ABSTRACT
CALIFORNIA LETTUCE RESEARCH BOARD

For the period
(April 1, 2010-March 31, 2011)

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 low desert production. In the 2010-2011 period, major efforts targeted resistance to lettuce big vein disease, lettuce drop caused by *Sclerotinia species*, Verticillium wilt, Fusarium root rot, lettuce dieback/tombusviruses, bacterial leaf spot, corky root, downy mildew, leafminer, lettuce aphid, tipburn, shelf-life of salad-cut lettuce, and multiple disease resistance. Minor programs addressed resistance to 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*. 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, leafminers, downy mildew, lettuce aphid, corky root, shelf-life of salad-cut lettuce and Verticillium wilt. Publications during 2010-2011 included reports of original research on Sclerotinia, Verticillium wilt, downy mildew, dieback, leafminer, tipburn, shelf-life, resistance to the fungicide triforine and nutrient content.

PROJECT REPORT
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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, 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 and B. Maisonneuve)

High level resistance to *Mirafiori lettuce big-vein virus*, the causal agent of big vein disease, is known in *L. virosa* accession (acc.) SAL012 (Hayes et al., 2008 Euphytica 164:493–500). Eight BCF_2 *L. sativa* x *L. virosa* acc. SAL012 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. Crossing to develop additional BC families from Salinas x SAL012 is in progress.

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 adapted to early spring plantings in the Salinas Valley. In a January planted field experiment in Soledad, CA, seven $F_{4:5}$ lines were selected for head type and resistance. These plants will be used for backcrossing to adapted iceberg cultivars. An additional 28 plants from 10 $BCF_{2:3}$ iceberg families were selected with good head type and no big vein symptoms for further field experiments in 2011.

We conducted an October planted low desert field experiment 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. In some experiments, the yields have been low due to heterogeneous maturity. In 2011, we will make single plant selections in an attempt to rectify this problem. These lines were backcrossed to cvs. Coyote and Winterselect, and 11 $F_{2:3}$ plants were selected for head type and resistance. A single breeding line with resistance from Pavane was selected for resistance, improved head type, and increased head size. Thirty plants from 13 $BCF_{2:3}$ iceberg families were selected to introgress resistance from Margarita.

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 compared to previous approaches. In 2010 we inbred 13 populations to the F_3 through F_6 generation. We are currently producing F_2 seed lots for 32 new crosses using the parents identified as lettuce drop resistant in previous research (Hayes et al. 2010. HortScience 45:333–341).

We are evaluating resistant crisphead and romaine breeding lines that incorporate diverse sources of resistance. We developed two iceberg lines (RH08-0491 and RH08-0492) from the cross Salinas 88 x (75-501-1 x Holborn Standard), both have disease incidences significantly less than Salinas and Glacier over multiple spring and summer planted replicated field experiments. We crossed both breeding lines to cvs. Hallmark and Tiber, and developed 13 $F_{2:3}$ families by selecting for head type in a non-infested field experiment. These lines will be evaluated for resistance in 2011. RH08-0492 was evaluated for horticultural characters to determine its suitability for release. RH08-0492 had excessively tall cores (> 8 cm) in two field experiments and we will not continue to evaluate RH08-0492 in 2011 for this reason. We continued evaluating four romaine breeding lines with resistance from the stem lettuce accession Balady Banha or the primitive romaine PI 207490 for potential release. Lettuce drop resistance has been consistently lower than the romaine cultivar Darkland. The breeding lines derived from Balady Banha may additional possess field resistance to downy mildew.

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. Details on the resistance in Eruption can be found in the publications: Hayes et al. 2010. HortScience, 45(3):333–341; and Hayes et al. 2010. Plant Breeding, doi:10.1111/j.1439-0523.2010.01822.x. In 2010, we evaluated the lettuce drop resistance of 180 $F_{3:4}$ families from crosses with Eruption to Darkland, Hearts Delight, or Clemente in replicated spring and summer planted field experiments. These families were concurrently grown at Spence farm to select for romaine type plants within resistant families. From this effort, 175 $F_{4:5}$ families were developed by selecting within 22 $F_{3:4}$ families. An additional 113 $F_{4:5}$ families were developed by selecting for head type within resistant families in September and November planted low desert experiments.

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 F_9 breeding lines were released in 2010 under the designation RH08-0472 and RH08-0475; additional details can be found in the publication: Hayes et al. 2011. HortScience, 46(3):501–504. In some environments the head size of RH08-0472 and RH08-0475 was larger than Salinas, although the arrival quality may by inferior to Salinas. Yield of RH08-0472 and RH08-0475 was greater than Salinas in infested grower field sites, and similar to Salinas in non-infested field experiments. These two breeding lines were backcrossed to Tiber, and 72 F_5 resistant breeding lines have been developed. 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 used several sources of race 1 resistance identified by this research to breed resistant romaine and leaf breeding lines. Many of these breeding lines combine resistance to race 1 isolates of *V. dahliae* with resistance to corky root, lettuce mosaic virus, and lettuce drop. We will not continue to evaluate these lines, but will maintain them should interest in Verticillium wilt resistant romaine and leaf cultivars increase.

We are working to develop race 2 resistant breeding lines using the currently available sources of resistance. Using PIs 204707, 171674, 226641, and 169511, F_2 seed from resistant × resistant and resistance × susceptible (commercial cultivars) crosses was produced.

d. Fusarium wilt, a.k.a. root rot (with Fresh Express)

The Japanese, semi-iceberg lettuce ‘Costa Rica No. 4’ and ‘Salinas’ are comparable for resistance in greenhouse tests in Salinas, but their levels of resistance were in previous years insufficient for the early fall desert plantings under high disease pressure (Table 1). Their levels of resistance held up much better in a Fall 2010 planting under moderate disease pressure and were statistically different from two romaine cultivars Conquistador and King Louie (Table 1). ‘Patriot’, which has been used as a susceptible check, was highly susceptible in this same test (Table 1). ‘Autumn Gold’ exhibited intermediate level of resistance in a greenhouse tests, but was susceptible in an early-Fall test under high disease pressure (Table 1). The F_3 families of King Louie × Autumn Gold exhibited high level resistance, but their frequency distribution differed from those of their parents (Figure 1).

e. Lettuce dieback (with R. Sideman, K. Subbarao)

The lettuce dieback disease is caused by two closely related soilborne viruses of the family *Tombusviridae* (TBSV and LNSV). Previous studies have provided no evidence that either chemical treatment of 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.

Based on the resistance screening, earliness of bolting, and overall phenotypic appearance, and shelf-life, two romaine breeding lines were developed in collaboration with R. Sideman and K. Subbarao and released into the public domain. SM09A and SM09B are F_8 romaine breeding lines of lettuce resistant to the dieback disease and with good shelf-life (Simko et al. 2010, HortScience 45: 670-672). 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* (Simko et al. 2009, BMC Plant Biology 9:135; Simko et al. 2010, Acta Horticulturae 859: 401-408) Testing of salad-cut lettuce in modified atmosphere packaging indicated slower decay in the two breeding lines compared to other dieback-resistant romaines.

We continue development of romaine and leaf-lettuce breeding lines that combine resistance to dieback with other desirable traits. Twenty-four new crosses were made to develop material with combined resistance to dieback and downy mildew, tipburn, verticillium wilt, and shelf-life. Material from early generations (F_1 - F_3) is grown in greenhouse and field conditions. The best lines will be screened for disease resistance in multiple field trials and analyzed with molecular markers linked to the *Tvr1* gene.

f. 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 2010, we developed 140 $BCF_{2:3}$ families for further breeding. Fifty-one families may combine resistance to BLS with resistance to race 1 isolates of *V. dahliae*.

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. Seven parents were selected, and F_2 seed is currently being produced.

g. 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 highly resistant to the disease. PI 491239 and PI 273597c had lower corky root severity than cultivars with the *cor* resistant gene in 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.

Twelve F_7 or F_8 breeding lines of 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 2010 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 2-4).

h. 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. Sixteen F_8 breeding lines were tested in a replicated field trial at the Spence Farm in Salinas in summer 2010 with control cultivars. The breeding lines all showed strong resistance to yellow spot and good horticultural traits, but they also had tipburn.

i. 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 (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 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 (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. Plans are in place to genotype the remaining two populations in 2011-2012. Field-based testing confirmed 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. 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. Results of a testing carried out on the Salinas 88 × La Brillante population indicate a QTL on LG2. This QTL also explains around 20% of the trait variation in the early stages of disease infection. The position of this QTL was independently confirmed through seedling assay in which seedlings of susceptible control (Cobham Green), both parents, and 89 inbred lines were inoculated with *Bremia* isolate 879 CAVIII (O. Ochoa).

Crosses were carried out to develop new breeding lines that would exploit field resistance observed in cvs. 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. Fifty 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. These selections were made from spring 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. Good level of resistance to downy mildew was observed in material originating from crosses with Balady Banha, Iceberg, and Grand Rapids.

2. Insect resistance

a. Leafminer (with Jianlong Bi)

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, Merlot, 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. Eight 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 2010 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 (Table 5-8). Some of these lines also showed resistance to corky root or downy mildew. These breeding lines will be evaluated again next year.

We counted leafminer stings and mines and conducted biochemical analyses (carotenoids, phenolics, sugars, protein, etc.) of 16 leafminer resistant and susceptible genotypes grown in growth chambers and in the field to study the mechanism of leafminer resistance in lettuce. Leafminer stings at baby leaf stage were highly correlated with stings at mature plant stage. We are finishing up the biochemical assays and conducting statistical analysis.

b. Lettuce aphid (with Yong-Biao Liu)

Controlled infestation (Table 9) and open field (Figure 2) tests confirmed partial resistance of PI 491093 (*L. serriola*) to lettuce aphid.

3. Adaptation and Quality

a. Adaptation to low desert environments

Four field experiments were conducted to breed slow bolting, big-vein resistant, and tipburn resistant lettuce for adaptation to fall, mid-winter, and late spring plantings in the Imperial Valley of CA or the Yuma Valley of AZ. Reports on breeding for slow bolting, resistance to big-vein and resistance 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 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. The lines are most similar to Autumn Gold, but with larger and heavier heads. They also have a tendency for protruding ribs, which can lower yields. In mid-September plantings, the core length is equivalent to Empire. In two November planted experiment, the lines had high yields and heavy heads relative to other commercial cultivars. We are currently planting these lines in diverse environments, including the Salinas Valley, to determine their breadth of adaptation. Our goal is to release these lines in late 2011.

Bolting resistance for fall plantings is also being investigated in romaine germplasm. $F_{2:3}$ plants from the cross Siskyou x Valmaine were selected for short cores and head weight. Other families from crosses with Tall Guzmaine, PIC714, Valmaine, and Medallion were discarded due to low head weight. F_2 populations are being developed from crosses between bolting resistant cultivar Blonde Lente a Monter and adapted romaine to develop additional breeding populations.

c. Tipburn

Three F_5 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. 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; the breeding lines do not appear to be well adapted for production under cooler temperatures. Our goal is to release these lines in late 2011.

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. By selecting closed top romaine type plants and families that do not have tipburn symptoms, we have developed 226 F_3 through F_5 families or breeding lines from fourteen romaine x iceberg crosses. Prior to 2010, breeding efforts for tipburn resistance targeted the low desert and coastal California as independent programs. However, analysis of genotype x environment interactions for tipburn in the low desert and coastal California indicated that this was probably not necessary (Jenni and Hayes, 2010, Euphytica, 171: 427–439). Therefore, we are currently evaluating all of our populations in both locations and combining tipburn data in order to improve selection efficiency. In a low desert December 2010 planted field experiment, 226 F_3 through F_5 families or breeding lines from fourteen romaine x iceberg crosses were evaluated for tipburn and romaine head type. Forty-four closed top romaine type families or lines had lower tipburn incidence than Green Forest. These same lines were planted in Salinas for additional evaluation.

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

The USDA has developed modified atmosphere packaging and controlled atmosphere chamber assays to detect genetic differences for shelf-life in salad-cut 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 2010, 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 are working to develop a greenhouse assay for shelf-life using seedlings grown in plug trays. This approach may be useful for developing cultivars useful for spring mix production. 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. However, subsequent experiments using 34 previously characterized cultivars were not correlated with results from field grown plants. Due to the small amount of material grown from plug trays, the number of replicate bags per cultivar was low in this experiment. This may explain the poor correlation.

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 2011. Material used for shelf-life evaluations originated from trials grown in Yuma, AZ (one trial in 2009) and Salinas, CA (two trials in 2010). Shelf-life data were 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 KeyGene. There was a highly significant ($p < 0.001$) correlation in a rate of material deterioration among three trials, with the correlation ranging from 0.60 to 0.86. Statistical analysis of data identified three QTLs located at LG 1, 4, and 9. In every trial the largest effect was observed for the QTL on LG4. This QTL explained about 27% to 69% of the total phenotypic variation of the trait (Figure 3). Work was initiated to evaluate shelf-life in the other mapping population where a large difference was previously observed between the two parental cultivars (Parade – intermediate shelf life, and Pavane – poor shelf life).

We continue screening accessions from all types of cultivated lettuce to identify material with good and poor shelf-life. Up to date we screened 160 cultivars and advanced breeding lines for their performance. Crosses were made to develop additional populations for genetic analysis.

e. Effect of transplanting on reduction of Verticillium wilt

To test whether transplanting can reduce Verticillium wilt disease in lettuce, same-aged plants of a susceptible cultivar ‘Sniper’ were directly seeded and transplanted as 3-week old seedlings into the same bed in a field infested with a Race 1 pathogen in summer and fall, 2010. Each treatment had 20 plants and was replicated six times. Disease incidence (% diseased plants), severity (0-5), and head weight were recorded at harvest maturity for each treatment.

For the summer experiment, plants were evaluated at harvest maturity on 7/9/10. Transplants had significantly lower Verticillium disease incidence and severity than direct-seeded plants, and there was no significant difference in head weight between the treatments (Table 10). However, because transplants were 5-6 days later in maturity than direct-seeded plants, they were evaluated again on 7/19/10. By then, although transplants still had lower disease incidence and severity than direct-seeded plants, the difference was not statistically significant anymore.

Because the transplants tend to be later in maturity, ‘Sniper’ plants were planted in greenhouse one week earlier than direct-seeded plants before being transplanted into the field for the fall experiment. Plants were rated on November 3, 2010 and there was no significant difference in disease incidence, severity, and head weight (Table 11). Transplants spent two weeks less time in the field than direct-seeded plants in the fall, and it seems that is not enough time to reduce Verticillium disease.

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 88 (BLS susceptible) x Little Gem and Clemente (BLS susceptible) x Little Gem are being developed. A single replicated greenhouse experiment was completed for the Salinas 88 x Little Gem population, and the disease severity scores were quantitatively distributed. An additional greenhouse experiment will be conducted. Leaf tissue was collected from $F_{5:6}$ Salinas 88 x Little Gem plants for DNA extractions.

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) x

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 conducted by UC-Davis identified three QTL on linkage groups 3 and 4. The Parade x Pavane populations was planted in a Salinas, CA field experiment in January and big vein incidence data was collected. The field data is significantly correlated with greenhouse data, and will be provided to UC-Davis for QTL mapping. We produced F_2 seed from intra-virosa resistant x susceptible crosses to determine the inheritance of complete resistance in *L. virosa*.

3. Verticillium wilt (with R. Michelmore)

We previously determined that resistance to race 1 isolates in the cross Salinas 88 (susceptible) x La Brillante (resistant) had segregation consistent with a single dominant gene in F_1, F_2 and RIL populations. Using a Salinas 88 x La Brillante recombinant inbred line population and Verticillium wilt resistance data from the USDA, UC-Davis mapped the gene to linkage group 9 coincident with an expressed sequence tag marker (QGD8I16.yg.ab1) that has sequence similarity with the *Ve* gene that confers resistance to *V. dahliae* race 1 in tomato. The publication describing this work was accepted to Theoretical and Applied Genetics, and is currently in press.

4. Use of triforine as a selectable marker for detecting hybrids (with R. Michelmore, M. Truco)

Lettuce is a highly inbred species with a compound autogamous floral structure that makes manual cross-hybridization less than 100% reliable. Therefore, accurate testing of F1 hybrids is necessary to eliminate offspring resulting from self-pollination. We evaluated the potential of triforine as a selectable marker in breeding programs.

Some lettuce cultivars are highly sensitive to triforine, an inhibitor of sterol biosynthesis found in some commercial systemic fungicides. First symptoms of a sensitive reaction are usually observed within 24 to 48 hours after treatment and include severe wilting, necrosis and rapid plant death. We mapped a single dominant gene (*Tr*) that confers sensitivity of lettuce to triforine to linkage group 1 of the integrated genetic map of lettuce. The occurrence of sensitivity is not uniform across horticultural types of lettuce. While over 80% of green-romaine lettuce cultivars tested were sensitive, most cultivars of all other lettuce types were insensitive to triforine. All accessions of wild *Lactuca* spp. were insensitive to triforine. Allelism tests using F1 and F2 progeny revealed that sensitive cultivars of all horticultural types likely carry the same *Tr* gene. The dominant allele for sensitivity found in cultivated lettuce probably had a monophyletic origin. The reaction to triforine can be used as a marker for detecting hybrids originating from a cross between phenotypically similar parents with different responses to triforine treatment. It also provides an indication of genotypes for which applications of triforine-containing fungicides are inappropriate.

Because all tested iceberg cultivars were insensitive to triforine, insensitivity to triforine cannot be used in this horticultural type as a phenotypic marker when testing success of pollination. However, triforine can be successfully used as a phenotypic marker for detecting hybrids between otherwise phenotypically similar green-romaine type cultivars, if they differ in their reaction to triforine. Though DNA-based molecular markers are informative for hybrid identification, the simple and inexpensive seedling or detached leaf triforine assay could be used in breeding programs when available. We compiled a list of 225 *Lactuca* accessions that were tested for their reaction to triforine. Detailed information can be obtained from I. Simko or accessed at Euphytica (Simko et al., 2011, Euphytica DOI 10.1007/s10681-011-0407-0).

5. Tipburn (with R. Michelmore and P. Hand)

We received a grant from the Arizona department of agriculture, specialty crop block grant program to evaluate existing recombinant inbred line populations for tipburn resistance in April harvested experiments in Yuma, AZ. The final report can be found at http://www.azda.gov/Main/Spring_Harvested_Lettuce.pdf. The populations evaluated were: Salinas 88 x La Brillante, (Valmaine x Salinas 88) x Salinas (developed by UC-Davis), and Saladin x Iceberg (developed by HRI, UK). Each population was evaluated for two years in field experiments with three replications occurring in 2008, 2009, and 2010. Broad sense heritability estimates for tipburn were low in all populations (H^2=0.14 to H^2=0.23), and indicate that selection for reduced tipburn incidence is relatively ineffective. Head closure, core height, head weight, and head maturity were significantly and positively correlated with tipburn incidence. The significance, magnitude and direction of correlations were population dependant. Reduced tipburn was typically associated with open heads, tall cores, low head weight and delayed head maturity. QTL analysis was conducted in the Salinas 88 x La Brillante and the (Valmaine x Salinas 88) x Salinas populations. Analysis of the (Valmaine x Salinas 88) x Salinas population indicates that tipburn resistance is likely associated with differences in plant morphology, and the results were consistent with the genetic correlations found for this population. Four significant QTL on chromosomes 2, 4, and 7 were detected, with reduced tipburn incidence (better resistance) inherited from the romaine parent Valmaine in all cases. All QTL for tipburn incidence were positioned at the same location as QTL with head closure, core height, head weight, or head maturity. Two QTL for tipburn incidence on chromosomes 1 and 4 were detected in Salinas 88 x La Brillante. These QTL were independent of plant morphology.

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 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. Through 2010, we have screened 770 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). However, all of these PIs have had at least a few symptomatic plants, and all but PI 171674 have had non-symptomatic plants that are nonetheless colonized by *V. dahliae*. A publication reporting the existence of partial resistance in lettuce to race 2 isolates of *V. dahliae* was published in HortScience (Hayes et al. 2011. HortScience 46(2):201–206. Complete resistance to race 2 has not yet been found, and we will continue to screen our germplasm collection in hopes of finding this trait. We are also intercrossing PIs with partial resistance to evaluate the progeny for transgressive segregants with greater levels of resistance.

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 mixes.

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 2010-2011 period, requests were made for Verticillium wilt resistant iceberg breeding lines (RH05-0336, RH05-0339, RH05-0340, RH08-0472, RH08-0475), bacterial leaf spot resistant iceberg breeding lines (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 2010, seed from 19 accessions of five wild *Lactuca* species were collected in the Russian Caucuses by Svetlana Litvinskaja and Ramazan Murtazaliev. 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, Wu BM, Subbarao KV (2010): A single recessive gene conferring short leaves in romaine x Latin type lettuce (*Lactuca sativa* L.) crosses, and its effect on plant morphology and resistance to lettuce drop caused by *Sclerotinia minor* Jagger. Plant Breeding. doi:10.1111/j.1439-0523.2010.01822.x.

Ramos SJ, Rutzke MA, Hayes RJ, Faquin V, Guilherme LRG, Li L (2010): Selenium accumulation in lettuce germplasm. Planta 233: 649-660.

Hayes RJ, Maruthachalam K, Vallad GE, Klosterman SJ, Subbarao KV (2011): Selection for resistance to Verticillium wilt caused by race 2 isolates of *Verticillium dahliae* in accessions of lettuce (*Lactuca sativa* L.). HortScience 46: 201-206.

Hayes RJ, Maruthachalam K, Vallad GE, Klosterman SJ, Simko I, Yaguang L, Subbarao KV (2011): Iceberg lettuce breeding lines with resistance to Verticillium wilt caused by race 1 isolates of *Verticillium dahliae*. HortScience 46: 501–504.

Mou B, Ryder EJ (2010): 06-857, a green leaf lettuce breeding line with resistance to leafminer and lettuce mosaic virus. HortScience 45: 666-667.

Simko I, Hayes RJ, Subbarao KV, Sideman RG (2010): SM09A and SM09B: Romaine lettuce breeding lines resistant to dieback and with improved shelf life. HortScience 45: 670-672.

Simko I, Hayes RJ, Truco MJ, Michelmore RW (2011): Mapping a dominant negative mutation for triforine sensitivity in lettuce and its use as a selectable marker for detecting hybrids. Euphytica DOI 10.1007/s10681-011-0407-0

Simko I, Pechenick DA, McHale LK, Truco MJ, Ochoa OE, Michelmore RW, Scheffler BE (2010): Development of molecular markers for marker-assisted selection of dieback disease resistance in lettuce (*Lactuca sativa*). Acta Horticulturae 859: 401-408.

F. Appendix of Tables and Figures

Table 1. Fusarium wilt ratings from previous greenhouse and field (high diseases pressure) tests and symptom severity ratings from a 2010 field test (intermediate disease pressure).

	Previous results		
Entry	Greenhouse[z]	Field	2010 field test
Patriot	S	S	3.0a[y]
Autumn Gold	I	S	1.7b
Conquistador	R	R	1.1c
King Louie	R	R	1.0c
BOS 9021	R	R	1.0c

Salinas	R	I-S	1.0c
Costa Rica No. 4	R	I-S	0.9c

[z]S = susceptible; I = intermediate; R = resistant

[y]Plants rated on a 1 to 4 scale where 1 = no apparent disease; 2 = slight-moderate stunting ; 3 = severe stunting; 4 = dead. Means followed by different letters are significantly ($P_{0.05}$) different.

Table 2. 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 2010.

Genotype	Corky root[z]	Plant Wt. (g)	Core length (cm)	Tipburn leaves[y]
Grand Rapids	8.0 A	336.8 BC	5.2 AB	0.8 A
Waldmann's Green	7.9 A	285.3 C	4.2 B	0.7 A
Shining Star	7.9 A	399.0 BC	5.7 AB	0.1 A
Two Star	7.4 A	348.3 BC	4.4 B	0.1 A
Glacier	5.7 B	809.7 A	6.5 A	0.0 A
MU06-831-1	5.6 B	530.3 B	6.0 AB	0.8 A
MU06-833-1	5.5 B	519.8 B	6.5 A	0.3 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 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 2010.

Genotype	Corky root[z]	Downy mildew	Plant Wt. (g)	Core length (cm)	Tipburn leaves[y]
Prizehead	7.8 A	3.8 A	289.0 D	3.4 DE	0.0 A
Big Red	7.8 A	4.0 A	462.1 BC	8.3 A	0.0 A
Lolla Rossa	7.2 B	0.3 D	86.1 E	3.3 E	0.0 A
Redina	7.2 B	1.0 BCD	276.0 D	8.4 A	0.0 A
Merlot	6.9 B	1.3 BCD	276.2 D	6.5 B	0.0 A
MU07-553-1	6.0 C	1.1 BCD	407.8 BCD	4.0 DE	0.1 A
MU06-810-1	5.9 C	1.3 BCD	520.0 B	4.8 CDE	0.5 A
MU09-404-1	5.9 C	0.8 CD	481.3 BC	4.1 DE	0.4 A
MU09-392-1	5.8 C	2.0 B	337.3 CD	5.0 BCD	0.0 A
MU09-488-1	5.8 C	1.0 BCD	491.5 BC	4.5 CDE	0.0 A
MU09-493-1	5.8 C	3.8 A	499.2 B	5.9 BC	0.6 A
MU09-392-2	5.7 C	1.8 BC	367.0 BCD	3.8 DE	0.0 A
Glacier	5.7 C	4.0 A	809.7 A	6.5 B	0.0 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 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 2010.

Genotype	Corky root[z]	Plant Wt. (g)	Core length (cm)	Tipburn leaves[y]
Red Hot Cos	7.9 A	528.3 A	7.0 AB	1.3 B
Red Eye Cos	7.7 A	529.8 A	6.4 B	2.6 B
MU09-447-1	5.9 B	606.7 A	8.6 A	7.2 AB
MU09-447-2	5.8 B	621.0 A	8.4 A	11.6 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 5. 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 2010.

Genotype[z]	Stings/20 cm^2[y]	Plant Wt. (g)	Core length (cm)	Tipburn leaves
Two Star	7.2 A	348.3 AB	4.4 B	0.1 B
Waldman's Green	5.2 B	285.3 BC	4.2 B	0.7 B
Shining Star	3.6 C	399.0 A	5.7 A	0.1 B
MU06-857	1.7 D	211.1 C	3.0 C	0.0 B
MU09-520 (*cor*)	1.5 D	392.4 AB	3.9 BC	1.6 A
MU09-456 (*cor*)	0.9 D	435.7 A	4.2 B	0.5 B

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

Table 6. 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 2010.

Genotype	Stings/20 cm^2[z]	Plant Wt. (g)	Core length (cm)	Downy mildew
Big Red	7.5 A	462.1 A	8.3 A	4.0 A
Prizehead	6.3 A	289.0 B	3.4 C	3.8 A
Lolla Rossa	2.7 B	86.1 C	3.3 C	0.3 C
MU07-838	1.7 B	348.9 B	5.3 B	2.5 B

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

Table 7. 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 2010.

Genotype	Stings[z]/20 $cm^{2,z}$	Plant Wt. (g)	Core length (cm)	Tipburn leaves
Green Forest	14.3 A	595.3 AB	6.5 AB	0.0 A
Darkland	10.3 B	454.7 B	5.2 B	0.0 A
Heart's Delight	8.8 BC	428.9 B	5.2 B	0.0 A
Clemente	7.3 C	688.6 A	7.1 A	0.5 A
MU08-617-1	1.1 D	661.8 A	6.2 AB	0.0 A

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

Table 8. 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 2010.

Genotype	Stings[z]/20 $cm^{2,z}$	Head Wt. (g)	Core length (cm)	Tipburn leaves
Sniper	13.8 A	737.8 A	4.5 A	0.0 B
Premier	13.1 AB	633.1 AB	4.2 A	0.0 B
Bronco	11.6 B	608.5 AB	4.6 A	0.0 B
MU07-810-1	6.4 C	455.1 C	4.5 A	0.8 A
MU06-859	4.3 D	484.5 BC	3.8 A	0.0 B

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

Table 9. Mean numbers of lettuce aphids per plant of susceptible 'Salinas', partial resistant PI 491093 (*L. serriola*), and high-level resistant IVT 280 (*L. virosa*) and 'Barcelona'; 28 days post-infestation; five aphids place on each plant in an aphid-proof cage in a field.

Entry	Mean ± std.dev.	Range
Salinas	121 ± 136	0 - 309
PI 491093	10± 6	1 - 15
IVT 280	0	–
Barcelona	0	–

Table 10. Disease incidence, severity, and head weight of direct-seeded (planted on 4/27/10) and transplanted (planted in greenhouse on 4/26/10 and transplanted into field on 5/18/10) 'Sniper' lettuce plants in a field in Salinas, Calif. infested with a Race 1 pathogen of Verticillium wilt disease.

Treatment	Disease incidence %[z]	Severity 0-5	Head weight, g
	----------------------Evaluated on 7/9/10------------------------		
Direct seed	76.0 A	3.6 A	1,235.3 A
Transplant	36.0 B	1.5 B	1,186.7 A
	----------------------Evaluated on 7/19/10------------------------		
Direct seed	75.8 A	3.7 A	1,215.3 A
Transplant	51.3 A	2.5 A	1,228.3 A

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

Table 11. Disease incidence, severity, and head weight of direct-seeded (planted on 8/11/10) and transplanted (planted in greenhouse on 8/4/10 and transplanted into field on 8/27/10) 'Sniper' lettuce plants in a field in Salinas, Calif. infested with a Race 1 pathogen of Verticillium wilt disease. Plants were evaluated on 11/3/10.

Treatment	Disease incidence %[z]	Severity 0-5	Head weight, g
Direct seed	61.7 A	2.8 A	759.7 A
Transplant	65.8 A	2.9 A	778.1 A

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

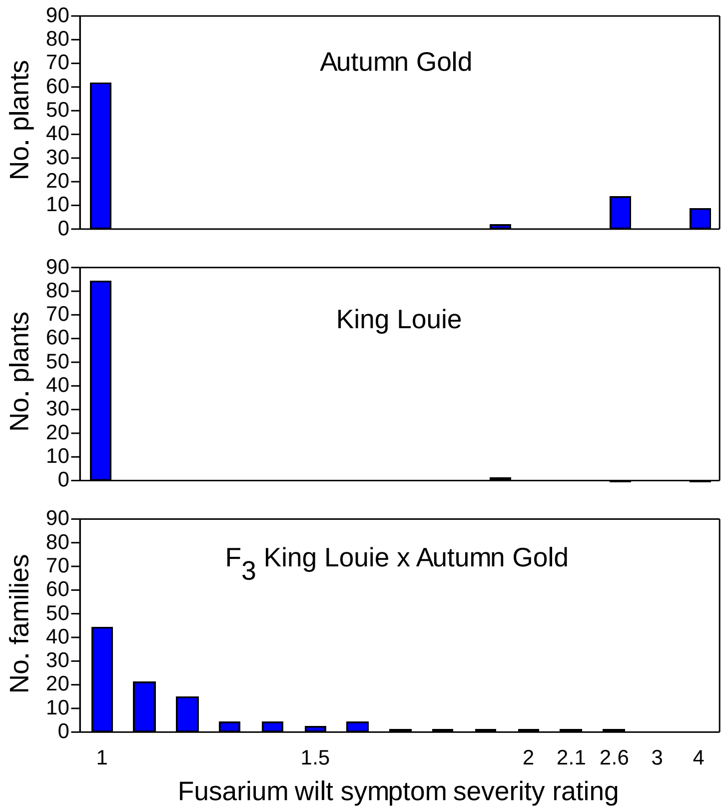

Figure 1. Frequency distributions of ‘Autumn Gold’, ‘King Louie’, and 100 F_3 families in a naturally infected field test for resistance to Fusarium wilt, plants rated on a 1 to 4 scale where 1 = no apparent disease; 2 = slight-moderate stunting; 3 = severe stunting; 4 = dead; Fall 2010.

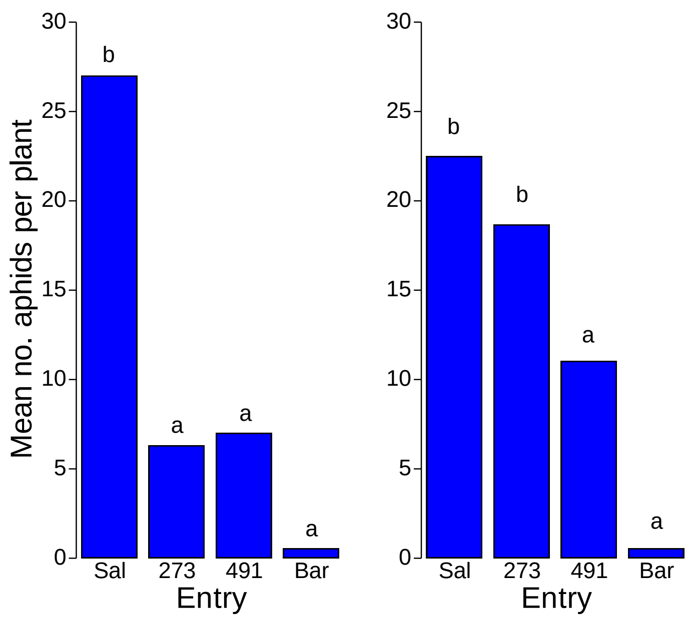

Figure 2. Mean number of lettuce aphids per plant of susceptible ‘Salinas’ and PI 273597C (*L. serriola*), partial resistant PI 491093 (*L. serriola*), and high-level resistant ‘Barcelona’

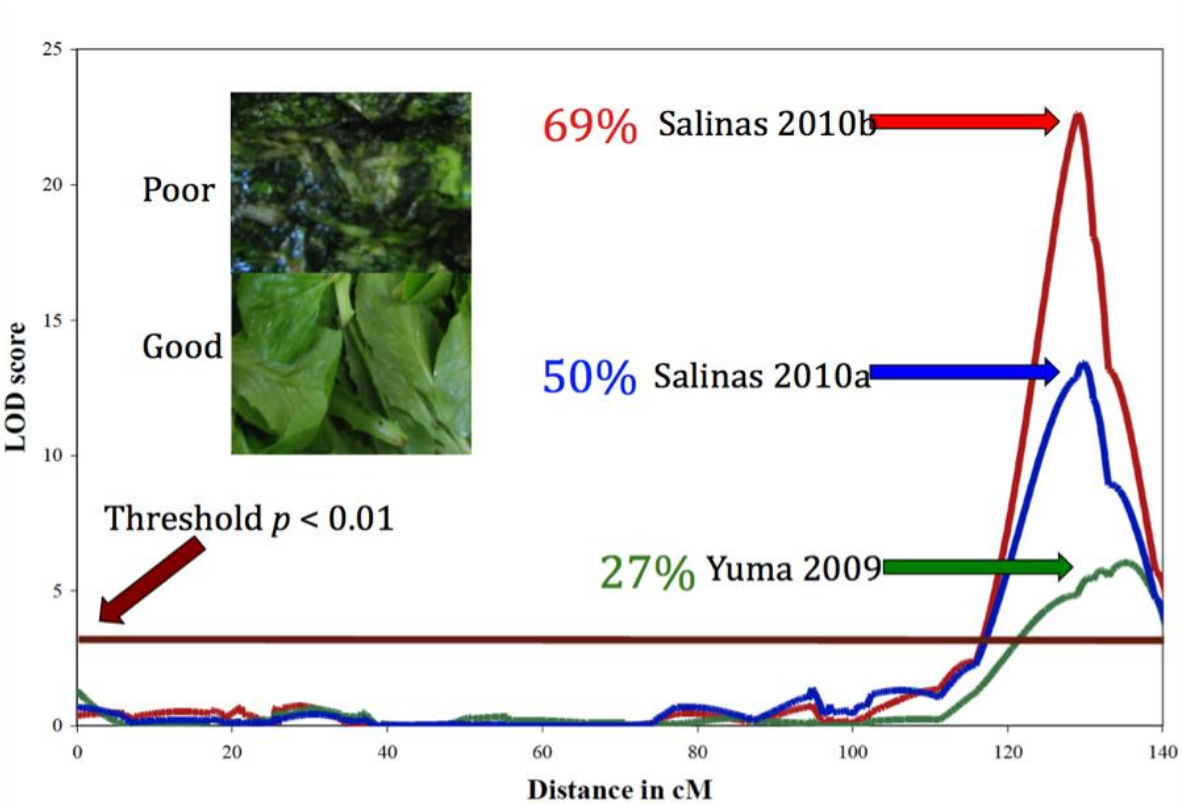

Figure 3. QTL for shelf-life on LG2. Individual peaks identify a size of LOD score for trials in Yuma, AZ (2009), and Salinas, CA (2010a, 2010b). Percentage values show a total phenotypic variation of the trait that is explained by the QTL.