that exhibited iceberg type characteristics were dug for seed production in a greenhouse at Salinas, but none survived to produce seed. Remnant seed of the families from which the selections were made will be tested in greenhouse tests for advancement to the next generation (F_5).

Table 2. Summary of Fusarium wilt tests in Huron and Yuma, 2014.

	Huron	Yuma
Planted:	22 Aug	17 Sep
Evaluated:	11 Nov	19 Nov
No. F_4 families	44	95
Mean symptom rating[z]		
95 F_4 families	1.0	1.5
Susceptible control	1.0	3.9
Resistant control	1.0	1.3

[z]rated using a 1 to 4 visual scale where 1 = no apparent disease, 2 = slight-moderate stunting, 3 = severe stunting and yellowing, and 4 = senesced.

e. Lettuce dieback

The lettuce dieback disease is caused by two closely related soilborne viruses of the family *Tombusviridae* – tomato bushy stunt virus (TBSV) and lettuce necrotic stunt virus (LNSV). Previous studies have provided no evidence that either chemical treatment or rotation with non-host crops can effectively reduce, remove, or destroy the virus in infested soil; thus resistant cultivars are the only known protection against the disease. While modern iceberg cultivars are resistant to dieback, susceptibility is widespread in romaine and leaf-type lettuce.

We continued developing romaine and leaf-lettuce breeding lines with combined resistance to dieback and other desirable traits. Crosses have been made to develop material with combined resistance to dieback and downy mildew, tipburn, Verticillium wilt, and extended shelf-life. Material were grown and tested in field conditions for resistance to downy mildew, and tipburn. The best lines were screened in multiple trials for disease resistance and post-harvest deterioration after minimally processing for salad. Resistance to dieback will be tested with the *Cntg10192* molecular marker that is closely linked to the *Tvr1* gene (Simko et. al. 2009, BMC Plant Biology 9:135). Inbred lines with resistance to dieback were released (for more information see Section D. Release of breeding lines)

f. Bacterial leaf spot (with C. Bull)

We were introgressing resistance from cultivar Little Gem into iceberg type lettuce using a greenhouse testing method. However, this work is no longer needed as research on the genetics of bacterial leaf spot resistance determined that resistance in Little Gem, Pavane, and La Brillante is likely due to the same gene. The breeding line RH08-0111 was released in 2013 for combining resistance to race 1 of Verticillium wilt with resistance to downy mildew and bacterial leaf spot. The performance of this line is described in Section D. Release of breeding lines. RH08-0111 was crossed to the cultivar Telluride, a cultivar carrying the *cor* gene for corky resistance. Progeny from this cross will be used to breed new cultivars with resistance to multiple disease and improved head weight.

We are breeding BLS resistance into cultivars suitable for spring mix production. Previous research determined that the greatest need for BLS resistance is in red romaine and red leaf types. We have developed and released two $F_{3:4}$ families from the cross of the resistant cultivar Reine de Glaces × Eruption. These lines were released under the numbers RH12-3370 and RH12-3371. These lines possess uniform levels of BLS resistance equivalent to that in Reine des Glaces, which results in lower disease than in the cultivars Vista Verde and Eruption. When packaged as salad, the shelf-life of these populations was as good or better than the cultivars Eruption and Annapolis. The populations are susceptible to leafminers and downy mildew. While these populations are uniform for BLS resistance and shelf-life, they are variable for leaf color, shape, savoy, undulation, and margin serration. Private and public breeding programs can select for the leaf morphology they desire without the need to select for BLS resistance or shelf-life. Detailed descriptions of these lines can be found in the publication Hayes et al. 2013. Hortscience 49: 18-24.

g. Corky root

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. PI 491239 and PI 273597c had lower corky root severity than cultivars with the *cor* resistance gene in soil from Watsonville that has *cor*-resistance breaking strains. The resistance from these lines is being incorporated into cultivated lettuces.

We continued to make crosses to transfer the *cor* resistance gene from 'Glacier' to green leaf, red leaf, romaine, and butterhead lettuce types, and to combine corky root resistance with resistances to other diseases and insects. F_2 to F_6 plants from these crosses were selected in the field for horticultural traits and resistances to corky root, downy mildew, leafminers, and tipburn. Backcrosses were used as necessary to restore horticultural types.

Thirteen F_7 to F_{10} breeding lines of butterhead, red leaf, and red romaine lettuce were tested in a replicated field trial at the USDA Spence Farm, Salinas from June to August 2013 for corky root resistance and horticultural traits. The corky root resistance of the breeding lines was similar to the resistant controls, while their plant weight, core length, tipburn, and downy mildew resistance were comparable or better than control cultivars (Tables 3-5).

Table 3. Mean values of corky root severity and head characteristics of butterhead lettuce breeding lines and cultivars evaluated in a trial at the USDA Spence Farm, Salinas, CA, summer 2013.

Genotype	Corky root[z]	Plant Wt. (g)	Core length (cm)	Tipburn leaves[y]
Dark Green Boston	7.9 A	425.2 DE	5.3 CDE	4.2 A
Bibb	7.7 A	443.0 DE	6.6 ABC	1.0 BC
Cobham Green	7.7 A	373.7 E	4.7 E	3.2 AB
Margarita	7.6 A	426.2 DE	4.4 E	0.8 BC
Glacier	6.0 B	904.2 A	7.9 A	1.7 ABC
MU12-903	5.7 BC	519.7 CD	5.0 E	0.1 C
MU12-902	5.2 CD	552.0 BC	5.2 DE	1.6 ABC
MU12-900	4.9 D	561.2 BC	6.5 BCD	1.0 BC
MU12-898	4.9 D	596.5 BC	7.4 AB	1.7 ABC
MU12-896	4.8 D	641.8 B	7.2 AB	1.3 BC

[z] 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 downy mildew severity and head characteristics of red leaf lettuce breeding lines and cultivars evaluated in a trial at the USDA Spence Farm, Salinas, CA, summer 2013.

Genotype	Corky root[z]	Downy mildew[y]	Plant Wt. (g)	Core length (cm)	Tipburn leaves[x]
Big Red	8.0 A	4.0 A	449.7 B	6.4 B	0.0 B
Prizehead	8.0 A	3.3 B	398.7 BC	5.3 BC	0.0 B
Red Fox	7.9 AB	2.5 C	275.0 DE	4.6 CD	0.0 B
Lolla Rossa	7.6 BC	1.5 E	152.3 F	3.7 D	0.0 B
Redina	7.3 CD	1.0 F	195.8 EF	6.5 AB	0.0 B
Merlot	6.9 D	1.0 F	174.7 F	4.8 CD	0.0 B
Glacier	6.0 E	4.0 A	904.2 A	7.9 A	1.7 A
MU12-1032	6.0 E	3.0 B	399.7 BC	4.6 CD	0.0 B
MU12-894	5.9 E	2.0 D	286.3 DE	3.9 CD	0.0 B
MU12-895	5.8 E	2.0 D	324.5 CD	4.2 CD	0.0 B

[z] Means in the same column followed by different letters indicate significant differences at $P < 0.05$.
[y] Scale from 0 (resistant) to 5 (highly susceptible)
[x] Number of leaves with tipburn in a head.

Table 5. Mean values of corky root severity and head characteristics of red romaine lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2013.

Genotype	Corky root[z]	Downy mildew[y]	Plant Wt. (g)	Core length (cm)	Tipburn leaves[x]
Green Forest	8.0 A	3.5 A	815.7 A	7.9 B	0.0 B
Red Hot Cos	8.0 A	3.5 A	594.0 B	6.1 B	0.3 B
Parris Island	7.9 A	3.0 B	813.8 A	8.6 B	0.1 B
Flashy Troutback	7.8 A	3.5 A	779.0 AB	17.0 A	3.6 A
MU12-884	6.3 B	1.5 E	655.3 AB	7.4 B	1.2 B
Heart's Delight	6.2 BC	3.0 B	677.0 AB	6.9 B	0.3 B
MU12-882	6.0 BC	2.5 C	654.9 AB	8.0 B	0.8 B
MU12-883	5.9 BC	2.0 D	691.1 AB	6.6 B	1.1 B
MU12-885	5.9 BC	1.5 E	660.5 AB	7.4 B	1.2 B
Clemente	5.8 C	3.3 AB	737.3 AB	8.0 B	0.1 B
MU12-889	5.3 D	3.5 A	731.3 AB	8.3 B	0.0 B

[z] Means in the same column followed by different letters indicate significant differences at $P < 0.05$.
[y] Scale from 0 (resistant) to 5 (highly susceptible)
[x] Number of leaves with tipburn in a head.

h. Downy mildew (quantitative resistance) (with R. Michelmore, M. Truco, O. Ochoa, R. Antonise, M. Pel)

Downy mildew (caused by the oomycete *Bremia lactucae*) is considered by some to be the most important disease affecting lettuce production world wide. Over 40 resistance genes (*Dm* genes) have been identified and introgressed into cultivated lettuce. Although *Dm* genes can be used in the resistance breeding programs they are race-specific and thus can be defeated by new isolates of the pathogen. Our research focuses on developing material with quantitative resistance. Material with this type of resistance (often called field resistance) is usually infected with the pathogen, but there are fewer and smaller lesions on fewer affected leaves, and disease progresses at a slower than on susceptible cultivars.

Five mapping populations have been being developed and currently tested in replicated field trials to detect quantitative trait loci for downy mildew resistance. Those populations originated from the crosses Salinas (susceptible) × Grand Rapids (resistant), PI 491224 (susceptible) × Iceberg (resistant), Grand Rapids × Iceberg, Salinas 88 (susceptible) × La Brillante (resistant), and Parade (intermediate) × Pavane (susceptible). Two populations based on crosses between Grand Rapids × Iceberg, Salinas 88 × La Brillante were previously genotyped with SNP (R. Michelmore's laboratory) and AFLP (KeyGene, The Netherlands) markers, while the Parade × Pavane population was genotyped with SNP markers. Field-based testing confirmed the presence of polygenes for resistance to downy mildew in all populations. Some lines in the Grand Rapids × Iceberg population exhibited either higher or lower levels of resistance than the two parents. The Salinas 88 × La Brillante mapping population has been tested in seven trials. The most consistent QTLs were detected on linkage groups (LG) 2, 4, and 7. QTLs on LG 1 and LG 9 were each detected in a single trial. The Parade × Pavane population has been tested only once, and a single QTL was detected on LG 9.

The Grand Rapids × Iceberg population has been tested in five field trials and two laboratory experiments. Field experiments were conducted from 2008 to 2013 in Salinas and The Netherlands. Three significant QTLs were detected in all environments. The QTLs located on linkage groups 2 (*qDM2.1*) and 5 (*qDM5.1*) were associated with disease resistance under both field and laboratory conditions. Resistance alleles at both QTLs originated from the cultivar Iceberg. The QTL on LG 9 (*qDM9.1*) was detected through simultaneous analysis of all experiments using a mixed-model approach. Alleles for elevated resistance at this locus originated from the cultivar Grand Rapids. Markers linked to the resistance alleles can aid future marker-assisted selection programs (Figure 2), thus complementing field-based phenotyping. We are developing additional mapping populations from crosses with highly susceptible accessions that will be used to map resistance genes not detected in the present studies. (Simko et al., 2013).

Crosses were made to develop new breeding lines that would exploit field resistance observed in the cultivars Balady Banha, Iceberg, Grand Rapids, Holborn's Standard, La Brillante, Merlot, and Primus. The hybrid plants were detected with molecular markers developed by our laboratory. Selections from multiple families will be evaluated in replicated trials for resistance to downy mildew, bolting, tipburn, and horticultural characteristics. These selections were made from spring and summer plantings in Salinas. Plants were selected that had a minimum number of lesions and were not bolting at the time of evaluation. The selected material will be evaluated for yield, size, uniformity, and tipburn resistance. A good level of

resistance to downy mildew was observed in material originating from crosses with Balady Banha, Iceberg, and Grand Rapids.

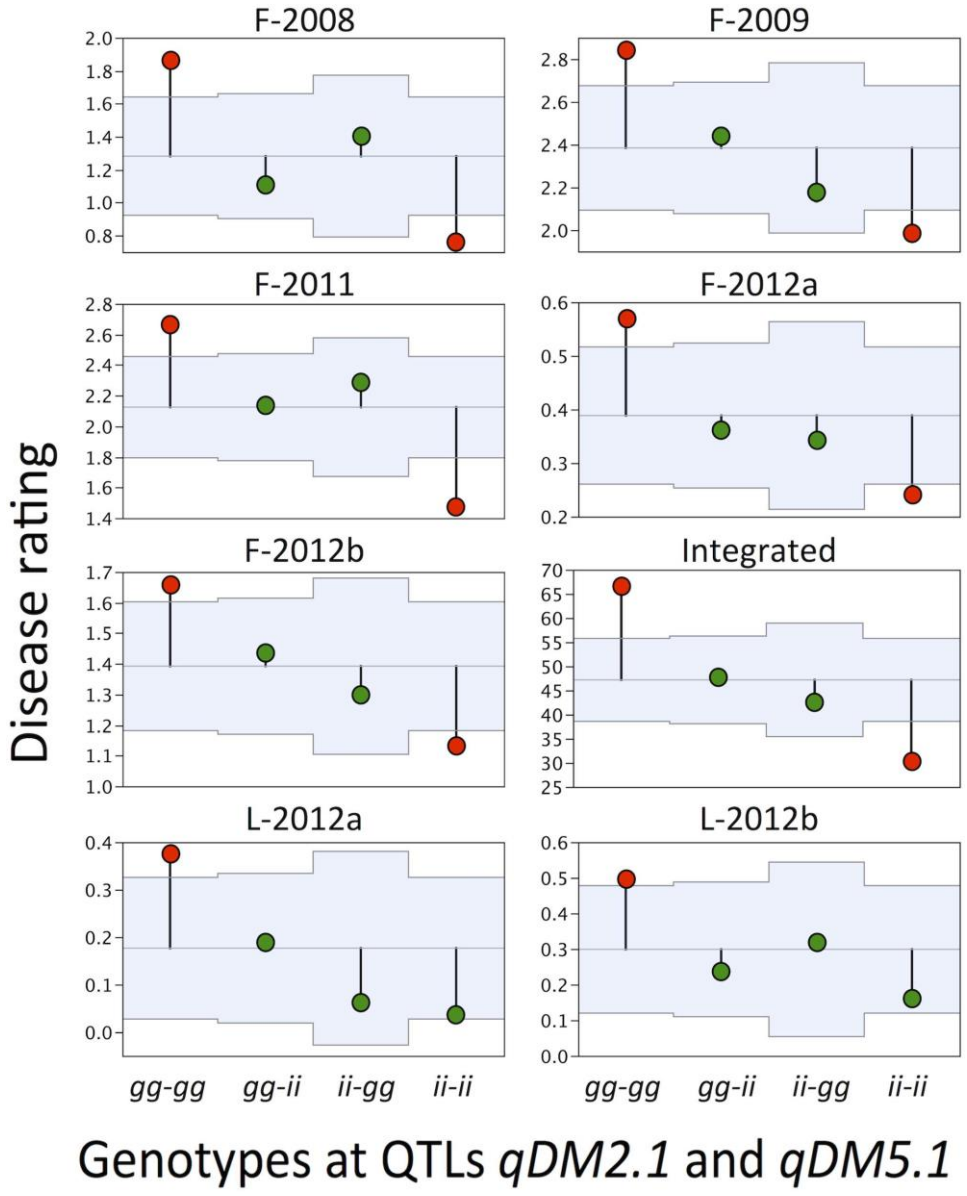

Figure 2. Testing potential of molecular markers linked to *qDM2.1* and *qDM5.1* for marker-assisted selection. The first two letters of genotypes show alleles at *qDM2.1* and the other two letters show alleles at *qDM5.1*. Values that are outside decision limits (marked in red) are significantly different ($P < 0.05$) from the overall mean. Data illustrate consistency of results in five field experiments (F-2008, F-2009, F-2011, F-2012a, and F-2012b), integrated rating of all experiments, and two laboratory experiments (L-2012a and L-2012b). Figure is from Simko et. al., 2013.

i. Powdery mildew (with G. Rauscher and R. Sideman)

Powdery mildew of lettuce is caused by the fungus *Golovinomyces cichoracearum sensu stricto* (formerly *Erysiphe cichoracearum* DC). The fungus may develop on both leaf surfaces, producing white, powdery spores. Affected leaves become slightly yellow, then brown, and eventually die, resulting in lower product quality. We mapped quantitative trait loci (QTLs) for resistance to powdery mildew under greenhouse conditions in an interspecific population derived from a cross between susceptible *L. sativa* cultivar Salinas and the highly susceptible *L. serriola* accession UC96US23. Four significant QTLs were detected on linkage groups LG 1 (*pm-1.1*), LG 2 (*pm-2.1* and *pm-2.2*), and LG 7 (*pm-7.1*); each explaining 35% to 42% of the phenotypic variation. The four QTLs are not located in the documented hotspots of lettuce resistance genes. Results of the field trials with $F_{2:3}$ and $F_{3:4}$ families derived from a Soraya (moderately resistant) × Salinas cross demonstrated effective transfer of resistance to powdery mildew in this material. An integrated rating approach allowed us to estimate relative levels of resistance in 80 cultivars and accessions tested in a total of 23 field and greenhouse experiments. Generally, very low resistance was observed in crisphead-type lettuces, while the highest relative resistance was recorded in leaf and butterhead types. The most resistant cultivars were Two Star (leaf), Clarion (butterhead), Sabine (butterhead), and Salad Bowl (leaf). The most susceptible cultivars were iceberg types: Autumn Gold, Grizzly, Silverado, Wolverine, and Yuma. More detailed information with relative resistance rating of 80 accessions is available in Simko et. al., 2014c. In the process of disease screening we developed a visual guide for estimating percentage of leaf area covered by powdery mildew (Figure 3).

Figure 3. Percentage ratings of leaf area covered by powdery mildew. Detailed information is in Simko et. al., 2014c.

2. Insect resistance

a. Leafminer

Crosses were made 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 to susceptible parents if necessary to combine leafminer resistance with desirable horticultural types. We also continued to make crosses to combine leafminer resistance with resistances to other diseases and insects. Crosses were also made among resistant sources to elevate the level of resistance.

F_2 to F_6 plants from crosses between leafminer resistant PI 169513, Red Grenoble, Merlot, Lolla Rossa, Bibb, and Tom Thumb and susceptible cultivars Salinas, Salinas 88, Tiber, Prizehead, and Lobjoits were selected in the field for leafminer and multiple resistances to other diseases, and some of them were backcrossed to the susceptible parents to combine leafminer and other resistances with desirable horticultural traits. Eleven promising F_7 to F_{10} breeding lines of green leaf, red leaf, and romaine lettuce were trialed at the USDA Spence Farm, Salinas from June to August 2013 with four replications, along with check cultivars. The breeding lines all had significantly lower leafminer sting density than susceptible cultivars and resistant controls, and the plant weight, core length, and tipburn incidence of many lines were similar to or better than commercial cultivars (Tables 6-8). Some of these lines also showed resistance to corky root, downy mildew, and excellent shelf life. These breeding lines will be evaluated again next year.

Table 6. Mean values of leafminer sting density, downy mildew rating, and head characteristics of green leaf lettuce breeding lines and cultivars evaluated in a trial at the USDA Spence Farm, Salinas, CA, summer 2013.

Genotype[z]	Stings/20 cm^2[,y]	Plant Wt. (g)	Core length (cm)	Downy mildew[x]
Two Star	125.7 A	362.5 ABC	5.1 B	4.0 A
Waldman's Green	77.0 B	388.8 ABC	7.7 A	3.8 A
Grand Rapids	55.2 C	285.7 C	5.1 B	1.5 BC
Shining Star	54.2 C	288.4 C	4.7 B	4.0 A
MU12-1141 (*cor*)	24.6 D	452.5 A	4.9 B	1.5 BC
MU11-365 (*cor*)	24.6 D	308.2 C	3.5 C	1.0 C
MU11-373 (*cor*)	22.0 D	333.3 BC	4.1 BC	1.5 BC
MU11-485 (*cor*)	16.5 D	424.0 AB	4.9 B	1.0 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 $P < 0.05$. [x] Scale from 0 (resistant) to 5 (highly susceptible).

Table 7. Mean values of leafminer sting density, downy mildew rating, and head characteristics of red leaf lettuce breeding lines and cultivars evaluated in a trial at the USDA Spence Farm, Salinas, CA, summer 2013.

Genotype	Stings/20 cm^{2,z}	Plant Wt. (g)	Core length (cm)	Downy mildew[x]
Prizehead	154.0 A	398.7 AB	5.3 AB	3.3 B
Big Red	142.0 A	449.7 A	6.4 A	4.0 A
Red Fox	117.4 B	275.0 C	4.6 BC	2.5 C
Merlot	52.8 C	174.7 D	4.8 BC	1.0 D
Lolla Rossa	46.2 C	152.3 D	3.7 C	1.5 D
MU12-1108	24.8 D	400.8 AB	5.9 AB	2.5 C
MU12-1161	18.9 D	332.9 BC	4.9 BC	3.0 BC

[z] Means in the same column followed by different letters are significantly different at $P < 0.05$.
[x] Scale from 0 (resistant) to 5 (highly susceptible).

Table 8. Mean values of leafminer sting density, and head characteristics of romaine lettuce breeding lines and cultivars evaluated in a trial at the USDA Spence Farm, Salinas, CA, summer 2013.

Genotype[z]	Stings[z]/20 cm^{2,y}	Plant Wt. (g)	Core length (cm)	Tipburn leaves
Green Forest	198.3 A	815.7 AB	7.9 BCD	0.0 B
Parris Island	175.3 AB	813.8 AB	8.6 BC	0.1 B
Red Hot	159.8 B	594.0 C	6.1 D	0.3 B
Heart's Delight	126.0 C	677.0 BC	6.9 CD	0.3 B
MU12-1208 (*cor*)	55.8 D	881.0 AB	11.3 A	2.1 A
MU12-1124	36.3 DE	980.3 A	10.8 A	0.1 B
MU12-1223 (*cor*)	33.3 DE	767.2 BC	9.9 AB	1.9 A
MU12-1126 (*cor*)	28.4 E	779.8 ABC	9.7 AB	1.1 AB
MU12-1030-1 (*cor*)	22.0 E	763.0 BC	7.5 CD	0.0 B
MU11-506	14.1 E	822.8 AB	6.9 CD	0.0 B

[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 $P < 0.05$.

b. Lettuce aphid

Two biotypes are known: Nr:0, which is known world wide, and Nr:1, which is known only in Europe. Resistance to biotype 0 (Nr:0) is controlled by a multiple allelic series of genes at a single locus. Complete (high-level) resistance in *L. virosa* PIVT 280 is controlled by the single dominant gene Nr^C that is being transferred to U.S. types by commercial breeders. Partial resistance in *L. serriola* PI 491093 is controlled by the single dominant allele Nr^P that is recessive to Nr^C but dominant to susceptibility, which is conditioned by the recessive allele *nr*. Partial resistance was expressed in open field tests, and in controlled-infestation field studies. Additional germplasm was obtained for testing: 55 CGN lettuce accessions reported to express resistance against European strains of biotypes Nr:0 and Nr:1. Seed of most of the accessions was increased in a greenhouse.

In an initial test of the CGN accessions, 'Barcelona', which was included as a resistant control, had ca. 40 aphids per plant compared with ca. 55 aphids on PI 491093 and ca. 81 aphids on 'Salinas' (Table 9). In this test, nine *L. virosa* accessions had ca. 2 aphids per plant, and 50 *L. serriola* accessions averaged 77 aphids per plant. Number of aphids per plant on the 50 *L. serriola* accessions ranged from zero to 318 in this test. Six *L. serriola* accessions had low numbers of aphids in the first test (2013-1) but had substantially higher numbers of aphids in the second test (2013-2).

The relatively high numbers of aphids on 'Barcelona' was unexpected (Table 9), but was observed in a subsequent test of a subset of eight of the CGN accessions when it had higher numbers of aphids than some of the CGN accessions (Table 10). Four seed lots of 'Barcelona' produced in a greenhouse at Salinas were evaluated along with 'Dynamite', which is also lettuce aphid-resistant, and IVT 280, which is the source of lettuce aphid resistance in these two cultivars. The seed lot designated Barcelona 5/12 had low numbers of aphid and was comparable to 'Dynamite' and IVT 280. Numbers of aphids on the other three seed lots ranged from 20 to 51, which was comparable to 'Salinas' (Table 11). These data suggest a seed mix-up rather than a change in the aphid biotype.

Table 9. Mean numbers of lettuce aphids on CGN candidate accessions for resistance to U.S. strain of lettuce aphid biotype Nr:0, 12 days post-infestation (2013-1-2).

Group	Genotype	n	Mean ±SE
PI 491093	Nr^P	1	54.4 ±13.3
Barcelona	Nr^C	1	39.2 ±14.4
Salinas	*nr*	1	80.9 ±7.2
CGN candidate accessions			
L. serriola	–	50	76.6 ±4.3
L. virosa	–	9	1.3 ±0.3

Table 10. Mean numbers of lettuce aphids per plant on three resistant and susceptible control entries and eight CGN accessions in two greenhouse tests.

		Test	
Entry	Species	2013-1[z]	2013-2[y]
Barcelona	*sativa*	54.4	191.0
Salinas	*sativa*	80.9	329.0
PI 491093	*serriola*	54.4	111.7
CGN9300	*serriola*	9.0	122.5
CGN9301	*serriola*	24.6	82.2
CGN10900	*serriola*	18.0	62.3
CGN10902	*serriola*	20.6	126.2
CGN13370	*serriola*	4.4	78.7
CGN15702	*serriola*	28.2	94.5
CGN13361	*virosa*	2.8	1.2
CGN16266	*virosa*	0.2	4.0

[z]12-days post-infestation; n = 5 for each entry
[y]17-days post-infestation; n = 6 for each entry

Table 11. Mean numbers of lettuce aphids on four seed lots of 'Barcelona', 'Dynamite, 'Salinas' and IVT 280 (2013-4).

Entry and pedigree	Mean[z]
Barcelona	
14139	50.9
Barcelona 5/12	8.1
Barcelona summer 02	71.7
Barcelona9-04	19.5
Dynamite	10.5
IVT 280	8.3
Salinas	59.2

[z]28 -days post-infestation; n = 10

3. Adaptation and Quality

a. Bolting resistance for fall plantings (with 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. A 2013 mid-September planted field experiment was conducted to select bolting-resistant romaine plants from segregating F_3 families, evaluate advanced inbred lines for resistance to pre-mature bolting and horticultural quality, and evaluate RIL populations for segregation of pre-mature bolting. This experiment had a high incidence of *Lettuce chlorosis virus* and was low yielding; across all cultivars only 30% of the plants had sufficient size and quality to be harvestable. The highest yielding cultivar was Valmaine (48%

harvestable), followed by Siskiyou (35%), Hearts Delight (32%), and Sunbelt (30%). Bolting resistance was measured as head weight divided by core length (g/cm). By this measure, Sunbelt (95 g/cm) and Siskiyou (88 g/cm) had the best bolting resistance. The best breeding line for yield (41% harvestable) and bolting resistance (92 g/cm) was derived from a cross between Siskiyou and Valmaine. Twenty F_4 families from Blonde Lente a Monter × Clemente, Blonde Lente a Monter × Green Towers, Blonde Lente a Monter × Siskiyou, Blonde Lente a Monter × Valmaine were evaluated for core length and horticultural quality. Six plants were selected from four F_4 families and allowed to self-pollinate to produce $F_{4:5}$ seed. Twenty-eight $F_{6:7}$ romaine breeding lines from Hearts Delight × Eruption that possess high level resistance to lettuce drop were evaluated for bolting resistance in this experiment. These breeding lines are from the same Hearts Delight × Eruption population described in section A. Cultivar and advanced breeding line development, 1. Disease resistances, b. Lettuce drop. All these lines bolted prematurely and were too small for commercial use in this planting slot. Additional crosses are needed to breed lettuce drop-resistant romaine with sufficient bolting resistance to fall planted low desert production.

Recombinant inbred line populations from Salinas 88 × PI 251246, Pavane × Parade, Western Red Leaf × Cool Guard, and Diplomat × Margarita populations were evaluated for plant development on a scale of 1 = rosette; 3 = bolting; 5 = flowering; 7 = open involucres with seed in a mid-September planted experiment in Yuma, AZ and a July planted field experiment in Salinas, CA. In total, these populations have been evaluated for plant development in five environments located in Yuma, AZ and three California locations. We will collect development data from two additional California experiments and then use the data to map QTL for plant development in the lettuce genome and determine which QTL are environmentally sensitive.

b. Tipburn

We are developing improved tipburn resistance in romaine cultivars adapted to coastal and desert production using the tipburn resistance found in modern iceberg cultivars. In multiple low desert and Salinas Valley field experiments, we developed inbred romaine breeding lines from Green Towers × Salinas with consistently less tipburn than Green Towers. All of these breeding lines are closed-top hearting-type romaine. In two replicated field experiments in Yuma, AZ five breeding lines had significantly fewer plants with tipburn compared to Green Towers (Table 12). In Salinas, CA, four breeding lines had percentages of plants with tipburn significantly lower than Green Towers. Tipburn was more frequent in Salinas compared to Yuma and three breeding lines (09-1498, 09-1513, 09-1526) had substantially greater amounts of tipburn in Salinas compared to Yuma. We have made single plant selections from these lines to determine if the horticultural characters can be further improved. We have an additional 22 F_5 and F_7 lines that have demonstrated low tipburn incidence, but more testing is needed to confirm this performance.

We are conducting selection for tipburn-resistant romaine in populations from diverse crosses. In a spring planted low desert experiment, tipburn-free, closed-top romaine type plants were selected from $F_{3:4}$ families derived from the crosses Clemente × Hallmark and Green Towers × Hallmark W. These families have the potential to combine tipburn, dieback, and corky root resistance.

Table 12. Percentage of plants with tipburn in seven romaine inbred breeding lines from Green Towers × Salinas and the romaine cultivar Green Towers in two replicated Yuma, AZ field experiments and two replicated Salinas, CA field experiments.

	Percentage of plants with tipburn	
Cultivar or breeding line	Yuma, AZ[z]	Salinas, CA[y]
Green Towers	39 a[2]	66 a
09-1498	10 b	73 a
09-1513	5 b	35 ab
09-1517	30 ab	23 b
09-1526	8 b	63 ab
09-1532	17 ab	35 b
09-1562	7 b	26 b
09-1565	10 b	24 b

[z]mean are from two replicated experiments in each location conducted in different years. Thirty or more plants per breeding line or cultivar were evaluated in each experiment.

[y]means with different letters are significantly different at $P < 0.05$ using the Tukey-Kramer multiple comparison procedure. Data were analyzed on the arcsine scale then backtransformed for reporting on tables.

B. Genetic studies

1. Shelf life of fresh-cut lettuce (with C. Galeano and R. Michelmore)

We previously identified a major QTL for shell-life on LG4 (*qSL4*) in the Salinas 88 × La Brillante and Pavane × Parade recombinant inbred line populations. The La Brillante and Pavane allele of this QTL results in rapid decay of fresh-cut lettuce. Dr. C. Galeano working at UC-Davis as part of the Specialty Crop Research Initiative Project 'Genes to Growers' has been working to clone the gene controlling this QTL. Cloning the gene will increase our understanding of the biology of the trait and aid the in development of markers useful for marker assisted selection. RILs with good and poor shelf-life were intercrossed and then self-pollinated to create F_2 populations segregating for *qSL4*. Approximately 1000 F_2 seed were sown in the greenhouse and the seedlings were used for DNA extraction and further analysis. Using markers from single-strand conformation polymorphism analysis, two co-dominant markers were identified flanking the *qSL4* and used for recombinant screening. A total of 81 recombinants were identified and 77 were transplanted to bigger pots for further analysis and seed production. The selected recombinants will be assayed for additional markers and evaluated for shelf-life.

2. Verticillium wilt (with G. Sandoya and R. Michelmore)

Race 1: We previously determined that resistance to race 1 isolates in La Brillante is due to the single dominant gene *Verticillium resistance 1* (*Vr1*) found on linkage group 9. However several

other sources of race 1 resistance are known. We conducted two allelism experiments with F_2 plants from crosses between 'La Brillante' to five different race 1 resistant cultivars in the greenhouse. The populations were La Brillante × Annapolis (283 progeny), La Brillante × Eruption (288 progeny), La Brillante × Little Gem (291 progeny), La Brillante × Merlot (284 progeny), and La Brillante × Pavane (297 progeny). Disease was assessed in a single race 1 infested field experiment with 238 F_2 plants from La Brilante × Merlot. The parents and the susceptible cultivar Salinas were included. No plants infected with race 1 were recovered in the F_2 progeny or parents. The disease incidence (DI) of 'Salinas' was 38 and 68% in the greenhouse experiments and was 70% in the field experiment. Race 1 in all of these cultivars is due to the *Vr1* gene, or a different but linked gene.

Race 2: We made progress in studying the genetics of partial resistance against race 2 of *V. dahliae*. A population of 99 F_3 families from the cross PI 171674 × PI 204707 was evaluated for resistance against race 2 in greenhouse experiments. PI 171674 and PI 204707 are partially resistant to race 2. We evaluated the families using an alpha lattice design with 3 replicates per experiment in two independent experiments. The parents and the susceptible cultivar Salinas was included in both experiments. Disease Severity (DS, 0= no root discoloration to 5=complete root discoloration), the percentage of the plant with foliar symptoms (FS), and days to first flower (DFF) were assessed on each plant. The frequency of plants with DS $\geq$ 1 was used to calculate the DI. The family means between the experiments were significantly correlated and the data was combined into a single analysis. The family means were quantitatively distributed and indicated the presence of segregation for DI, DS, FS and DFF. Family means ranged from 7 to 67% for DI, 0.2 to 3 for DS, 6 to 65% for FS, and 91 to 155 for DFF. The parents significantly differed from each other and 'Salinas' for all traits having means of 17, 52 and 70% for DI; 0.8, 2.3 and 3.4 for DS; 13, 30 and 51% for FS, and 158, 108 and 178 for DFF. The presence of segregation and high-level resistance in PI 171674 × PI 204707 makes this population useful for identifying quantitative trait loci for resistance. Families with high-level resistance are being used to initiate breeding of iceberg cultivars with race 2 resistance.

A population of 99 F_3 families from a cross between susceptible Salinas and the partially resistant PI 169511 was evaluated in a single experiment using the same methods used for the PI 171674 × PI 204707 population. No significant differences were found among F_3 families for DI, DS and FS. The parents were also not significantly different from each other with PI 169511 and Salinas having a DI of 39 and 50%, DS of 1.8 and 2.4, and FS of 22 and 31% respectively. This population was highly diseased and it appears that the level of partial resistance in PI 169511 was inadequate for this testing environment.

C. Molecular markers

1. Shelf-life (with R. Michelmore and M. Truco)

We continue developing molecular markers that can be used for marker-assisted selection of germplasm with good shelf-life. Analysis of the sequenced lettuce genome yielded 35 scaffolds with high similarity to the AFLP marker that is closely linked to *qSL4*. We have developed primers that amplify 80 genomic regions from these 35 scaffolds. Amplification was first conducted on four cultivars with very good or very poor shelf-life of salad-processed lettuce (two cultivars in each group). Sequencing revealed single nucleotide polymorphisms (SNPs) among

the four cultivars. Twenty amplicons that showed perfect matches between SNPs and phenotypic data were used in the subsequent analyses. These 20 genomic regions were sequenced across eighteen cultivars with different quality of shelf-life. We developed assays for the high-resolution DNA melting analysis and KAPS genotyping. The marker-trait analysis was subsequently performed on 92 accessions from different horticultural types of lettuce. Neither one of the markers showed a perfect match with shelf-life phenotypes, however a combination of two markers flanking *qSL4* was able to distinguish accessions with very fast tissue decay after processing for salad.. In 2014 and 2015 we will be testing these markers on twelve sets of F_2 families originating from crosses between parents with very fast decay and slow or intermediate speed of decay. Testing of this material will allow us to determine the accuracy of the markers and their potential for marker-assisted selection.

2. Genotyping by sequencing (C. Galeano, L. Froenicke, J. Duitama Castellanos, A. Kozik, S. Reyes Chin-Wo, and R. Michelmore)

Next-generation sequencing applied to crop plant genomes is providing abundant data enabling and facilitating the discovery and use of millions of single nucleotide polymorphisms (SNPs) in diverse genomes. In particular, genotyping by sequencing (GBS) is an affordable application of NGS for the purposes of discovering and genotyping SNPs in a variety of crop species and populations. We have developed a double enzyme digestion (*Hin*dIII and *Nla*III) protocol and pipeline data analysis for lettuce. A RIL mapping population of the cross Salinas 88 × La Brillante was pooled at 96-plex following our GBS library protocol. The library was sequenced and a total of 83,318 high quality SNPs were identified as polymorphic between the parents and evaluated in the RIL population. Redundant genotyping information was condensed per scaffold producing a total of 4,452 scaffolds. After filtering based on missing data and number of SNPs per scaffold, a total of 1,798 scaffolds were selected to build the linkage map. The final linkage map consisted of a total of 1,777 scaffolds mapped to 15 linkage groups, with a total map length of 1,840 cM. The GBS approach used illustrates a powerful method for developing high-density maps at low cost. Linkages between developed makers are shown in Figure 4.

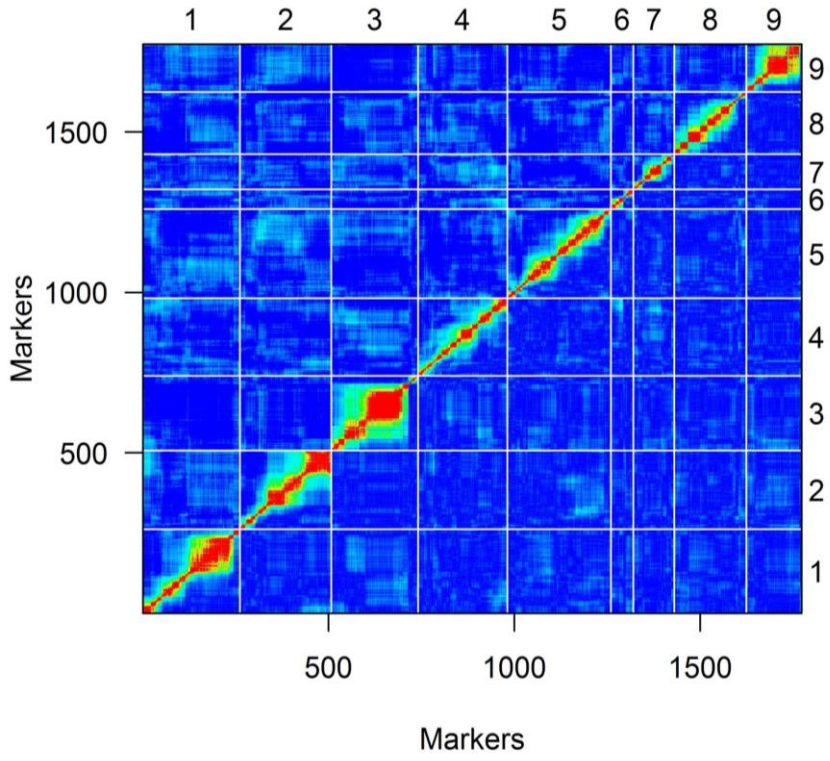

Figure 4. Pairwise recombination fraction and logarithm of the odds (LOD) score. Heatmap colors represent strength of linkage in both recombination frequency (upper left) and LOD score (lower right) between all pair of markers. Detailed information is in Galeano et. al., 2014.

D. Release of breeding lines

We are releasing sixteen breeding lines of lettuce (*Lactuca sativa* L.). Five (SM13-I1, SM13-I2, SM13-I3, SM13-I4, and SM13-I5) of the six iceberg breeding lines can be used for whole head or salad blend production; the sixth iceberg line (RH08-0111) does not form heads that meet the modern iceberg standard (Table 13, Figure 5). This breeding line, however, can be used in breeding programs because it combines high level resistances to downy mildew, bacterial leaf spot, Verticillium wilt race 1, and lettuce dieback. Two (SM13-R2, SM13-R3) of the four romaine breeding lines are suitable for whole head, salad blend, and spring mix production. One breeding line (SM13-R1) is suitable for whole head production only. The fourth romaine line (RH08-0464) can be used in breeding programs due to its good resistance to downy mildew. SM13-R1, SM13-R2, and SM13-R3 are resistant to dieback. Five leaf lettuce breeding lines (SM13-L1, SM13-L2, SM13-L3, SM13-L4, and SM13-L5) have very high levels of polygenic resistance to downy mildew and can be used for spring mix and salad blend production. The sixth leaf lettuce breeding line (SM13-L6) has resistance to Verticillium wilt race 1 and is suitable for whole head, spring mix, and salad blend production. The leaf breeding lines are resistant to dieback with the exception of SM13-L1. Limited samples of seeds are available for distribution to all interested parties for research or commercial purposes. Written requests should be sent to the first or the second author. Detailed description of the breeding lines is in Simko et. al., 2014a.

Figure 5. Phenotypes of 16 released breeding lines from three horticultural types. The bottom row shows selected lines before shipping to Maryland for evaluations of whole head decay (Figure is from Simko et. al., 2014a).

Table 13. Pedigree, description, and potential use of six iceberg, four romaine, and six leaf lettuce new breeding lines. Detailed information is in from Simko et. al., 2014a.

Line	Pedigree	Description	Advantage[a]	Disadvantage[a]	Potential use
RH08–0111	F_8: Salinas 88 × La Brillante	Green, slightly ruffled leaves with undulation	Improved resistance to downy mildew, bacterial leaf spot, and Verticillium wilt race 1	Small, soft heads that do not meet the modern iceberg standard	Salad blend, breeding programs
SM13-I1	F_6: [(Salinas × Iceberg) × (Iceberg × Calmar)] × Glacier	Green, slightly ruffled leaves with undulation	Improved resistance to downy mildew	Susceptible to tipburn	Whole head, salad blend
SM13-I2	F_6: [(Salinas × Iceberg) × (Iceberg × Calmar)] × Glacier	Green, slightly ruffled leaves with undulation	Improved resistance to downy mildew	Relatively rapid decay of whole heads	Whole head, salad blend
SM13-I3	F_6: [(Salinas × Iceberg) × (Iceberg × Calmar)] × Glacier	Green, slightly ruffled leaves with undulation	Improved resistance to downy mildew	Relatively rapid decay of whole heads	Whole head, salad blend
SM13-I4	F_6: [(Salinas × Iceberg) × (Iceberg × Calmar)] × Glacier	Green, slightly ruffled leaves with undulation	High percentage of harvestable heads, improved resistance to downy mildew	Susceptible to tipburn	Whole heads, salad blend
SM13-I5	F_8: (Iceberg × Salinas) × (Iceberg × Salinas)	Green, scalloped leaves with undulation	Improved resistance to downy mildew		Whole heads, salad blend
RH08–0464	F_6: Baladý Banha × Darkland	Dark green smooth leaves with slight undulation	Improved resistance to downy mildew	Long core; small percentage of harvestable heads; susceptible to tipburn, bacterial leaf spot, Verticillium wilt and dieback, rapid decay of whole heads	Breeding programs
SM13-R1	F_7: PI 491224 × Parris Island Cos	Green smooth leaves with undulation	Low tipburn incidence, resistant to dieback	Rapid decay after processing for salad	Whole head
SM13-R2	F_8: PI 491224 × Parris Island Cos	Green smooth leaves with undulation	Slightly more resistant to leafminer, slow decays of whole heads, resistant to dieback		Whole head, spring mix, salad blend
SM13-R3	F_8: Darkland × PI 491214	Green smooth leaves with undulation	Low tipburn incidence, slow decay of whole heads, slow decay after processing for salad, resistance to dieback	Susceptible to leafminer	Whole head, spring mix, salad blend
SM13-L1	F_7: Grand Rapids × Iceberg	Light green ruffled leaves with undulation	Relatively large head, improved resistance to downy mildew, slow decay of whole heads	Long core (early bolting), susceptible to dieback	Whole head, spring mix, salad blend
SM13-L2	F_7: Grand Rapids × Iceberg	Light green ruffled leaves with undulation	Low tipburn incidence, improved resistance to downy mildew, slow decay of whole heads, resistant to dieback		Whole head, spring mix, salad blend
SM13-L3	F_7: Grand Rapids × Iceberg	Light green ruffled leaves with undulation	Improved resistance to downy mildew, resistant to dieback	Susceptible to tipburn, relatively rapid decay of whole heads	Spring mix, salad blend
SM13-L4	F_7: Grand Rapids × Iceberg	Light green ruffled leaves with undulation	Improved resistance to downy mildew, resistant to dieback	Susceptible to tipburn, low percentage of harvestable heads, relatively rapid decay of whole heads	Spring mix, salad blend
SM13-L5	F_7: Grand Rapids × Iceberg	Light green ruffled leaves with undulation	Improved resistance to downy mildew, resistant to dieback	Relatively rapid decay of whole heads	Spring mix, salad blend
SM13-L6	F_7: Lolla Rossa × PI 491224	Dark green/reddish smooth leaves with undulation	Slow decay of whole heads, resistant to dieback and Verticillium wilt race 1	Less resistant to downy mildew than other five new breeding lines of leaf lettuce	Whole head, spring mix, salad blend

[a]Advantages and disadvantages as compared with commercial standard cultivars for the respective horticultural type.

E. Germplasm evaluation, maintenance and use

1. Screening

a. Bacterial leaf spot (with C. Bull)

In limited screening using *Xanthomonas campestris* pv. *vitians* (*Xcv*) isolates from C. Bull's collection, the HR resistance gene found in Little Gem, Pavane and La Brillante was effective against five isolates and defeated (resulting in susceptible reaction) by a single Canadian isolate (BS3127). We screened diverse *Lactuca* accessions in the USDA, Salinas germplasm collection for resistance to BS3127 by infiltrating *Xcv* into leaves of seedlings using a needleless syringe and assessing for HR 24 to 30 hours of infiltration. This is a pre-emptive effort to identify resistance should BS3127 or a similar isolate be found in California. We detected an HR reaction in ten *L. serriola* accessions. Two greenhouse experiments were conducted with these accessions along with La Brillante, Little Gem, Pavane, Batavia Reine des Glaces, and Vista Verde to assess disease severity against BS3127. *Xcv* was sprayed onto four-week-old seedling and disease severity was evaluated seven days later on a 0 = no disease to 5 = severe disease scale. Seven of the *L. serriola* accessions were highly resistant to *Xcv* BS3127 (mean DS < 0.5). La Brillante, Little Gem, Pavane, Batavia Reine des Glaces, and Vista Verde were susceptible with mean DS > 2.0.

b. Verticillium wilt (with G. Sandoya, K. Subbbarao, and R. Michelmore)

Previous research identified two races of *V. dahliae* capable of causing disease in lettuce. La Brillante and other germplasm are resistant to race 1. We have confirmed partial resistance (disease incidence significantly lower than Salinas) in four accessions (PIs 169511, 171674, 204707, 226641). However, all of these PIs have had at least a few symptomatic plants, and all but PI 171674 have had non-symptomatic plants that are nonetheless colonized by *V. dahliae*. No sources of complete resistance to race 2 are known. We are screening PIs for resistance to race 2 by conducting un-replicated greenhouse experiments to identify candidate sources of resistance, which are then tested in replicated greenhouse experiments to confirm resistance. In all experiments, plants are assessed for disease symptoms after they have flowered, and asymptomatic plants are tested for *V. dahlia* colonization by plating stem sections on semi-selective NP10 media. To date, we have screened over 850 *L. sativa* and *L. serriola* accessions in un-replicated experiments using race 2 *V. dahliae* isolate VdLs17.

In 2013 a replicated experiment with 34 previously tested cultivars was planted in the greenhouse and inoculated with the *V. dahliae* isolate VdLs17. Salinas and PI 171674 were used as the susceptible and partial resistant controls respectively. In this experiment we identified PI 358038 as having significantly less disease than the susceptible control Salinas. The level of resistance in PI 358038 was similar to PI 171674. Greenhouse testing for resistance and population development with this accession are ongoing.

The race specificity of germplasm with partial resistance to race 2 is unknown. We conducted a single greenhouse experiment with La Brillante (race 1-resistant), four accessions with partial resistance to race 2 (PIs 169511, 171674, 204707, and *L. serriola* 11G9991), and Salinas to evaluate their resistance against a representative race 1 (VdLs16) and race 2 (VdLs17)

isolate. Accession 11G9991 was previously selected by UC-Davis for resistance against race 2 isolates. The accessions showed partial resistance to race 1 and race 2 with DI means of 35, 30, 25, and 50% against race 1 and 25, 45, 10 and 30% against race 2 in PIs 169511, 204707, 171674 and 11G9991 respectively. Salinas had a mean DI of 50% against race 1 and 85% against race 2. In these experiments La Brillante was completely resistant to race 1 (0% of DI) but susceptible to race 2 (70% of DI). The same cultivars and accessions plus PI 226641 were planted in a race 1-infested field in two consecutive experiments. PIs 169511, 171674, 204707, 226641 and 11G9991 reached a DI of 30, 38, 35, 58, and 20% respectively. Salinas had 80% DI. These findings suggest that the gene(s) conferring partial resistance in PI 169511, PI 171674, PI 204707, PI 226641 and accession 11G9991 are effective against both races.

We screened diverse iceberg cultivars to determine if any can delay disease symptoms past market maturity. We evaluated 34 iceberg cultivars from different origins (United States and Europe) along with the race 1-resistant breeding line RH11-1798 and the susceptible Salinas as controls. Experiments were conducted in an artificially infested race 1 field at the USDA-ARS in Salinas, California over two years. We evaluated DS (0 = no root discoloration to 5=complete root discoloration) and the frequency of plants with DS $\geq$ 1 was used to calculate the DI. Foliar symptoms (FS) were evaluated on a scale of 0 = completely healthy to 5 = all cap leaves wilted and/or dead. Head maturity (HM) was rated on a scale of 0 = open heads to 5 = splitting, over mature heads. FS and HM were assessed at three time-points. We found genetic variation for DI, DS, FS and HM. The median FS of the cultivars Climax (0.4), Anuenue (0.8), Desert Storm (0.9), Batavia Reine des Glaces (1.1) and Bubba (1.4) were lower than other cultivars despite having a high percentage of plants with root discoloration (up to 80% DI). The resistant control had a median FS of 0.1 with 0% DI. The FS of Salinas was 3.3 with a DI of 80%. We have crossed the cultivars that delay foliar symptoms past market maturity with those that quickly develop wilting to study the genetics of this trait.

2. Collection and distribution

We have distributed publicly available accessions, cultivars and populations to various research groups and seed companies worldwide through individual requests and the Organic Seed Partnership program. Released USDA germplasm has been distributed to parties providing written requests. From 2013 through 2014, requests were made for bacterial leaf spot resistant baby leaf populations (RH12-3370 and RH12-3371), Verticillium wilt resistant iceberg breeding lines (RH05-0336, RH05-0339, RH05-0340, RH08-0472, RH08-0475), bacterial leaf spot resistant icebergs (RH04-0157-3, RH07-0370M, RH07-0373M, RH07-0379M, RH07-0380M, RH07-0386M, RH07-0387M), dieback resistant romaine lines SM09A and SM09B, crisphead lettuce breeding lines with resistance to corky root and lettuce mosaic virus (04-0344, 04-0350, 04-0353, 04-0363, 04-0368, 04-0375, and 04-379), leaf lettuce breeding lines with corky root resistance (06-831 and 06-833), and sixteen inbred lines released in 2014 (SM13-I1, SM13-I2, SM13-I3, SM13-I4, SM13-I5, RH08-0111, SM13-R1, SM13-R2, SM13-R3, RH08-0464, SM13-L1, SM13-L2, SM13-L3, SM13-L4, SM13-L5, and SM13-L6).

E. Evaluation of advanced breeding lines

To fully characterize the lines we develop prior to release, we have developed an extensive adaptation, disease resistance, and quality testing network. These testing resources enable us to characterize and breed multiple resistant cultivars, and to develop a thorough profile of a breeding line's strengths and weaknesses before it is released. Ultimately, this should increase adoption and use of USDA germplasm. Several field trials were planted and evaluated in the Salinas Valley and Yuma. We are indebted to numerous growers and shippers for their cooperation in providing space and resources for our trials. Most evaluation methods we use are documented in previous germplasm release publications: Mou et al. 2007, HortScience 42:701-703; Simko et al. 2010, HortScience 45:670-672; Hayes et al. 2011, HortScience 46:501-504; Simko et al. 2012, Crop Science 52:2131-2142. Arrival quality is being conducted by harvesting, cooling, and shipping lettuce cartons to Dr. Yaguang Luo with the USDA in Beltsville, MD. Whole heads are assessed for quality on a 1 (high quality) through 10 (low quality) scale after 7, 14, and 21 days storage at 5°C. Cooling and shipping is conducted in coordination with Taylor Farms. Data will be accumulated over years and communicated when lines are released. In 2013, we characterized 14 iceberg, 6 leaf, and 4 romaine USDA or UCD breeding lines for yield, horticultural characters, shelf-life of salad and arrival quality, and for resistance to Verticillium wilt, lettuce drop, downy mildew, tipburn and pre-mature bolting.

F. Recent publications relevant to this project

Galeano CH, Froenicke L, Duitama Castellanos JA, Kozik A, Reyes Chin-Wo S, Simko I, Hayes R, Michelmoe R (2014): Development of high-density intra-specific linkage map using genotyping by sequencing (GBS) in lettuce. Plant and Animal Genome conference, San Diego, CA, #P686.

Hayes RJ, Trent MA, Mou B, Simko I, Gebben S, and Bull CT (2014) Baby leaf lettuce germplasm enhancement: developing diverse populations with resistance to bacterial leaf spot caused by *Xanthomonas campestris* pv. *vitians*. Hortscience, 49: 18-24.

Kwon S, Simko I, Hellier B, Mou B, Hu J (2013): Genome-wide association of 10 horticultural traits with expressed sequence tag-derived SNP markers in a collection of lettuce lines. Crop Journal, 1: 25-33.

Lafta A, Mou B (2013): Evaluation of lettuce genotypes for seed thermotolerance. HortScience 48: 708-714.

Mou B (2013): Lettuce. In: (Wehner TC, Mou B, eds.) Vegetable cultivar descriptions for North America, List 27. HortScience 48: 252-257.

Samtani, JB, Rachuy JS, Mou B, Fennimore SA (2014): Evaluation of tribenuron-methyl on sulfonylurea herbicide tolerant lettuce germplasm. Weed Technology (*in press*)

Simko I (2013): Marker-assisted selection for disease resistance in lettuce. In: (Varshney R, Tuberosa R, eds.) Translational genomics for crop breeding: Volume 1 Biotic Stress. Wiley-Blackwell.

Simko I, Atallah AJ, Ochoa OE, Antonise R, Galeano CH, Truco MJ, Michelmore RW (2013): Identification of QTLs conferring resistance to downy mildew in legacy cultivars of lettuce. Scientific Reports, 3: 2875.

Simko I, Hayes RJ, Bull CT, Mou B, Luo Y, Trent MA, Atallah AJ, Ryder EJ, Sideman RG (2014a): Characterization and performance of 16 new inbred lines of lettuce. HortScience, 49: 679-687.

Simko I, Hayes RJ, Mou B, McCreight JD (2014b): Lettuce and spinach. In: (Smith S, Diers, B, Specht J, Carver B, eds.) CSSA Special Publications, Yield Gains in Major U.S. Field Crops 33. ASA, CSSA, AND SSSA, Madison, WI.

Simko I, Rauscher G, Sideman RG, McCreight JD, Hayes RJ (2014c): Evaluation and QTL mapping of resistance to powdery mildew in lettuce. Plant Pathology, 63: 344-353.