

**PROJECT REPORT  
CALIFORNIA LETTUCE RESEARCH BOARD**

**For the period  
April 1, 2012 - March 31, 2013**

PROJECT TITLE: Lettuce Breeding, USDA-ARS

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Growers, shippers, seedsmen- All districts

**ABSTRACT:**

In the 2012-2013 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)

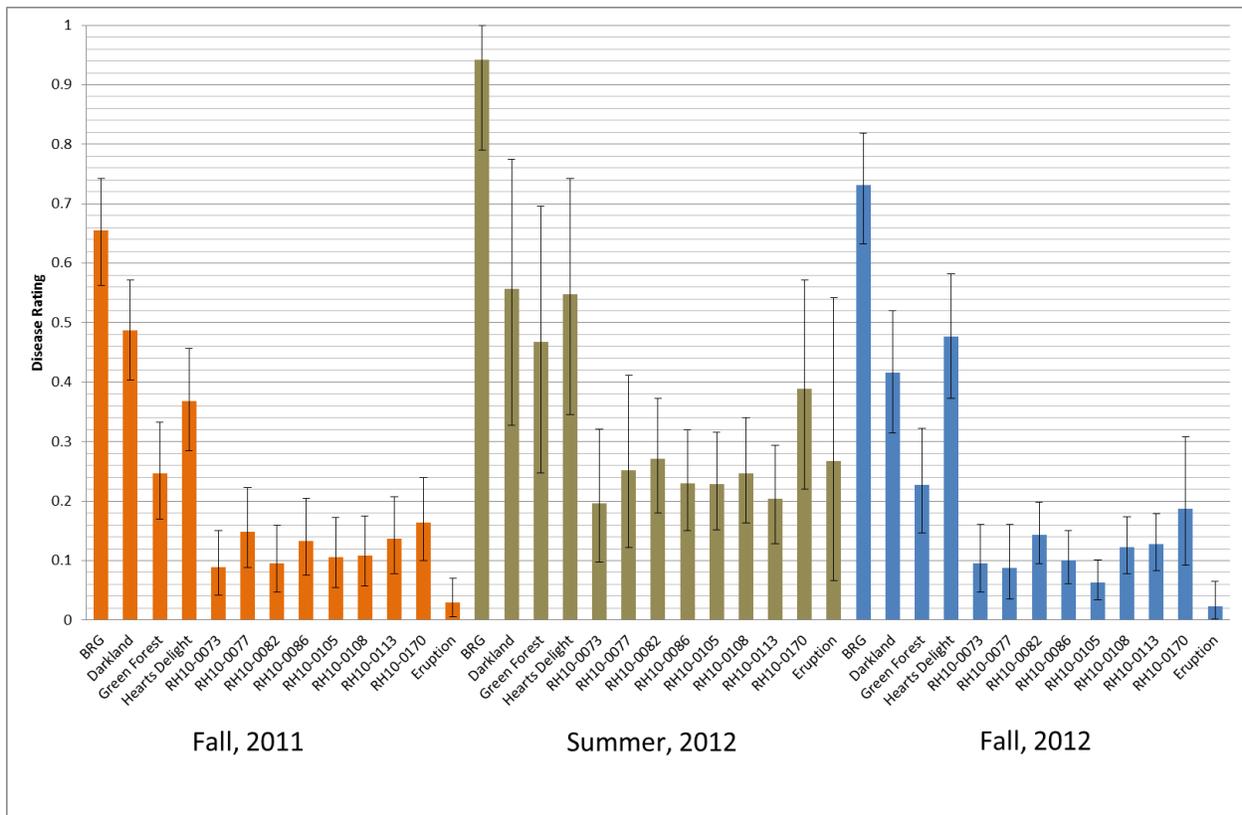
A high level of partial resistance to big-vein is available in the butterhead cultivar Margarita; we are introgressing this resistance into iceberg breeding lines. In a January planted field experiment in Soledad, CA, we evaluated fourteen BCF<sub>3:4</sub> iceberg type lines derived from crosses between Margarita and iceberg cultivars, along with the check cultivars Margarita, Pacific, and Tiber. Margarita had zero big-vein, while Pacific and Tiber had 23% and 33% respectively. Thirteen of the fourteen BCF<sub>3:4</sub> iceberg families had greater big-vein incidence than Pacific. One BCF<sub>3:4</sub> had 16% big-vein, but had unacceptable horticultural quality. A January planting in Salinas, CA with 48 BCF<sub>3:4</sub> families was attempted, but the planting was abandoned due to extensive bird damage. We will evaluate additional families and lines in an attempt to identify recombinant breeding lines that combine resistance and good horticultural quality.

High level resistance to *Mirafiori lettuce big-vein virus*, the causal agent of big vein disease, is known in *L. virosa* accession SAL012 (Hayes et al. 2008, Euphytica 164:493–500). We continued crosses to develop BC families from Salinas x SAL012 but made no substantial progress towards developing these populations.

##### 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 2012 we inbred 184 iceberg lines from Hallmark x (Salinas 88 x (75-501-1 x Holborn Standard)) to the F<sub>5</sub> generation, 129 romaine lines from Darkland x RH07-0731-4-3 to the F<sub>6</sub> generation, and 161 lines from Reine des Glaces x Eruption to the F<sub>4</sub> generation. Seven new F<sub>2</sub> populations were developed using resistance from Eruption and PI226641.

We are introgressing resistance from the cultivar Eruption into romaine breeding lines. 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. Details on the resistance in Eruption can be found in the publications: Hayes et al. 2010, HortScience 45:333–341; and Hayes et al. 2010, Plant Breeding 130:388-393. In three replicated field experiments, eight inbred romaine breeding lines with resistance from Eruption routinely demonstrated significantly lower mortality than Darkland and Hearts Delight (Fig. 1). Breeding lines with significantly less lettuce drop than Green Forest was observed in two experiments (Fig. 1). Core length was evaluated in one experiment to determine each lines propensity for early bolting; all lines had shorter cores than Green Forest, Hearts Delight, and Darkland (data not shown).



**Figure 1.** Lettuce drop disease rating for eight inbred romaine breeding lines from Hearts Delight x Eruption and the cultivars Reine des Glaces (BRG), Darkland, Hearts Delight, Green Forest, and Eruption in three replicated field experiments. Disease ratings are the backtransformed means calculated from analysis of proportion mortality data on the arcsine scale. Disease rating is positively correlated with disease incidence.

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

In 2012 a population of twelve inbred resistant iceberg breeding lines from Tiber x ((Pacific x La Brillante) x Tiber) were evaluated in a race 1 infested field site at the USDA, Salinas, CA, an infested grower's field in Salinas, CA, and a non-infested grower's field site in Soledad, CA. Disease data were collected at the two infested sites, horticultural quality was evaluated at the two grower sites. The breeding lines demonstrated resistance (0 – 6% disease incidence) at both Salinas, CA locations, while greater than 70% disease incidence was observed in Salinas and Tiber. Eight lines were selected with improved horticultural quality for evaluation next season.

We made progress on developing iceberg breeding lines that combine resistance to race 1 isolates of *V. dahliae* with resistance to corky root, bacterial leaf spot, and downy mildew. In 2012 we developed F<sub>1</sub> and F<sub>2</sub> populations segregating for genes conferring resistance to these diseases.

We are working to develop race 2 resistant breeding lines using the currently available sources of resistance. Using PIs 204707, 171674, 226641, and 169511, breeding populations from resistant × resistant and resistance × susceptible-commercial-cultivar crosses were

produced. We continue to screen diverse accessions for resistance to race 2 isolates. Additional details can be found in genetics and germplasm evaluation section of this report.

#### d. Fusarium wilt

Three races of Fusarium wilt are known in Japan; only race 1 is present in California and Arizona. Tests of resistant and susceptible cultivars and about 100 F<sub>3</sub> King Louie x Autumn Gold families in Fall 2010 and Fall 2012 seasons were highly and significantly correlated ( $r = 0.68$ ,  $P < 0.001$ ). The F<sub>3</sub> families expressed high-level resistance and segregated for plant type; 53 families selected for advancement to F<sub>4</sub>.

#### 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 developing resistant cultivars is 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 shelf-life. Material from the F<sub>2</sub> – F<sub>5</sub> generations were grown and tested in field conditions for resistance to downy mildew, and tipburn. The best lines will be screened again 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).

#### f. Bacterial leaf spot (with C. Bull)

We were introgressing resistance from Little Gem into iceberg type cultivars using a greenhouse testing method. However, this work is no longer needed. Research on the genetics of bacterial leaf spot resistance determined that resistance in Little Gem, Pavane, and La Brillante are due to the same gene (see section B. Genetic studies, 1. Bacterial Leaf Spot). Iceberg type lines from the Salinas 88 x La Brillante RIL population were identified as bacterial leaf spot resistant. They are currently being evaluated for multiple characters in order to characterize the lines for release.

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. Through 2011 and 2012, three independent replicated greenhouse experiments were conducted to select two F<sub>3</sub> romaine type families (BL27 and BL22) from the cross Batavia Reine de Glaces (resistant cvr) x Eruption. The resistance in these families was equal in level and uniformity to Batavia Reine de Glaces (Table 1). Therefore, these families appear to be genetically fixed for resistance genes from Batavia Reine de Glaces. The families are variable

for leaf color, shape, savoy, undulation, and margin serration. After release to private and public breeding programs, these institutions will be able to select for the leaf morphology they desire without the need to select for BLS resistance.

**Table 1.** Bacterial leaf spot mean and variance of resistant cultivars La Brillante and Batavia Reine des Glaces, the susceptible cultivar Eruption, and two F<sub>3</sub> families from Batavia Reine des Glaces x Eruption evaluated in three replicated greenhouse experiments.

Entry	Bacterial leaf spot disease severity					
	Experiment 1		Experiment 2		Experiment 3	
	mean	variance	mean	Variance	mean	variance
La Brillante	0.1	0.1	0.7	0.1	1.8	0.8
Batavia Reine des Glaces	0.8	0.3	1.3	0.4	2.6	1
BL27	0.7*	0.2 <sup>NS</sup>	1.4*	0.4 <sup>NS</sup>	2.2*	0.6 <sup>NS</sup>
BL22	0.9*	0.3 <sup>NS</sup>	1.6*	0.6 <sup>NS</sup>	2.5*	0.6 <sup>NS</sup>
Eruption	1.6	0.5	2.9	0.1	3.7	1.9

Disease severity rated 0 = no symptoms to 5 severe symptoms

\*/ mean sig. lower than Eruption and not significantly different from Reine des Glaces

NS/ variance among plants not significantly different from Reine des Glaces

#### g. Corky root

We 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 *cor* resistant 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<sub>2</sub> to F<sub>6</sub> 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.

Fourteen F<sub>7</sub> or F<sub>8</sub> breeding lines of butterhead, green leaf, red leaf, and red romaine lettuce were tested in a replicated field trial at the USDA Spence Farm in Salinas from June to August 2012 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 2-4).

**Table.** Mean values of corky root severity and head characteristics of butterhead lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2012.

Genotype	Corky root <sup>z</sup>	Plant Wt. (g)	Core length (cm)	Tipburn leaves <sup>y</sup>
Cobham Green	7.9 A	313.8 DE	4.1 BCD	2.9 B
Bibb	7.9 A	393.7 CD	4.8 AB	0.2 C
Dark Green Boston	7.9 A	359.2 CDE	4.9 AB	6.8 A
Margarita	7.8 A	307.0 E	3.8 CD	0.0 C
MU11-534	6.0 B	521.3 A	4.6 ABC	0.0 C
MU10-482	5.7 BC	414.2 BC	3.6 D	0.0 C
MU10-481-1	5.4 CD	483.5 AB	4.9 AB	0.0 C
MU10-479-1	4.8 DE	406.4 BC	4.9 AB	0.2 C
MU10-480-1	4.8 E	426.7 BC	5.2 A	0.1 C

<sup>z</sup> Means in the same column followed by different letters indicate significant differences at  $P < 0.05$ .

<sup>y</sup> Number of leaves with tipburn in a head.

**Table 3.** 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 Spence Farm in Salinas, Calif. in summer 2012.

Genotype	Corky root <sup>z</sup>	Downy mildew	Plant Wt. (g)	Core length (cm)	Tipburn leaves <sup>y</sup>
Big Red	7.8 A	3.8 A	360.4 BC	5.3 A	0.0 B
Red Fox	7.8 A	3.5 AB	394.4 B	5.3 A	0.0 B
Lolla Rossa	7.6 A	1.0 E	171.8 E	4.3 AB	0.0 B
Prizehead	7.8 A	3.0 B	372.7 BC	5.2 A	0.0 B
Redina	7.5 A	1.3 DE	247.5 DE	5.6 A	0.0 B
Merlot	6.8 B	1.8 CD	201.0 E	4.4 AB	0.0 B
MU09-486-1	6.1 C	1.5 CDE	320.7 BCD	3.2 B	0.0 B
MU11-329	6.0 C	3.0 B	603.0 A	5.6 A	0.2 A
MU11-332	5.8 C	2.0 C	303.0 CD	3.1 B	0.0 B

<sup>z</sup> Means in the same column followed by different letters indicate significant differences at  $P < 0.05$ .

<sup>y</sup> Number of leaves with tipburn in a head.

**Table 4.** 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 2012.

Genotype	Corky root <sup>z</sup>	Plant Wt. (g)	Core length (cm)	Tipburn leaves <sup>y</sup>
Red Hot Cos	7.9 A	782.0 A	7.2 ABC	1.5 A
Flashy Troutback	7.9 A	760.5 A	8.3 AB	1.6 A
MU11-448	6.2 B	477.8 B	4.4 F	0.0 B
MU11-449-1	6.2 B	618.7 AB	5.2 EF	0.3 B
MU11-450	6.1 B	677.0 AB	6.0 CDE	1.4 A
MU11-452-1	6.0 B	765.5 A	6.9 BCD	0.5 AB
Heart's Delight	5.9 B	866.3 A	8.6 A	0.0 B

<sup>z</sup> Means in the same column followed by different letters indicate significant differences at  $P < 0.05$ . <sup>y</sup> 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 oomycete *Bremia lactucae*) is considered the most important disease affecting lettuce production. 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 slower rate of disease progress than on susceptible cultivars.

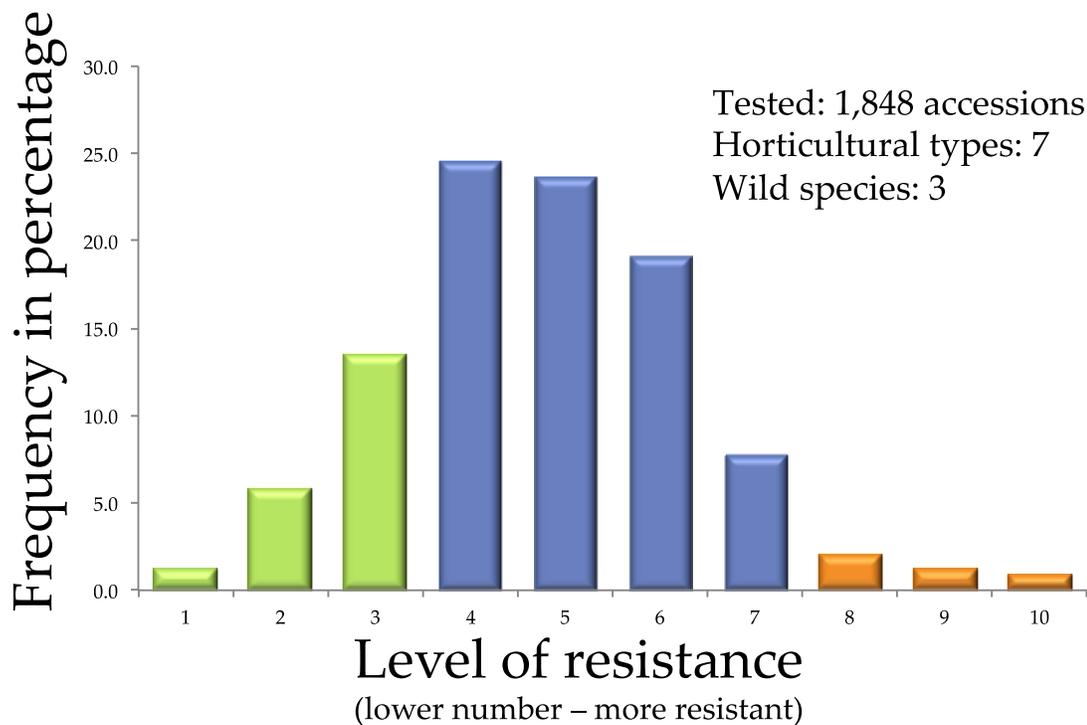
Five mapping populations have been being developed and currently tested in replicated field trials to detect quantitative trait loci for downy mildew resistance. Those populations originate from the crosses Salinas (susceptible) × Grand Rapids (resistant), PI491224 (susceptible) × Iceberg (resistant), 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 already genotyped with SNP (R. Michelmore's laboratory) and AFLP (KeyGene, The Netherlands), while the Parade × Pavane population was genotyped with SNP markers only. Field-based testing confirmed the presence of polygenes for resistance to downy mildew in all populations. In the Grand Rapids × Iceberg population lines with both higher and lower level of resistance than either one of the two parental cultivars were identified. 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 detected in a single trial each. 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 during the 2008 – 2012 period in Salinas and The Netherlands. Results were similar in all environments, detecting the effects of two significant QTLs. The QTLs located on linkage groups 2 (*dm-2.1*) and 5 (*dm-5.1*) were associated with disease resistance under both field and laboratory conditions. Resistance alleles

at both QTLs originate from cultivar Iceberg. The alleles for stable resistance to downy mildew will be introgressed into modern lettuce cultivars.

Crosses were carried out to develop new breeding lines that would exploit field resistance observed in 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 is being 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.

Over 1,800 accessions were tested in replicated and unreplicated trials for their resistance to downy mildew (Fig. 2). Among accessions tested in at least two trials the highest level of resistance was observed in 04G642 that harbors the *Dm17* gene. This result shows that *Dm17* still provides resistance against most of the downy mildew races detected in the Salinas-growing region. The highest level of polygenic resistance was recorded for Batavia-type cultivars, Holborn Standard tested in 14 trials and Iceberg tested in 37 trials. High level resistance was also observed in leaf-type cultivars Lolla Rossa and Grand Rapids (Simko et al. 2012, Crop Science 52:2131-2142). Generally, very high susceptibility was detected in iceberg and romaine type cultivars. This indicates that to achieve improvement in polygenic resistance to downy mildew, novel genes (or alleles) need to be introgressed into these types from other lettuce types or wild species.



**Figure 2.** Distribution of resistance to downy mildew in 1,848 accessions tested in field experiments.

In order to combine downy mildew disease progress data across multiple trials, we have developed approaches to calculate the area under the disease progress steps (Simko and Piepho 2012, *Phytopathology* 102:381-389). In addition, we have developed statistical approaches to integrate heterogeneous phenotypic data collected from multiple trials into a single, overall rating (Simko and Piepho 2011, *Trends in Plant Science* 16:235-237; Simko et al. 2012, *Crop Science* 52:2131-2142).

#### 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 a lower quality of product. 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 between 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<sub>2:3</sub> and F<sub>3:4</sub> 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. The more detailed information with relative resistance rating of 80 accessions is available upon request from Simko.

## 2. Insect resistance

### a. Leafminer (with C. Bull)

Crosses were made to transfer leafminer resistance from wild lettuce species into iceberg and mixed lettuce types. BC<sub>1</sub>F<sub>2</sub> to BC<sub>1</sub>F<sub>6</sub> 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. Crosses were also made among resistant sources to elevate the level of resistance.

F<sub>2</sub> to F<sub>6</sub> plants from crosses between leafminer resistant 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. Twelve promising F<sub>7</sub> or F<sub>8</sub> breeding lines of green leaf, red leaf, romaine, and crisphead lettuce were trialed at Spence Farm

in Salinas from June to August 2012 with four replications, along with check cultivars. The breeding lines all had significantly lower leafminer sting density than cultivars and resistant controls, and the plant weight, core length, and tipburn of many lines were similar to or better than commercial cultivars (Tables 5-8). Some of these lines also showed resistance to corky root, downy mildew, and excellent shelf life. Several breeding lines were resistant to bacterial leaf spot disease in two inoculated greenhouse tests (Figure 3 and Table 9). These breeding lines will be evaluated again next year.

**Table 5.** Mean values of leafminer sting density, downy mildew rating, shelf-life (Area under Decay Progress Stairs), and head characteristics of green leaf lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2012.

Genotype <sup>z</sup>	Stings/20 cm <sup>2,y</sup>	Plant Wt. (g)	Core length (cm)	Downy mildew	Shelf life AUDPS
Two Star	5.5 A	491.7 A	4.9 BC	3.9 A	97.2
Waldman's Green	5.1 A	429.5 AB	7.5 A	4.0 A	54.4*
Shining Star	3.6 B	391.8 AB	5.7 B	4.0 A	50.8*
Grand Rapids	3.5 B	340.4 B	4.8 BC	1.9 BC	46.4*
MU09-520-2 ( <i>cor</i> )	1.2 C	509.4 A	4.5 C	2.5 B	17.9*
MU11-373 ( <i>cor</i> )	1.0 C	438.0 AB	4.3 C	1.8 C	30.9*
MU11-485-1 ( <i>cor</i> )	0.7 C	401.8 AB	4.2 C	1.5 C	51.3*

<sup>z</sup> Some breeding lines have the *cor* gene and are resistant to corky root. <sup>y</sup> Means in the same column followed by different letters indicate significant differences at  $P < 0.05$ . \*Not significantly different from long shelf-life control, Bronco (AUDPS = 15.6). Shelf-life rated for four weeks at weekly intervals on a 0 = no decay to 10 = high decay scale. Higher AUDPS indicates shorter-shelf-life.

**Table 6.** Mean values of leafminer sting density, downy mildew rating, shelf-life (Area under Decay Progress Stairs), and head characteristics of red leaf lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2012.

Genotype	Stings/20 cm <sup>2,z</sup>	Plant Wt. (g)	Core length (cm)	Downy mildew	Shelf life AUDPS
Big Red	7.8 A	360.4 A	5.3 A	3.8 A	125.2
Prizehead	4.4 B	372.7 A	5.2 A	3.0 B	113.8
Red Fox	4.0 BC	394.4 A	5.3 A	3.5 AB	78.0
Lolla Rossa	3.9 BC	171.8 B	4.3 A	1.0 D	70.8
Merlot	2.1 CD	201.0 B	4.4 A	1.8 C	24.8*
MU10-558	1.5 D	430.9 A	5.1 A	3.3 AB	46.4*
MU07-838	1.4 D	334.2 A	5.5 A	3.0 B	77.0
MU11-365-1( <i>cor</i> )	0.7 D	384.8 A	3.7 A	1.0 D	31.1*

<sup>z</sup> Means in the same column followed by different letters are significantly different at  $P < 0.05$ . \*Not significantly different from long shelf-life control, Bronco (see table 5 for more information).

**Table 7.** Mean values of leafminer sting density, shelf-life (Area under Decay Progress Stairs), and head characteristics of romaine lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2012.

Genotype	Stings <sup>z</sup> /20 cm <sup>2,z</sup>	Plant Wt. (g)	Core length (cm)	Tipburn leaves	Shelf life AUDPS
Green Forest	16.9 A	851.3 B	7.9 A	0.0 B	37.3*
Parris Island Cos	16.5 A	1,157.7 A	8.6 A	0.0 B	36.4*
Heart's Delight	16.3 A	866.3 B	8.6 A	0.0 B	25.7*
Red Hot Cos	10.8 B	782.0 B	7.2 A	1.5 A	71.4
MU11-570-1 ( <i>cor</i> )	2.7 C	935.3 AB	8.7 A	0.2 B	64.6
MU11-572-3 ( <i>cor</i> )	2.6 C	771.9 B	7.6 A	0.2 B	53.4*
MU11-572-2 ( <i>cor</i> )	2.0 C	940.7 AB	8.4 A	0.0 B	25.7*
MU11-572-1 ( <i>cor</i> )	1.9 C	893.7 B	8.5 A	0.1 B	29.4*
MU11-506	1.4 C	958.2 AB	7.8 A	0.2 B	28.0*

<sup>z</sup> Means in the same column followed by different letters indicate significant differences at  $P < 0.05$ . \*Not significantly different from long shelf-life control, Bronco (see table 5 for more information).

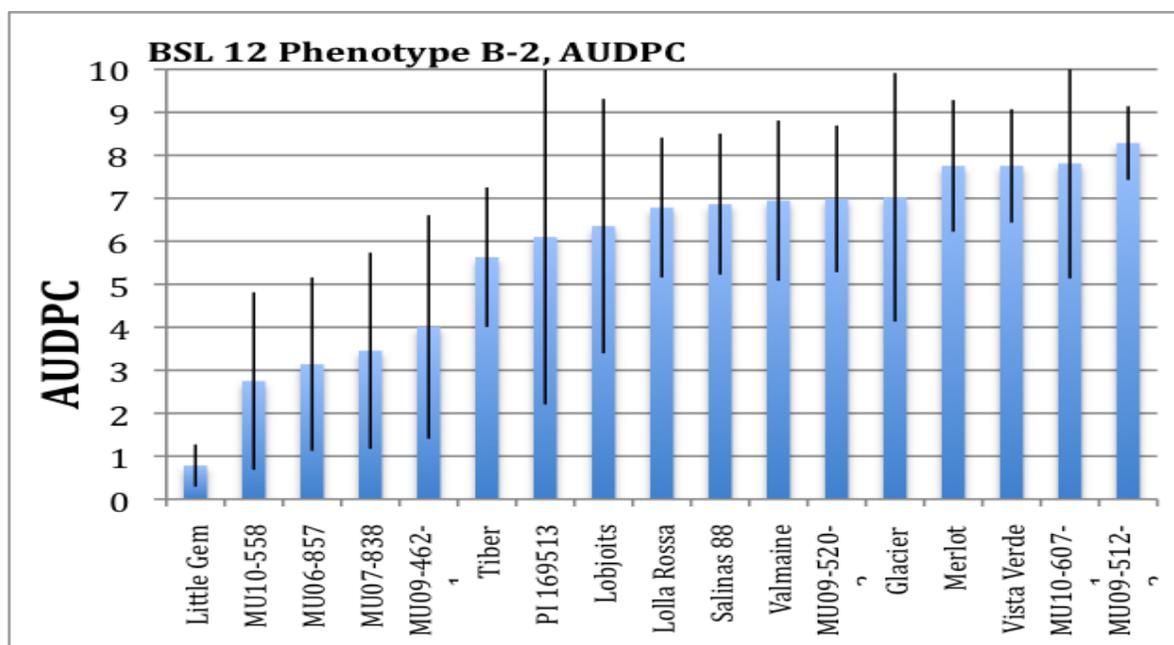
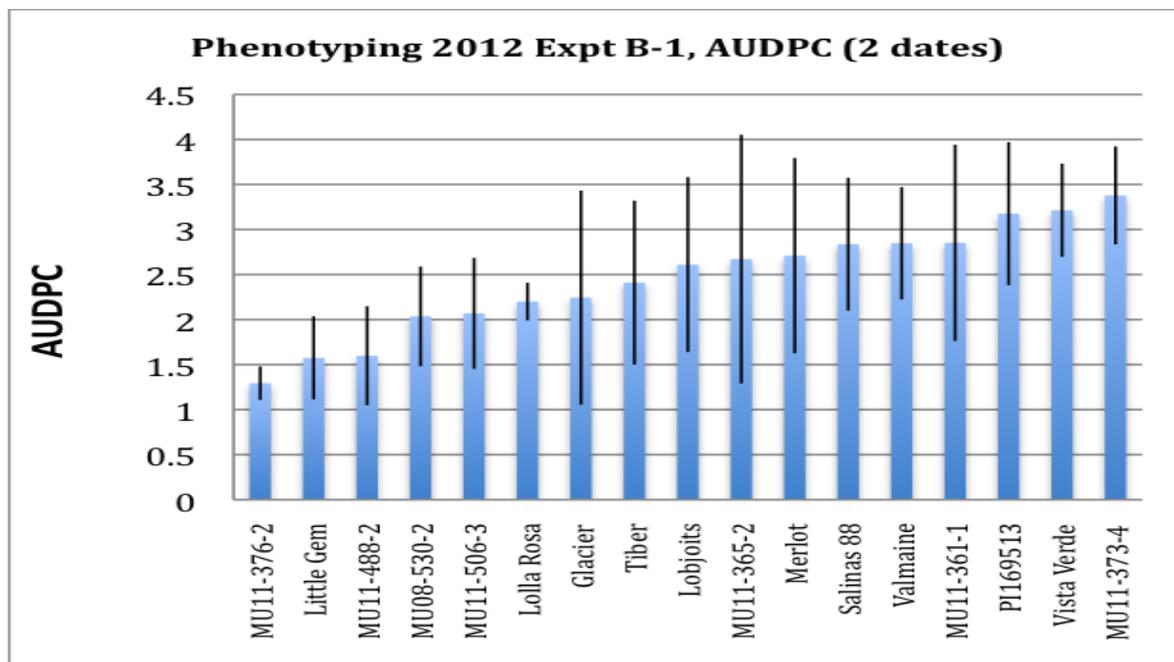
**Table 8.** Mean values of leafminer sting density, shelf-life (Area under Decay Progress Stairs), and head characteristics of crisphead lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2012.

Genotype	Stings <sup>z</sup> /20 cm <sup>2,z</sup>	Plant Wt. (g)	Core length (cm)	Tipburn leaves	Shelf life AUDPS
Premier	16.2 A	1,005.3 A	6.4 A	0.0 B	42.8*
Bronco	15.8 A	905.3 AB	6.1 A	0.0 B	15.6*
Iceberg	8.7 B	517.2 C	7.3 A	0.0 B	35.8*
MU09-467-1	3.2 C	798.2 B	6.4 A	1.1 A	50.6*

<sup>z</sup> Means in the same column followed by different letters indicate significant differences at  $P < 0.05$ . \*Not significantly different from long shelf-life control, Bronco (see table 5 for more information).

**Table 9.** Pedigree and resistance to bacterial leaf spot (BLS) disease for seven leafminer-resistant breeding lines tested in two inoculated greenhouse experiments.

Line	Pedigree	Resistance reaction
MU06-857	(Lolla Rossa x Salinas 88) F8 = MU08-530-2	Resistant
MU09-512-2	(Lolla Rossa x Tiber) F10 = (MU11-365-2)	Susceptible
MU09-520-2	(Glacier x Merlot) F8 = (MU11-361-1)	Susceptible
MU10-607-1	(Glacier x Merlot) F10 = (MU11-373-4)	Susceptible
MU07-838	(PI 169513 x Lobjoits) F8 = (MU11-376-2)	Resistant
MU10-558	(Lolla Rossa x Tiber) F11 = (MU11-488-2)	Resistant
MU09-462-1	(Valmaine x Lolla Rossa) F9 = (MU11-506-3)	Moderately resistant



**Figure 3.** Area under disease progress curve (AUDPC) of bacterial leaf spot disease for breeding lines and cultivars evaluated in two inoculated greenhouse tests.

## 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. High-level (complete) 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 has been obtained for testing: 55 CGN lettuce accessions reported to express resistance against European strains of biotypes Nr:0 and Nr:1 were obtained for testing against a California strain of biotype Nr:0.

## 3. Adaptation and Quality

### a. Bolting resistance for fall plantings (with D. Still)

We developed three F<sub>7</sub> iceberg breeding lines from the cross 87-714-8 x Autumn Gold with resistance to premature bolting for fall plantings in the low desert. We have completed evaluation of these lines for mid-September plantings as well as for diverse planting dates. We are currently summarizing data in order to release these lines.

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 2012 mid-September planted field experiment was conducted to select bolting resistant romaine plants from segregating F<sub>3</sub> families, evaluate advanced inbred lines for resistance to pre-mature bolting and horticultural quality, and evaluate RIL populations for segregation of pre-mature bolting. Among the commercial cultivars in this experiment, the lowest incidence of bolting was observed in cultivar Siskiyou (16% bolted plants) while the greatest incidence was observed in cultivar Green Towers with over 70% plants bolted. Twelve F<sub>3</sub> families from Blonde Lente a Monter x Green Towers and Blonde Lente a Monter x Siskiyou had fewer bolting plants than cultivar Siskiyou. Single plant selections were made from these families to develop F<sub>4</sub> families for further evaluation.

Five inbred breeding lines derived from crosses using the cultivars Siskiyou, Medallion, Valmaine and Eruption were compared to the cultivars Siskiyou, Sunbelt, Green Towers, King Henry, Medallion, Valmaine, and Hearts Delight in a replicated experiment. The shortest cores (greatest bolting resistance) was observed in Siskiyou (6 inches) while the cultivars with the highest leaf weight (head weight excluding the core) was observed in Sunbelt (3.0 lbs / plant). The weight of leaves observed in Sunbelt was significantly greater than all other lines in the experiment. The mean core length of Sunbelt was over 9 inches. One breeding line had a core length similar to Siskiyou (6.5 inches) and the second highest leaf weight (2.0 lbs / plant). This breeding line will be evaluated again next season.

Four RIL populations were evaluated for plant development on a scale of 1 = rosette; 3 = bolting; 5 = flowering; 7 = open involucre with seed. Data were collected at weekly intervals beginning on November 18 and continuing until February 10. Segregation for the rate of plant

development was observed within each RIL population. These evaluations will continue next season and at two additional California locations to collect the data needed to map QTL for this trait.

#### b. Tipburn

Three F<sub>5</sub> iceberg breeding lines from Salinas x Vanguard 75 were developed by Dr. Ed Ryder (USDA, retired) via selection for the absence of tipburn symptoms and iceberg type head characteristics. We are currently summarizing data in order to release these lines.

We are increasing our focus on developing improved tipburn resistance in romaine cultivars adapted to coastal and desert production using tipburn resistance found in modern iceberg cultivars. In multiple low desert and Salinas Valley field experiments, we developed five inbred romaine breeding lines from Green Towers x Salinas with consistently lower tipburn symptoms than Green Towers. All of these breeding lines are closed-top hearting type romaine. Two breeding lines have the potential to combine tipburn resistance with dieback resistance. Tipburn-free closed-top romaine type plants were selected from F<sub>3</sub> families derived the crosses Clemente x Hallmark, Darkland x Sniper, and Green Towers x Hallmark W. These families have the potential to combine tipburn, dieback, and corky root resistance.

#### c. Shelf-Life of Processed Lettuce & Spring Mix (with C. Galeano and R. Michelmore)

We previously identified a major QTL for shelf-life (*sl-4.1*) on LG4 in the Salinas 88 x La Brillante recombinant inbred line population. We have been working to clone the gene controlling this QTL. The core linkage map developed by UC-Davis of the population *L. sativa* cv. Salinas x *L. serriola* US96UC23 (Salinas x Serriola) was used to identify scaffolds of genomic DNA sequence located around *sl-4.1* and to develop and map six simple sequence repeat (SSR) markers near *sl-4.1*. These anchor markers delineated a common region between the Salinas 88 x La Brillante map and the Salinas x *L. serriola* map of 20 cM and 2 cM respectively. Using data from whole genome sequencing of lettuce, we determined that the *sl-4.1* locus is around 10Mbp. Based on gene prediction models, 53 putative Arabidopsis proteins were identified within this interval. Of them, 18 are involved in catalytic processes. More research is needed to determine if these genes are involved in shelf-life.

The RIL population Pavane x Parade was previously evaluated for shelf-life and we identified a major QTL at LG4. We were able to align the Pavane x Parade map with the core linkage map from Salinas x *L. serriola* and the Salinas 88 x La Brillante map to determine that the QTL in Pavane x Parade and Salinas 88 x La Brillante are likely the same. RILs from Pavane x Parade and Salinas 88 x La Brillante were selected for crossing to develop new populations for fine mapping.

## B. Genetic studies

### 1. Bacterial Leaf Spot (with C. Bull)

We previously discovered a hypersensitive response (HR) in the cultivar Little Gem to Xcv (the bacterium that causes BLS). The HR response is a rapid leaf necrosis at the site of pathogen inoculation, which is a well-known mechanism of disease resistance. In this research, hypersensitive responses were detected by infiltrating a suspension of Xcv into the leaf tissue; lettuce genotypes with the HR developed necrosis in less than 48 hours. Genotypes without a HR develop a typical BLS lesion. In 2012, a HR was discovered in the cultivars La Brillante and Pavane. Infiltration of Salinas 88 x La Brillante RILs resulted in 47 with HR : 43 without HR, a good fit to a 1:1 segregation ratio ( $\chi^2$ , 1 *df* = 0.2, *P* = 0.7), which indicates a single gene controlling the HR. Infiltration of RILs from Clemente x Little Gem also had segregation consistent with a single gene (49 HR : 44 not HR). Cultivars expressing HR were intercrossed and the resulting F<sub>2</sub> progeny were infiltrated to determine the segregation of HR. All F<sub>2</sub> progeny expressed HR, indicating that the same gene or a closely linked gene confers HR in La Brillante, Little Gem, and Pavane. The Salinas 88 x La Brillante population has an existing molecular marker linkage map (Hayes et al. 2011, Theoretical and Applied Genetics 123:509-517), which was used to map the new gene to linkage group 2 of lettuce.

A field experiment and two greenhouse experiments were conducted to determine the effect of HR in Salinas 88 x La Brillante RILs on bacterial leaf spot disease severity. The greenhouse experiments were inoculated by spraying 4-week old seedlings until runoff with a mixed solution of Xcv isolates BS339, BS340, and BS347. The field experiment had natural infection. The amount of disease in RILs expressing the HR was less than half the amount observed in RILs that do not express HR, indicating that the gene for HR in La Brillante confers a high level of resistance to bacterial leaf spot.

### 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. We developed F<sub>2</sub> populations from crossing La Brillante with other sources of race 1 resistance. Preliminary results indicate that Annapolis, Merlot, Little Gem, Pavane and Eruption also carry *Vr1* or a separate resistance gene closely linked to *Vr1*. More testing is needed to confirm this finding.

Race 2: We made progress in developing populations to study the inheritance of partial resistance to race 2 *V. dahliae* using the currently available sources of resistance. Using nine sources of partial resistance, F<sub>3</sub> and F<sub>2</sub> populations from 19 resistant x resistant and resistant x susceptible-commercial-cultivar crosses were developed. Greenhouse experiments to evaluate segregation of resistance in two populations comprised of 99 F<sub>3</sub> families were initiated in 2012 and are ongoing.

## C. Molecular markers

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

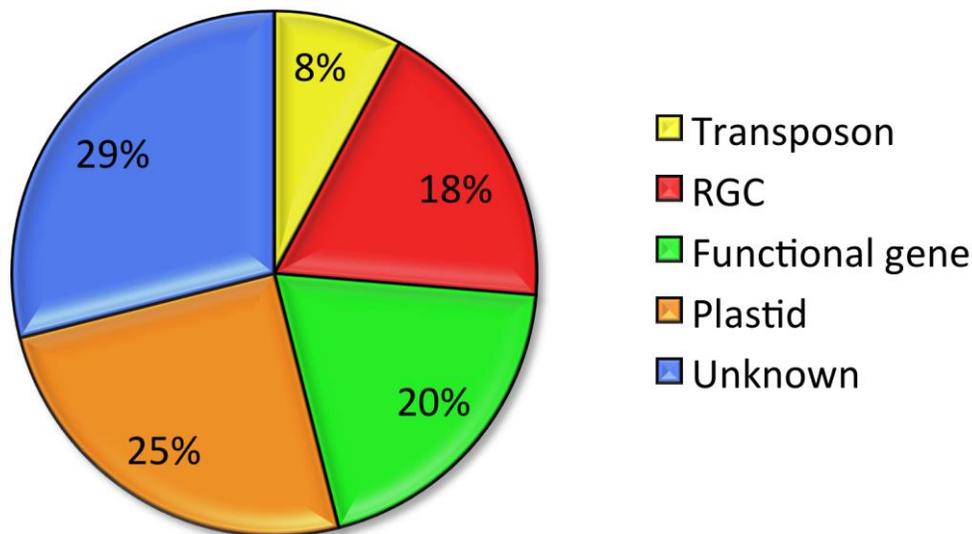
We continue developing molecular markers that can be used for marker-assisted selection of germplasm with good shelf-life. Analysis of sequenced lettuce genome yielded 35 scaffolds with high similarity to the AFLP marker that is closely linked to *sl-4.1*. We have developed primers that amplify 70 genomic 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 match 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. SNPs from two genomic regions, named M18 and M28, showed 100% match with observed phenotypes. We developed a probe-based assay for the high-resolution DNA melting analysis to distinguish haplotypes of the two genomic regions. Next, the marker-trait analysis was performed on 75 accessions from different horticultural types of lettuce (Table 10). Overall, the M28 marker showed somewhat better match with phenotypes than the M18 marker. If lettuce accessions were classified into three groups with good, intermediate, and poor shelf life; the M28 marker was able to accurately determine shelf-life in all 21 accessions with poor shelf-life. It also showed high accuracy (~95%) in detecting accessions with good shelf life (37 out of 39 were determined accurately). In the group of accessions with intermediate quality of shelf-life, 7 haplotypes were associated with good shelf-life and 8 haplotypes were associated with poor shelf-life. Among the most popular horticultural types (iceberg, romaine, and leaf) the haplotype-trait association worked well for iceberg and leaf cultivars, and was not accurate in only two out of 31 romaine accessions. Analyses with a larger number of accessions will be performed in 2013 to determine accuracy of these two markers in different types of lettuces.

### 2. Microsatellite-based molecular markers

Fingerprinting of plant cultivars is frequently carried out with SSR markers (microsatellites) because they are co-dominant, multi-allelic and thus more informative than dominant-types of markers. However, development of SSR markers is costly and time-consuming and therefore only a very limited number of SSR markers are publicly available for lettuce. Previously, we developed a set of EST-SSR markers (Simko 2009, Journal of Heredity 100:256-262) from approximately twenty thousand unigenes of *L. sativa* and its close wild relative prickly lettuce (*L. serriola* L.). We employed the method of enriched microsatellite libraries to develop 97 genomic SSR markers. Fifty-four genomic SSR markers were placed on the molecular linkage map of lettuce. Distribution of markers in the genome appeared to be random, with the exception of a possible cluster on linkage group 6. Any combination of 32 genomic SSRs was able to distinguish genotypes of all 36 accessions. Fourteen of the newly developed SSR markers originate from fragments with high sequence similarity to resistance gene candidates (RGCs) and RGC pseudogenes (Fig. 4). Genomic SSR markers can be used for fingerprinting lettuce cultivars and alignment of linkage maps.

**Table 10.** Association of haplotypes at markers M28 & M18 with shelf life of salad-processed lettuce.

Type	Shelf life	Number of accessions	M28 haplotypes		M18 haplotypes	
			G	P	G	P
Batavia	Good	3	3	-	2	1
	Intermediate	1	-	1	-	1
	Poor	1	-	1	-	1
Butterhead	Intermediate	1	1	-	1	-
	Poor	6	-	6	1	5
Iceberg	Good	10	10	-	10	-
Latin	Intermediate	2	2	-	2	-
	Poor	3	-	3	-	3
Leaf	Good	5	5	-	5	-
	Intermediate	4	-	4	3	1
Romaine	Good	21	19	2	18	3
	Poor	10	-	10	-	10
Romaine-red	Intermediate	3	1	2	3	-
Stem	Intermediate	4	3	1	1	3
	Poor	1	-	1	1	-
Total	Good	39	37	2	35	4
	Intermediate	15	7	8	10	5
	Poor	21	-	21	2	19



**Figure 4.** Homology of sequences containing microsatellites. Transposon group contains sequences similar to transposable elements (both transposons or retrotransposons). RGC group contains sequences similar to resistance gene candidates or RGC pseudogenes. Functional gene group contains sequences that are similar to proteins with known function, but not those in the RGC group. Plastid group includes sequences similar to chloroplast or mitochondrion (Figure from Rauscher and Simko 2013, BMC Plant Biology 13:11)

## D. Germplasm evaluation, maintenance and use

### 1. Screening

#### a. 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; no sources of resistance to Race 2 are known. We are screening PIs for resistance to Race 2 by conducting unreplicated 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. Through 2013, we have screened 824 accessions using race 2 *V. dahliae* isolate VdLs17. Of these, 120 accessions are being treated as candidate sources of resistance that require further testing. Partial resistance has been confirmed in four accessions (PIs 169511, 171674, 204707, 226641) which are being used to develop populations for breeding and genetics research. Five additional PIs are being tested in a race 1 field experiment. Greenhouse testing for resistance and population development are ongoing.

We screened diverse iceberg cultivars to determine if any exhibit an ability to delay disease symptom expression past market maturity. In the summer of 2012 we evaluated thirty diverse iceberg cultivars in a race 1 infested field at the USDA-ARS station in Salinas, CA. The iceberg breeding line RH11-1798, which carries the *Verticillium resistance 1* gene for race 1 resistance, was included as a resistant control. A sample of 10 plants of each cultivar was assessed for disease severity at three different time-points (before, during and after market maturity) over a three week period. The disease severity (DS) scale used was on a 0 to 5 scale, where 0 = no root vascular discoloration, 1 = 1 to 25% of the vascular tissue exhibiting discoloration; 2 = 26 to 50%, 3 = 51 to 75%, and 4 = 76 to 100% discoloration in the absence of foliar symptoms; and 5 = 100% discoloration and the presence of foliar symptoms typical of Verticillium wilt. Most cultivars developed disease symptoms prior to or at harvest maturity. The cultivars Anuenue (DS = 0.74), Honcho II (DS = 1.7), and Batavia Reine des Glaces (DS = 2.0) had mean disease severity at the third time-point that was significantly lower than the susceptible cultivar Salinas (DS = 3.9); these cultivars did not have DS significantly higher than RH11-1798 (DS=0.0). Market maturity and disease severity were not correlated in this population (data not shown). We will conduct a 2013 field experiment to determine if we can confirm these results. Crosses between cultivars displaying delayed disease symptoms and a susceptible cultivar are under way.

#### b. Spring Mix Lettuce

Seventeen lettuce lines with unique phenotypes (e.g., leaf shape, coloration) were increased in the greenhouse for evaluation as potential components of spring mixe salads. Five of eight lettuce mutants compared favorably with eight lettuce cultivars for leaf number in a Fall planting.

### c. Wound induced leaf edge browning (WILE)

Chopped samples of 13 lettuce cultivars from one harvest date were stored in normal atmospheres at 10°C (50°F) and evaluated for browning reaction 24-hr and 10-days thereafter. Differences in browning were observed within heads and among cultivars as n 2012.

## 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 2012 through 2013, requests were made for *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), and leaf lettuce breeding lines with corky root resistance (06-831 and 06-833).

## 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 2012, we characterized 14 iceberg, seven leaf, and four romaine USDA or UCD breeding lines for yield, horticultural characters, shelf-life of salad and arrival quality, and for resistance to big vein, dieback, *Verticillium* wilt, lettuce drop, downy mildew, tipburn and pre-mature bolting.

## F. Recent publications relevant to this project

Lafta A, Mou B (in press): Evaluation of lettuce genotypes for seed thermotolerance. *HortScience*.

- Mou B (2012): Nutritional quality of lettuce. *Current Nutrition & Food Science* 8: 177-187.
- Rauscher G, Simko I (2013): Development of genomic SSR markers for fingerprinting lettuce (*Lactuca sativa* L.) cultivars and mapping genes. *BMC Plant Biology*, 13: 11.
- Simko I, Hayes RJ, Kramer M (2012): Computing integrated ratings from heterogeneous phenotypic assessments: A case study of lettuce post-harvest quality and downy mildew resistance. *Crop Science*, 52: 2131-2142.
- Simko I, Rauscher G, Sideman RG, McCreight JD, Hayes RJ (in press): Evaluation and QTL mapping of resistance to powdery mildew in lettuce. *Plant Pathology*.