ABSTRACT
CALIFORNIA LETTUCE RESEARCH BOARD

for the period
(April 1, 2009-March 31, 2010)

PROJECT TITLE: Lettuce Breeding, USDA-ARS

PROJECT INVESTIGATORS: R. Hayes, I. Simko, B. Mou, J. D. McCreight, USDA/ARS Crop Improvement and Protection Unit, Salinas, CA

SUMMARY:

Our objectives are to incorporate resistance to several diseases, insects, and physiological defects into iceberg, romaine, and mixed lettuce cultivars and breeding lines adapted for coastal and desert production. In the 2009-2010 period, major efforts targeted resistance to lettuce big vein disease, lettuce drop / *Sclerotinia species*, Verticillium wilt, Fusarium root rot, lettuce dieback/tombusviruses, bacterial leaf spot, corky root, downy mildew, leafminer, lettuce aphid, tipburn and multiple disease resistance. Minor programs addressed resistance to powdery mildew and yellow spot. In all programs, horticultural traits, adaptation, and resistance to tipburn are essential.

We confirmed resistance in previously identified germplasm to Lettuce Drop, Verticillium wilt, Fusarium wilt, Yellow Spot, dieback, and lettuce aphid. New candidate sources of resistance were identified to race 2 isolates of *Verticillium dahliae* and pre-mature bolting. Selections were taken from breeding populations and advanced breeding lines were evaluated as part of breeding for resistance to big vein disease, lettuce drop, Verticillium wilt, dieback, bacterial leaf spot, corky root, leafminer, tipburn, and pre-mature bolting.

Genetic studies concurrent with breeding programs are being conducted to determine the inheritance of resistance to big vein disease, bacterial leaf spot, dieback, lettuce mosaic virus, leafminers, downy mildew, lettuce aphid, corky root, and Verticillium wilt. Publications during 2009-2010 included reports of original research on Sclerotinia, Verticillium wilt, downy mildew, dieback, leafminer, tipburn, shelf-life, and nutrient content.

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COOPERATING PERSONNEL:

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Growers, shippers, seedsmen- All districts

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, 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 and B. Maisonneuve)

Hybrid materials between *Lactuca virosa* accession IVT280 and several European cultivars provided by B. Maisonneuve, INRA, Montfavet, France have demonstrated a high level of partial resistance to big vein disease. In January 2009 planted field experiments, seven iceberg and three romaine type $BC_2F_{7:8}$ lines were selected for head type and resistance. Higher levels of resistance are known in *L. virosa* accession SAL012 (Hayes et al., 2008 Euphytica 164:493–500). Eight BCF_2 *L. sativa* × *L. virosa* populations were tested in replicated greenhouse experiments for big vein resistance, and seven had significant fewer symptomatic plants than Clemente, Salinas, Great Lakes 65 and Pacific. However, all populations had at least one symptomatic plant. Seed was produced from single plant selections for additional testing. Additional *L. sativa* × *L. virosa* BC families are in development.

A high level of partial resistance to big vein is available in the cultivar Margarita and in a diversity of *L. sativa* PIs; we are introgressing this resistance into iceberg and romaine type breeding lines. In a January planted field experiment, 81 $BCF_{2:3}$ iceberg families with resistance from Margarita were developed by selecting plants with good head type and no big vein symptoms. An additional 30 $F_{4:5}$ iceberg lines were selected from the cross Sniper × Margarita.

We conducted low desert field experiments to develop big vein resistant icebergs for that region. Two F_9 lines have been identified with resistance similar to Pacific and Winterselect, but with increased head coverage. The head size of these lines is smaller than Coyote. Additional lines with resistance from Pavane were selected for backcrossing to improve head type and size. F_2 populations were developed to introgress resistance from Margarita.

b. Lettuce drop (with K. Subbarao)

We are initiating 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 than the current pedigree method, identifying resistant lines in 3 ½ years instead of 4 – 5 years. In 2008, twelve populations were inbred to the F_3 through F_5 generation.

We are developing resistant crisphead and romaine breeding lines using diverse sources of resistance. Over four field experiments through 2009, we have identified a romaine breeding line with significantly less mortality than the susceptible cultivar Darkland. The source of resistance is PI207490. Two iceberg lines from Salinas 88 × (75-501-1 × Holborn Standard) have been developed with significantly less disease than Salinas and Glacier. One line was advanced for further evaluation of horticultural characters to determine its suitability for release; the other line is not suitable for commercial production. All these lines were backcrossed to adapted cultivars to develop populations with resistance and improved head type.

We are working with a new source of resistance found in the small statured Latin type cultivar Eruption. This cultivar is not early bolting and has demonstrated resistance in repeated *S. minor* infested field experiments. We have also demonstrated that its resistance is due to something other than small size and upright growth habit (Hayes et al., 2010, HortScience 45:333–341). In 2009, 14 romaine type F_3 families from romaine cvr. × Eruption crosses were identified with significantly lower mortality than Darkland, Clemente, and Hearts Delight in spring and fall field experiments. In a non-infested field experiment, 168 romaine type plants were selected from these resistant families. The resulting $F_{3:4}$ families will be evaluated in infested and non-infested field experiments in 2010.

c. Verticillium wilt (with K. Subbarao and S. Klosterman)

Advanced iceberg breeding lines with race 1 resistance to Verticillium wilt from La Brillante are being developed that combine yield, quality, and resistance to other lettuce diseases. Spring and fall race 1 infested field experiments and non-infested field experiments were conducted to select resistant iceberg breeding lines. Two F_8 lines were identified with disease incidence significantly less than Salinas, and not significantly different than La Brillante. The head type was similar to Salinas in non-infested field experiments. In addition, 68 F_4 families were developed through single plant selection within resistant F_3 families. Crossing is in progress to generate iceberg families segregating for race 1 resistance and corky root resistance.

Substantial effort was devoted to identifying variation for Verticillium wilt resistance between and within the different lettuce types. Resistance to race 1 was identified in red leaf, romaine, Latin, and Batavia cultivars (Hayes et al., 2007, Plant Disease 91:439 – 445). We are using several sources of race 1 resistance identified by this research to breed resistant cultivars. By selecting for head type in non-infested field experiments in 2008, we developed three F_5 iceberg breeding lines with resistance from Merlot. These lines have been backcrossed for further breeding. Eighty-nine romaine type families were developed with resistance from Annapolis, Defender, Eruption, and Little Gem. Several of these combine Verticillium wilt resistance with corky root and lettuce mosaic resistance, or have demonstrated potential for lettuce drop resistance.

We are working to develop race 2 resistance with the currently available sources of resistance. Using PIs 204707, 171674, 226641, and 169511, F_1 seed from resistant × resistant and resistance × susceptible (commercial cultivars) crosses were created. F_2 seed will be produced in 2010.

d. Powdery mildew

Powdery mildew resistant iceberg breeding lines adapted to mid-winter low desert plantings are being developed using the butterhead cultivars Big Boston and Soraya as sources of resistance. In a low desert November 2009 planting, BCF_2, $BCF_{2:3}$ and $BCF_{3:4}$ families were evaluated for head type and reduced powdery mildew severity. Over two evaluation dates spanning 10 days, we did not find any breeding lines that combined good head type and reduced disease severity. More specifically, plants and lines with low disease were either immature or had open growth habits. We are reassessing the feasibility of using resistance from butterhead sources to develop resistant iceberg cultivars.

e. Fusarium wilt, a.k.a. root rot (with T. Gordon, S. Koike, M. Matheron)

The Japanese, semi-iceberg lettuce 'Costa Rica No. 4' and 'Salinas' are comparable for resistance in greenhouse tests in Salinas, but the level of resistance is insufficient for the early fall desert plantings when the daytime temperature exceeds 100 ºF. A group of romaine cultivars previously identified to have levels of resistance potentially useful for the early desert plantings were crossed with susceptible and resistant cultivars. Three F_2 were advanced to the F_3: King Louie × Costa Rica #4, King Louie × BOS 9091, and King Louie × Autumn Gold.

f. Lettuce dieback (with R. Sideman)

The lettuce dieback disease is caused by soilborne viruses of the family *Tombusviridae*. Resistance to the disease is high in iceberg-type cultivars, but is very limited in leaf lettuces and almost non-exist in modern romaine type cultivars. USDA-ARS previously released three romaine breeding lines with resistance to the disease. However, recent evaluations have shown that all three breeding lines and most of the romaine accessions bearing the *Tvr1* resistance gene have a very short shelf-life. Therefore, in collaboration with R. Hayes, we are testing all resistant advanced breeding lines for shelf-life. The field experiment was carried out at 'Carr Lake' in Salinas, CA. Plants were checked weekly for disease symptoms in order to discriminate between plants dying due to dieback and unrelated causes. The percentage of plants that showed typical dieback symptoms (or were dead due to dieback) was recorded at harvest maturity.

Based on the resistance screening, earliness of bolting, and overall phenotypic appearance, and shelf-life, two breeding lines were released into the public domain. SM09A and SM09B are F8 romaine breeding lines of lettuce resistant to the dieback disease and with good shelf-life (Simko et al. 2010). SM09B was selected from a cross between 'Darkland' and PI491224, while SM09A was developed from 'Green Towers' × ('Darkland' × PI491224). Resistance to the disease in both breeding lines is derived from PI491224, a primitive romaine-type lettuce that is highly perishable when processed for salad. In replicated field trials, the two breeding lines showed complete resistance to dieback. Field observations were confirmed through the analysis of molecular markers closely linked to the dieback resistance gene *Tvr1*.

Testing of salad-cut lettuce in modified atmosphere packaging indicated slower decay in the two breeding lines compared to other dieback-resistant romaines Both SM09A and SM09B produce closed heads of acceptable size, with a core length about 20% longer and with 10% more leaves than cvs. Darkland and Green Towers. SM09A is light-green, while SM09B has a medium-green color. Limited testing for yield indicates that the proportion of harvestable heads (0.92 for SM09A and 0.70 for SM09B) is similar to other romaine cultivars (with the exception of ‘Bandit’) (Simko et al. 2010).

g. Bacterial leaf spot (with C. Bull)

We are introgressing resistance from Little Gem into iceberg type cultivars using a greenhouse testing method. This approach uses a limited amount of time and space, and facilitates the evaluation of a large number of progeny. In 2009 we backcrossed selected lines from Little Gem × Sniper to the cultivars Pacific and Sniper. Selection of F_2 seedling for resistance was initiated in March of 2010.

We are refocusing the BLS resistance project towards developing resistance in cultivars suitable for spring mix. In 2009, two independent greenhouse experiments were conducted with 36 lines covering eight leaf types used in spring mix production (See C. Bull bacteriology report for more details). Seven parents were selected, and crossing to develop breeding populations is in progress.

h. Corky root (with C. Bull)

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 to be highly resistant. PI 491239 and PI 273597c had lower corky root severity than cultivars with the *cor* resistant gene when grown in resistance breaking soil from Watsonville. The resistance from these lines is being incorporated into cultivated lettuces.

We continued to make crosses to transfer the resistant gene cor 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.

Twenty F_7 or F_8 breeding lines of green leaf, red leaf, and butterhead lettuce were tested in a replicated field trial at the Spence Farm in Salinas from June to August 2009 for corky root resistance and horticultural traits. The corky root resistance of the breeding lines was similar to the resistant control ‘Glacier’, while their plant weight, core length, tipburn, and downy mildew were comparable or better than control cultivars (Tables 1-3).

i. Yellow spot

We continued to study "yellow spots" disorder of lettuce, especially on romaine lettuce, and identified some putative resistant and susceptible genotypes. We are making some crosses and selections for high level of resistance to the disorder.

j. Downy mildew (quantitative resistance) (with R. Michelmore, M. Truco, O. Ochoa)

Downy mildew (caused by oomycete *Bremia lactucae*) is considered the most important disease affecting lettuce production. A large number of 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 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.

Three mapping populations are being developed and tested in replicated field trials to detect quantitative trait loci for downy mildew resistance. Those populations originate from the crosses Salinas (susceptible) × Grand Rapids (field resistant), PI491224 (susceptible) × Iceberg (field resistant), and Grand Rapids × Iceberg. The Grand Rapids × Iceberg population is being genotyped with SNP molecular markers in the R. Michelmore's laboratory (UC Davis). Field-based testing confirmed presence of polygenes for resistance to downy mildew in all three 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. Preliminary results from two trials show the effect of a significant QTL originating from cv. Iceberg. This locus explains 20% of the total phenotypic variation of the trait.

Over fifty crosses were performed to develop new breeding lines that would exploit field resistance observed in cvs. Iceberg, Grand Rapids, Holborn's Standard, La Brillante, Merlot, and Primus. The hybrid plants were detected with molecular markers developed by our laboratory. Forty-five selections that were made from F_3 to F_7 families will be evaluated in replicated trials. Selection of material was based on resistance to downy mildew, bolting observations, and other horticultural characteristics. The selections were made from spring planting in Spence and summer planting in Salinas. Plants were selected with minimum number of lesions and non-bolting at the time of evaluation. The selected material is being evaluated for yield, size, uniformity, and tipburn resistance.

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 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_2 to F_6 plants from crosses between leafminer resistant PI 169513, Red Grenoble, Lolla Rossa, Bibb, and Tom Thumb and good horticultural types Salinas, Salinas 88, Tiber, Prizehead, and Lobjoits were selected in the field, and some of them were backcrossed to restore horticultural traits. Nine promising F_7 or F_8 breeding lines of crisphead, romaine, red leaf, and green leaf lettuce were trialed at Spence Farm in Salinas from June to August 2009 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 commercial cultivars (Tables 4-7). These breeding lines will be evaluated again next year.

b. Lettuce aphid (with Yong-Biao Liu)

High-level or complete resistance to the lettuce aphid (*Nasonovia ribis-nigri*) found in IVT280, an accession of *L. virosa* and a distant relative of lettuce, is being transferred by commercial breeders from European lettuce cultivars to U.S. lettuce types. New sources of resistance may be helpful in delaying emergence of resistance breaking biotypes of lettuce aphid, and will be necessary in the event the lettuce aphid overcomes the resistance derived from IVT280. Complete (high-level) resistance in *L. virosa* accessions PI 274378 and SAL112 is comparable to that of IVT280. SAL162 (*L. virosa*) and PI 491093 (*L. serriola*) have partial resistance to lettuce aphid.

A series of controlled-infestation, greenhouse tests elucidated genetic relationships among these different sources of resistance to lettuce aphid. Complete resistance in PI 274378 and SAL112 was allelic to that in IVT280. Complete resistance in PI 274378D was dominant to partial resistance in SAL162. Partial resistance in PI 491093 segregated as expected of a dominant trait in F2 (Salinas × PI 491093).

Unexpectedly high numbers of lettuce aphids per plant (>100) were observed on PI 491093 in two greenhouse tests. Data from one test suggest that plant age at the time of infestation may be a factor: lettuce aphid reproduced on younger plants (<10 days post-planting at time of infestation) than older plants (42 days post-planting). Other factors may also affect reproduction of lettuce aphid on lines with partial resistance.

3. Adaptation and Quality

a. Adaptation to low desert environments

Three field experiments were conducted to breed bolting and tipburn resistant lettuce for adaptation to fall and late spring plantings in the Imperial Valley of CA or the Yuma Valley of AZ. Reports on breeding for resistance to bolting and tipburn can be found in those sections of this report.

b. Bolting resistance for fall plantings

We developed three F_7 iceberg breeding lines from the cross 87-714-8 × Autumn Gold with bolting resistance for fall plantings in the low desert. The lines are most similar to Autumn Gold, but with larger and heavier heads. They also have a tendency for protruding ribs. In mid-September plantings, the bolting resistance was equivalent to Empire. We increased seed of these lines for further testing.

Bolting resistance for fall plantings is also being investigated in romaine germplasm. F_2 families from crosses between Green Towers, Siskyou, Tall Guzmaine, PIC714, and Valmaine were selected for head type and short cores. Seed from nine selected plants are currently being produced for further testing. Crosses are being conducted between the bolting resistant cultivar Blonde Lente a Monter and modern romaine cultivars to develop additional breeding populations.

c. Tipburn

Three F_5 iceberg breeding lines from Salinas × Vanguard 75 were developed by Dr. Ed Ryder (USDA retired) via selection for the absence of tipburn symptoms and iceberg type head characteristics. These lines are also resistant to *Lettuce mosaic virus* (LMV). In three mid-April evaluated low desert experiments, the breeding lines tended to have heavier, larger diameter heads with taller cores than the control cultivars, although these differences were not significant. The tipburn incidence was equivalent or lower than the commercial cultivars. In two mid-march evaluated low desert experiments, the breeding lines tended to have smaller heads than other commercially available cultivars.

We are increasing our focus on developing improved tipburn resistance in mixed type cultivars adapted to coastal and desert production using tipburn resistance found in modern iceberg cultivars. In a low desert December 2009 planted field experiment, more than 330 F_4 families from Green Towers × Salinas were evaluated for tipburn and romaine head type. More than 70 single plant selections were made, and grown to seed for further testing. In a Salinas Valley field experiment, 89 F_3 and F_4 families from 12 crosses were developed by selecting for closed-top type romaine plants with no tipburn symptoms.

d. Shelf-Life of Processed Lettuce & Spring Mix (with Michelmore)

The USDA has developed modified atmosphere packaging and controlled atmosphere chamber assays to detect genetic differences for shelf-life in shredded whole head field grown lettuce (Hayes and Liu, 2008. J. Amer. Soc. Hort. Sci. 133:228–233). We currently use these methods in our breeding program to select against poor shelf-life while introgressing disease resistance from un-adapted sources. In 2009, we assessed the shelf-life of advanced breeding lines in the dieback, verticillium wilt, and lettuce drop resistance breeding programs. Lines with poor shelf-life will not be released. We began development of a greenhouse based assay using seedlings that might enable breeding for extended shelf-life (rather than eliminating lines with poor shelf-life), and be more useful for developing cultivars targeted for spring mix. Initial experiments with five good, intermediate, and poor cultivars using 5-week old seedlings grown in plug trays were consistent with results using field grown material. Additional experiments are in progress using 34 previously characterized cultivars.

Understanding the genetics of shelf-life of processed lettuce in modified atmosphere packages may enable the efficient breeding of cultivars with extended shelf-life. Assessments using field grown plants of the recombinant inbred line (RIL) population Salinas 88 (good shelf-life) × La Brillante (poor shelf-life) was initiated in 2009 and will continue through 2010. Shelf-life data will be used to determine the segregation of shelf-life, and to locate QTL for shelf-life on a genetic map of SNP markers from UC-Davis and AFLP markers provided by Key Gene. We also assessed the shelf-life of commonly used parents, this data was used to identify additional RIL populations for testing that should segregate for shelf-life. Crosses were made to develop additional populations for genetic analysis.

e. Wound-Induced Leaf Edge Browning

The objective is to evaluate lettuce cultivars for genetic variation for resistance to wound-induced leaf edge browning and the potential for genetic gain through selection for resistance to browning of lettuce at ambient temperature. Such resistance could add to long shelf-life of whole and chopped (salad) products. Preliminary tests were to initiate a study of resistance to browning of lettuce leaves at ambient temperature. Heads bisected or quartered with a lettuce knife exhibited browning on the cut surface. Leaf pieces from chopped heads exhibited browning on the cut edges and well into the veins away form the edges. Browning on chopped leaves left open (exposed to sun and air in place) was similar to that on leaves held in closed, food grade plastic containers and held in the dark.

B. Genetic studies

1. Bacterial Leaf Spot (with C. Bull)

The inheritance of bacterial leaf spot from the cultivar Little Gem is being investigated. Previous research indicated that resistance from Little Gem is conditioned by multiple genes. Recombinant inbred line populations from Salinas × Little Gem and Clemente × Little Gem are being developed, and were advanced to the F_6 and F_5 generations respectively. Greenhouse testing for resistance in the Salinas × Little Gem RILs will begin in 2010.

2. Big Vein (with R. Michelmore)

We are collaborating with UC-Davis to determine the inheritance of big vein resistance in *L. sativa*. Eighty F_6 recombinant inbred lines (RILs) from the cross Parade (susceptible) × Pavane (resistant) were developed by Ed Ryder, and were subsequently tested over three years for resistance in greenhouse experiments. Molecular marker genotyping and QTL analysis was conducted by UC-Davis, and identified three QTL on linkage groups 3 and 4. Salinas 88 × La Brillante RILs were tested in a greenhouse experiment, but segregation for resistance was not observed. We are also producing F_2 seed from intra- *L. virosa* resistant × susceptible crosses to determine the inheritance of complete resistance in *L. virosa*.

3. Leafminer (with G. Rauscher, R. Michelmore, and M. Truco)

To study the inheritance of leafminer sting density and downy mildew resistance, crosses were made between a *L. saligna* line PI 509525 (low sting density, downy mildew resistant) and cultivars 'Da Ye Wo Sun' and Bibb (high sting density, downy mildew susceptible). Progeny plants were transplanted in the field and the sting density and downy mildew severity on each plant were recorded. Plant tissue was also collected from each plant for DNA isolation and genotyping. Some tentative molecular markers were identified in a BSA analysis of (PI 509525 × Bibb) F_2 population.

4. Verticillium wilt (with R. Michelmore)

We previously determined that resistance to race 1 isolates in the cross Salinas 88 × La Brillante had segregation consistent with a single dominant gene in F_1, F_2 and RIL populations. Molecular marker analysis, development of a genetic map, and QTL analysis of the Salinas 88 × La Brillante RILs identified a large effect QTL on chromosome 9 while minor QTL were located on chromosomes 5 and 8. A full-length publication on this work will be completed in 2010.

5. Marker-assisted selection for dieback resistance (with L. McHale, M. Truco, O. Ochoa, R. Michelmore, and B. Scheffler)

A single dominant gene on chromosome 2 (*Tvr1*) was found to be responsible for the dieback resistance observed in modern iceberg lettuces. A combination of classical linkage mapping and association mapping allowed us to pinpoint the location of the *Tvr1* resistance gene on chromosomal linkage group 2 (Simko et al. 2009). Nine molecular markers, based on expressed sequence tags (EST), were closely linked to *Tvr1* in the mapping population, developed from crosses between resistant (Salinas and Salinas 88) and susceptible (Valmaine) cultivars.

Sequencing of these markers from a set of 68 cultivars revealed a relatively high level of nucleotide polymorphism (θ = 6.7 × 10-3) and extensive linkage disequilibrium (r^2 = 0.124 at 8 cM) in this region. However, the extent of linkage disequilibrium was affected by population structure and the values were substantially larger when the analysis was performed only for romaine (r^2 = 0.247) and crisphead (r^2 = 0.345) accessions. The association mapping approach revealed that one of the nine markers (Cntg10192) in the *Tvr1* region matched exactly with resistant and susceptible phenotypes when tested on a set of 200 *L. sativa* accessions from all horticultural types of lettuce. The marker-trait association was also confirmed on two accessions of *L. serriola* – a wild relative of cultivated lettuce. The combination of three single-nucleotide polymorphisms (SNPs) at the Cntg10192 marker identified four haplotypes.

Three of the haplotypes were associated with resistance and one of them was always associated with susceptibility to the disease. We have successfully applied high-resolution DNA melting (HRM) analysis to distinguish all four haplotypes of the Cntg10192 marker in a single analysis. Marker-assisted selection for dieback resistance with HRM is now an integral part of our breeding program (Figure 1) that is focused on the development of improved lettuce cultivars (Simko et al. 2009).

6. Development of SSR markers (with G. Rauscher)

Microsatellites or simple sequence repeats (SSRs) are short, tandemly repeated motifs of DNA ubiquitous in all analyzed eukaryotic genomes. SSR-based molecular makers are frequently used in plant genetics due to their high reproducibility, codominant inheritance, and high information content. Though SSRs are markers of choice in many plant species, only a very limited number of SSR markers are publicly available for lettuce. The use of microsatellites has been limited in plants by the costs involved in isolating large numbers from the target species.

We have applied two methods to develop microsatellite markers for lettuce; *in silico* approach that uses analysis of expressed sequence tags (ESTs) from the Compositae Genome Project database (CGPDB), and development of anonymous SSRs from genomic DNA through the library enrichment method. Using the two methods we have developed a set of 61 EST-SSR markers and 139 anonymous SSR markers. Most of the markers were successfully placed on the lettuce molecular linkage map. Markers are distributed on all nine linkage groups. Currently we are testing marker polymorphism on a set of lettuce accessions from different horticultural types. The SSR markers identified in this work are already being used for cultivar genotyping, analysis of population structure in association studies, and QTL mapping.

C. Germplasm evaluation, maintenance and use

1. Screening

a. Verticillium wilt

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 (isolate VdLs17) by conducting unreplicated greenhouse experiments to indentify 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 tested 513 accessions using this approach and 466 were determined to be susceptible (Table 8). An additional 43 putatively resistance accessions are being re-tested. Four accessions have demonstrated a repeatable level of partial resistance over four independent replicated experiments (Table 9). All of these PIs have had at least a few symptomatic plants, and all but PI171674 has had non-symptomatic plants that were nonetheless colonized by *V. dahliae*.

b. Spring Mix Lettuce

Seventeen lettuce leaf mutants were evaluated in a preliminary greenhouse test for potential for use in Spring mixes. Difference in size and growth rate were apparent in comparison with 12 lettuce cultivars. Seed were produced from the mutant stock for use in 2010 studies.

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. In the 2009-2010 period, requests were made for Verticillium wilt resistant breeding lines (RH05-0336, RH05-0339, and RH05-0340), bacterial leaf spot resistant icebergs (RH07-0370M, RH07-0373M, RH07-0379M, RH07-0380M, RH07-0386M, RH07-0387M, and RH04-0157-3), and dieback resistant romaine lines SM09A and SM09B.

Exploration and collection of wild *Lactuca* was sponsored through the USDA, Plant Exchange Office. In 2009, seed from 233 plants of four wild *Lactuca* species from 56 sites were collected in Armenia by Dr. Kamilla Tamanyan, and from the Republic of Georgia by Dr. Marine Mosulishvili. Seed was distributed to UC-Davis for race 2 Verticillium wilt resistance screening, and are also being regenerated and characterized in Salinas, CA.

D. Field trials and cooperation

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.

E. Recent publications relevant to this project

Hayes RJ, BM Wu, BM Pryor, P Chitrampalam, and KV Subbarao (2010). Assessment of resistance in lettuce (*Lactuca sativa* L.) to mycelia and ascospore infection by *Sclerotinia minor* Jagger and *S. sclerotiorum* (Lib.) de Bary. HortScience 45: 331-341.

Jenni S and RJ Hayes (2010). Genetic variation, genotype × environment interaction, and selection for tipburn resistance in lettuce in multi-environments. Euphytica 171: 427–439.
Klosterman SJ and RJ Hayes (2009). A soilless Verticillium wilt assay using an early flowering lettuce line. Plant Disease 93: 691-698.
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F. Appendix of Tables and Figures

Table 1. 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 2009.

Genotype	Corky root[z]	Head Wt. (g)	Core length (cm)	Tipburn leaves[y]
Salinas	7.9 A	477.4 B	3.0 CDE	0.0 E
Margarita	7.5 AB	148.6 F	1.5 F	0.0 E
Dark Green Boston	7.3 B	178.5 EF	3.8 BC	0.4 BCDE
Cobham Green	7.2 B	154.7 F	2.0 EF	1.7 AB
MU08-497-1	6.0 C	261.5 CD	3.8 BC	0.5 BCDE
MU08-517-1	5.8 C	187.8 EF	2.6 DEF	1.5 ABC
MU08-499-1	5.8 CD	267.2 CD	4.1 ABC	0.3 CDE
Glacier	5.7 CD	622.4 A	4.4 AB	0.1 DE
MU08-518	5.5 CD	207.5 E	5.1 A	1.4 ABCD
MU08-516-1	5.2 D	220.9 DE	3.8 BCD	2.6 A

[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 2. 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 2009.

Genotype	Corky root[z]	Downy mildew	Plant Wt. (g)	Core length (cm)	Tipburn leaves[y]
Prizehead	7.9 A	3.7 A	374.5 CDE	4.3 CD	0.3 AB
Big Red	7.8 AB	3.1 ABC	333.7 EF	5.0 BC	0.5 A
Merlot	7.2 BC	3.0 BC	257.7 G	6.8 B	0.0 B
Lolla Rossa	7.1 C	3.2 ABC	110.7 H	2.6 D	0.0 B
Redina	6.7 C	2.7 CD	306.2 FG	11.2 A	0.0 B
MU08-533-1	6.1 D	3.1 BC	474.5 B	6.5 B	0.1 B
MU08-510-1	5.8 DE	2.4 D	317.8 EF	3.1 D	0.0 B
MU08-496-1	5.8 DE	3.0 BC	394.9 CD	5.1 BC	0.0 B
MU08-508-1	5.8 DE	2.2 D	337.5 DEF	3.3 CD	0.0 B
Glacier	5.7 DE	3.6 AB	622.4 A	4.4 CD	0.1 B
MU06-810-1	5.7 DE	2.8 CD	427.5 BC	4.2 CD	0.0 B
MU08-564-1	5.4 E	1.5 E	337.0 DEF	3.0 D	0.0 B

[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 3. Mean values of corky root severity and head characteristics of green leaf lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2009.

Genotype	Corky root[z]	Plant Wt. (g)	Core length (cm)	Tipburn leaves[y]
Grand Rapids	8.0 A	320.5 C	4.9 BC	0.5 AB
Waldmann's Green	8.0 A	393.7 C	9.1 A	0.3 AB
Shining Star	7.9 AB	448.3 BC	7.2 AB	0.3 AB
Two Star	7.7 B	449.4 BC	5.1 BC	0.0 B
MU06-831-1	5.9 C	602.2 AB	6.8 ABC	0.0 B
Glacier	5.7 C	622.4 A	4.4 C	0.1 B
MU06-833-1	5.4 D	641.3 A	7.9 A	1.3 A

[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 leafminer sting density and head characteristics of green leaf lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2009.

Genotype[z]	Stings/20 cm^{2,y}	Plant Wt. (g)	Core length (cm)	Tipburn leaves
Two Star	143.8 A	449.4 B	5.1 BC	0.0 A
Waldman's Green	103.3 B	393.7 BC	9.1 A	0.3 A

Shining Star	96.5 B	448.3 B	7.2 AB	0.3 A
MU08-530-1	32.8 C	329.2 C	4.3 C	0.0 A
MU08-522-1 (*cor*)	30.1 C	614.0 A	6.9 AB	0.1 A
MU06-844-1 (*cor*)	30.0 C	567.3 A	5.1 BC	0.0 A

[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 $P < 0.05$.

Table 5. Mean values of leafminer sting density and head characteristics of red leaf lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2009.

Genotype	Stings/20 cm^2	Plant Wt. (g)	Core length (cm)	Tipburn leaves
Big Red	145.9 A	333.7 B	5.0 AB	0.5 A
Prizehead	130.5 A	374.5 AB	4.3 B	0.3 AB
Lolla Rossa	62.0 B	110.7 C	2.6 C	0.0 B
MU08-595	44.2 B	372.0 AB	5.4 AB	0.0 B
MU08-540-1	35.8 B	430.3 A	6.7 A	0.0 B

[z] Means in the same column followed by different letters are significantly different at $P < 0.05$.

Table 6. Mean values of leafminer sting density and head characteristics of romaine lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2009.

Genotype	Stings[z]/20 cm^2	Plant Wt. (g)	Core length (cm)	Tipburn leaves
Green Forest	316.3 A	698.5 B	6.9 B	0.0 B
Heart's Delight	291.1 AB	737.2 B	7.0 B	0.3 AB
Darkland	284.6 AB	708.8 B	6.6 BC	0.0 B
Clemente	254.7 B	1,021.0 A	8.4 A	0.9 A
MU08-617-1	36.0 C	722.7 B	6.1 C	0.1 B

[z] Means in the same column followed by different letters indicate significant differences at $P < 0.05$.

Table 7. Mean values of leafminer sting density and head characteristics of crisphead lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2009.

Genotype	Stings[z]/20 cm^2	Head Wt. (g)	Core length (cm)	Tipburn leaves
Premier	213.9 A	585.7 A	3.4 B	0.0 B
Sniper	206.7 A	600.3 A	3.7 B	0.0 B
Bronco	202.2 A	543.8 A	4.0 B	0.0 B

MU08-533-1	99.3 B	364.3 B	3.1 B	0.0 B
MU08-520-1	63.3 C	569.2 A	9.0 A	2.1 A

[z] Means in the same column followed by different letters indicate significant differences at $P < 0.05$.

Table 8. Plant Introductions tested for resistance to *Verticillium dahliae* isolate VdLs17.

Susceptible PIs										PIs being retested
120938	171671	178923	226514	273617	285650	342443	342518	358028	503602	179295
120957	171672	178924	227366	274355	285652	342444	342521	358031	503604	206965
120962	171674	179296	229761	274358	285654	342446	342522	358032	503606	226513
121935	171675	179297	229762	274364	285656	342447	342524	358035	503608	253468
140392	171676	181882	234624	274369	288244	342448	342526	358037	503610	269500
140394	172914	181883	236396	274371	289016	342449	342527	358039	503612	271476
140395	172915	181884	249536	274372	289017	342452	342528	358041	503614	273431
140398	172916	181946	250020	274373	289018	342453	342529	368624	503616	273582
141680	172917	181947	250427	274376	289019	342454	342530	368626	503618	273589
142871	172918	182208	250428	274379	289020	342456	342532	368628	503620	274359
142872	172919	182209	250430	274380	289022	342458	342534	368630	503622	274366
146071	172920	182210	251246	274416	289024	342460	342540	368632	503626	278073
146078	173710	183234	251501	274564	289026	342461	342541	370471	503628	278074
162787	173909	183684	251623	274807	289028	342462	342543	370473	503630	278080
164937	174224	184113	251790	274808	289030	342463	342547	372859	503632	289015
164938	174225	184786	251798	274898	289031	342464	342548	372895	503634	324242
164939	174229	184787	253229	274900	289032	342465	342549	372907	503636	342442
164940	175734	184788	253467	274901	289034	342466	342550	373912	503638	342450
165063	175735	184789	253468	278064	289035	342467	342552	373913	503640	358001
165492	175736	187238	255568	278067	289036	342468	342553	373916	503642	358009
167128	175737	187239	257288	278068	289037	342469	342554	378140	507926	358011
167140	175738	190906	258814	278069	289038	342473	342555	379354	507928	358018
167148	175739	198733	258816	278070	289040	342474	342556	379355	507930	358032
167150	176578	202349	261615	278072	289042	342477	342558	379357	508476	358038
167235	176579	204584	261616	278076	289044	342479	344074	379358	508479	368626
167391	176581	204585	261617	278078	289046	342482	344366	379363		368630
167393	176583	204706	261618	278079	289047	342483	344368	381933		368632
169493	176585	204708	261651	278082	289048	342484	344369	381937		379354
169494	176587	204753	261654	278084	289050	342485	344448	413677		379359
169495	176588	206963	263869	278086	289054	342486	348424	418990		391601
169496	176589	206964	264670	278088	289056	342492	358000	419140		491013
169497	176590	206966	268405	278090	289058	342493	358002	419212		491015
169499	176592	207490	269498	278091	289060	342494	358003	490999		491022
169500	176594	207490	269499	278092	289062	342495	358004	491000		491031
169501	176979	211118	269501	278094	289063	342497	358005	491002		491050
169503	176980	211600	269503	278096	289064	342498	358006	491004		491064
169505	176981	211601	269504	278098	289065	342501	358007	491005		491068
169507	177415	212015	271475	278100	289096	342502	358008	491020		491070
169508	177416	212099	271937	278102	320467	342503	358012	491022		491078
169509	177418	220172	271940	278104	321012	342505	358013	491027		491080
169510	177419	220524	273205	278106	322480	342506	358014	491030		491082
169512	177420	220665	273574	278107	323940	342507	358015	491052		491210
169513	177422	221936	273585	278108	323941	342508	358016	491064		491212
169514	177423	221936	273595	278109	339262	342509	358017	491066		
169515	177424	222253	273596	278110	342054	342510	358019	491078		
171665	177425	222724	273600	278111	342420	342512	358020	491080		
171666	177426	222994	273606	278112	342439	342514	358022	491082		
171668	178921	223379	273612	281876	342440	342515	358024	491084		
171669	178922	223380	273616	284702	342441	342516	358026	491220		

Table 9. Median and maximum Verticillium wilt disease incidence (DI) and relative marginal effect of DI from four independent greenhouse experiments inoculated with race 2 *Verticillium dahliae* isolate VdLs17.

Entry	Description	Disease Incidence (percent symptomatic plants)		
		Median	Maximum	RME[1]
PI204707	Early bolting dark green primitive, Cos-like habit; Collected in Malatya, Turkey	0	75	0.35 L, S
PI171674	Light green slow bolting Cos; Collected in Gumusane, Turkey	0	75	0.41 S
PI226641	Medium green Cos; Collected in Ahwaz, Iran	0	67	0.42 S
PI169511	Early bolting green leaf; Collected in Ezine, Turkey	0	100	0.44 S
La Brillante		45	80	0.66
Salinas 88		60	100	0.73

1/ relative marginal effect calculated from rank analysis of disease incidence data
S = significantly less disease than Salinas 88 at $P < 0.05$; L = significantly less disease than La Brillante at $P < 0.05$.

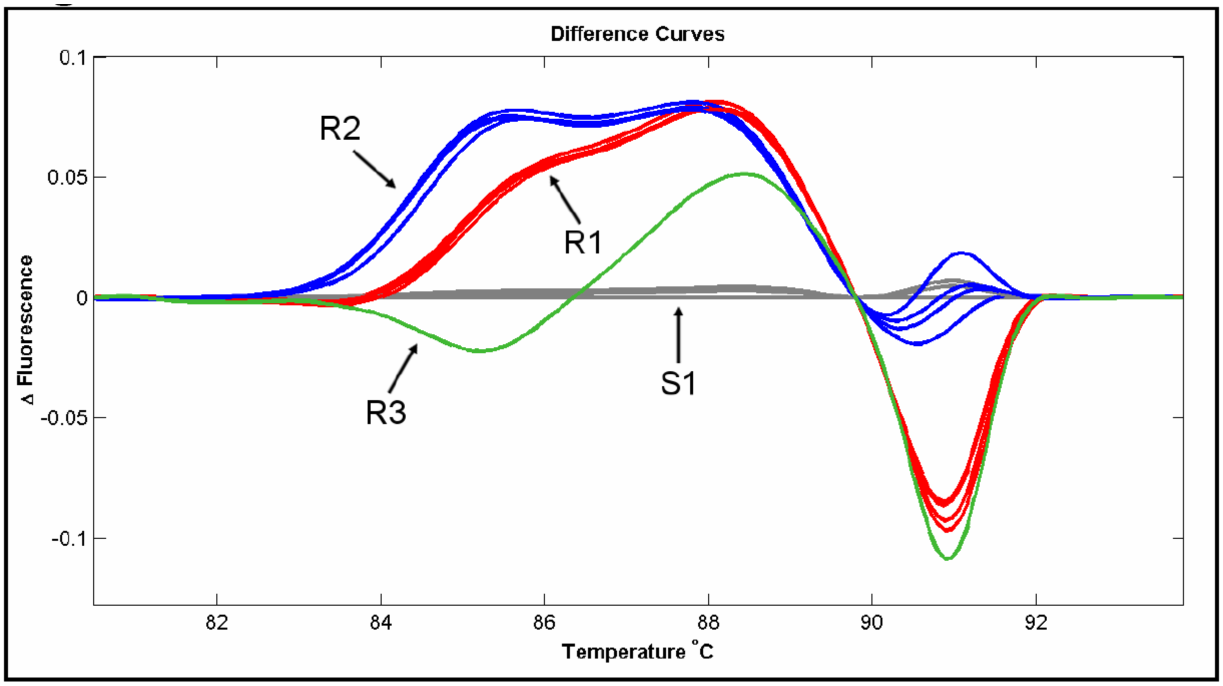

Figure 1. The differences in shapes of melting curves illustrate the detection of four homoduplexes corresponding to haplotypes R1, R2, R3, and S1. For example, the haplotype R1 was detected in iceberg cv. Salinas, haplotype R2 in primitive romaine-like accession PI491224, R3 in *L. serriola* accession UC96US23, and S1 in romaine cv. Valmaine (Simko et al. 2009).