CALIFORNIA LEAFY GREENS RESEARCH PROGRAM
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REPORT

Project Title: Lettuce Breeding and Genetics (USDA)

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Abstract:

The lettuce industry of California requires continued development of improved, adapted cultivars to meet new disease and insect problems, changes in the market, and changes in growing procedures. The lettuce breeding and genetics project aims to incorporate valuable traits into crisphead, mixed lettuce, and spring mix cultivars and breeding lines that are adapted to coastal California and low desert production conditions. In parallel, we develop information and tools to increase the utility of our germplasm. In 2019 and 2020, we emphasized developing resistance to Verticillium wilt, Fusarium wilt, Impatiens necrotic spot virus, bacterial leaf spot, leafminer, lettuce aphid, as well as extending fresh-cut salad shelf-life. In all programs, horticultural traits and adaptation were considered essential.

Objectives:

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

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Procedures and Results:

VERTICILLIUM WILT

In August 2019, we transplanted 18,240 Salinas 88 seedlings that had been inoculated three times with *Verticillium dahliae* Race 1 Isolate VdLs16 into 0.5 acres of Field C at the Salinas USDA-ARS station. In 2020, we will repeat this field inoculation so we may have Race 1-infected field trials by 2021.

We are working to combine resistance to *V. dahliae* race 1 with resistance to Fusarium wilt race 1. Crosses were made between an advanced *V. dahlia*-resistant iceberg selection and a Fusarium wilt-resistant selection. Twenty-three Verticillium wilt-resistant F_2 selections from a field test in a "Verticillium wilt sick field" plants produced F_3 families, 11 of which were evaluated for Fusarium wilt resistance in greenhouse tests; 71 FW–VR F_4 families were produced for Verticillium tests in 2020.

We continue to screen for resistance to *V. dahlia* Race 2 under controlled conditions. Three populations are under evaluation: Population A combines resistance to Race 1 and Race 2, Population B combines two sources of Race 2 resistance to identify more resistant progeny, and Population C introgresses Race 2 resistance into an iceberg-type lettuce.

FUSARIUM WILT (in collaboration with M. Matheron, R. Michelmore, and T. Turini)

Six iceberg type, Fusarium wilt (FW) resistant F_5 families were released as breeding lines in 2019: 15631, 15632, 15633, 15634, 15669, 15670 (PI 691904 through PI 691909, respectively).

They were derived from a cross of susceptible iceberg cultivar Autumn Gold with the FW-resistant romaine cultivar King Louie. The lines are freely available from the USDA–ARS, Western Regional Plant Introduction Station, Pullman, WA upon request: https://npgsweb.ars-grin.gov/gringlobal/search.aspx?

Selections were done in naturally-infected field tests in Yuma. The lines performed well in two naturally-infected FW field tests in Salinas Valley. They are variable for iceberg lettuce type (Figure 1). FW resistance selection in each cycle of selection was assessed on a 1 (no wilting) to 4 (dead) scale of foliar reaction. Roots were sampled in each test to confirm foliar reactions (Figure 2).
Five of the released FW-resistant breeding lines were included in two early-Fall, commercial field tests planted in Yuma, AZ in collaboration with the Yuma Center of Excellence for Desert Agriculture. Data for the five lines, their parents and two commercial check cultivars, one susceptible and one resistant, are summarized in Table 1.

For the central California coastal districts, Fusarium wilt resistance is being combined with resistance to Verticillium wilt race 1 (See the Verticillium wilt race 1 section above).

Figure 1. Variation in head formation within Fusarium wilt -resistant breeding line 15631; naturally infected field test, Salinas Valley, 2018.

Figure 2. Reactions of roots to Fusarium wilt infection: susceptible 'Patriot' (left) showing typical root symptoms and FW-resistant breeding line 15633; naturally infected field test, Salinas Valley, 2018.

Table 1. Summary of disease reactions of five Fusarium wilt-resistant breeding lines in a naturally-infected commercial field test, Fall 2019; collaborative with Yuma Center of Excellence for Desert Agriculture.

	Percent	Disease reaction[z]	
Entry	Diseased	Asymptomatic	Diseased
FW-resistant lines			
15631 (PI 691904)	0	85	0
15632 (PI 691905)	1	78	1
15633 (PI 691906)	0	101	0
15634 (PI 691907)	1	86	1
15669 (PI 691908)	0	93	0
Parents			
Autumn Gold (S)	41	17	12
King Louie	36	16	9
Commercial lines			
El Guapo (susceptible)	66	107	204
Meridian (resistant)	7	361	26

[z] Rated on a 1 to 4 scale of foliar symptoms, where 1 = no apparent disease symptoms, 2 = slight-moderate stunting, 3 = severe stunting, 4 = senesced, i.e., dead.

IMPATIENS NECROTIC SPOT VIRUS (in collaboration with D. Hasegawa and W. Wintermantel)

The thrips-vectored orthotospovirus, *Impatiens necrotic spot virus* (INSV), causes economic damage due to spotted wilt disease on lettuce in coastal California. INSV has become more widespread and severe in the Salinas Valley in recent years, associated with large populations of its thrips vector.

To validate last year's results and determine if resistance is segregating, 20 seedlings each of Eruption, Conquistador, and *E. sonchifolia* were rub-inoculated with INSV and moved to a greenhouse with viruliferous thrips. After four weeks, plants were given a final visual evaluation of INSV (presence or absence of symptoms) and thrips damage (rated 0-5) symptoms and 0.5 g leaf tissue was taken from each plant for determination of virus infection and titer by enzyme-linked immunosorbent assay (ELISA) using the Agdia INSV ELISA detection kit per manufacturer's recommendations. Samples were considered to be positive if the ELISA absorbance readings were 2.5x higher than the mean of negative controls (Table 2).

California's Monterey County harvests 100,000 acres of lettuce (Lactuca sativa L.) annually at a value of $1.2 billion. Impatiens necrotic spot virus (INSV) and its vector, Western Flower Thrips (Frankliniella occidentalis), caused substantial economic losses in numerous commercial fields beginning in 2006 even though only 5.7% of plants exhibited symptoms in affected fields. Numbers of infected fields and disease incidence within fields have risen significantly in recent years, with incidence as high as 80% in some fields. Even so, natural infection was too sporadic and incidence was too low for reliable evaluation of lettuce germplasm for resistance to INSV or thrips. In 2018 and 2019, high incidence and symptom severity of INSV in field trials at the USDA-ARS Spence Farm in Salinas provided a unique opportunity to evaluate 500 entries of experimental and commercial germplasm for resistance in four experiments with diverse germplasm. In both years, numbers of symptomatic plants per plot were recorded weekly for four weeks. Tipburn and 10 horticultural traits were assessed at plant maturity and harvest. INSV incidence was negatively correlated with head maturity (r = -0.54, P0.05) and closure (r = -0.62, P0.05) (Figure 3). Maturity is a measure of head firmness, ranging from 0 (very soft and yielding) to 5 (very firm). Closure is a measure of head openness, ranging from 0 (completely open head with leaves flat on the ground and meristem visible) to 5 (closed head with leaves overlapping on top of each other). Severe INSV infection in 2019 was observed in a field trial consisting of 585 commercial entries, wild relatives, and breeding material. Numbers of symptomatic plants per plot were recorded weekly for four weeks. Thrips damage was rated on one leaf from each of three plants per plot on a scale from 0 (no feeding scars) to 5 (>100 feeding scars). INSV incidence was not well correlated with thrips damage (r = 0.06, P0.05), but identified three groups of germplasm with respect to INSV incidence and thrips infestation for further evaluation: low INSV + low thrips symptoms, high INSV + high thrips symptoms, and, perhaps most interesting, low INSV symptoms + high thrips damage (Figure 4).

Table 2. Number (n) of plants positive (+) and negative (-) for symptoms of infection and ELISA virus titer of *Impatiens necrotic spot virus* and thrips damage (0-5 scale) of *Emilia sonchifolia*, Conquistador, and Eruption after manual inoculation and viruliferous thrips.

Entry	Description	N	INSV	ELISA	Thrips
E. sonchifolia	Susceptible	20	0-, 20+	0-, 20+	5.0
Conquistador	Susceptible	20	0-, 20+	0-, 20+	4.9
Eruption	Resistant	20	17-, 3+	6-, 14+	3.0

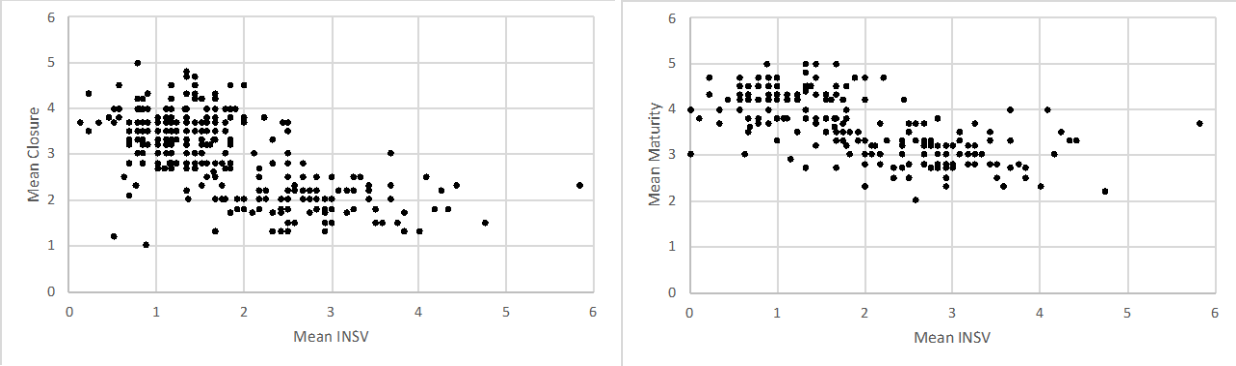

Figure 3. Scatter plot of A. mean closure and mean Impatient necrotic spot virus and B. mean maturity and mean Impatient necrotic spot virus

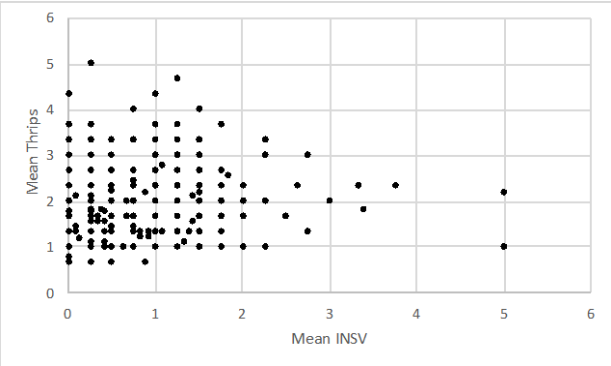

Figure 4. Scatter plot of mean thrips and mean Impatient necrotic spot virus.

BACTERIAL LEAF SPOT (in collaboration with J. Sthapit and G. Sandoya)

Bacterial leaf spot (BLS) disease of lettuce is caused by bacteria *Xanthomonas campestris* pv. *vitians* (*Xcv*). In baby leaf lettuce, seeds are planted in extremely high density in the field, which enhances the occurrence of BLS. It can cause significant economic damage making the crop unmarketable, mainly during cool and wet weather conditions. Cultivating resistant cultivars is the most sustainable and reliable strategy to manage BLS as it will reduce quality loss and minimize economic damage to the baby leaf industry. Identification of resistant germplasm will provide essential information and facilitate in breeding for BLS resistance.

Four hundred and ninety-eight lettuce accessions were evaluated for resistance to BLS with three BLS isolates (BS0347, BS2861, BS3127) at USDA-ARS, Crop Improvement and Protection Research Unit, Salinas, CA and three isolates (L7, L44, and Sc8B) at Everglades Research and Education Center, University of Florida, Belle Glade, FL with collaboration with Dr. Germán Sandoya. Seeds were planted in plug trays with each accession planted in three or four wells. For germination, the planted trays were put in growth chambers in dark at 10°C for 2 days. Then growth chamber program was set to 16 hours of light at 20°C. Three weeks after planting, the plants were moved to greenhouse and spray inoculated with one *Xcv* isolate. Inoculated plants were kept in high humidity with water mister systems installed on two walls of the

greenhouse to facilitate disease development. Plants were evaluated for BLS disease severity in about two to three weeks after inoculation using 0 to 5 rating scale where 0 = no disease, 1 = little disease, a few lesions of < 3 mm in size, 2 = individual disease lesions > 3 mm or more than 10 lesions covering < 10% of leaf area, 3 = large coalesced lesions covering 10 to 20% of leaf area, 4 = lesions covering 20 to 50% of leaf area, and 5 = lesions covering greater than 50% of leaf area.

Least square means (LS means) for disease severity were calculated for each accession in each experiment. LS means values were used for further statistical analyses and genome-wide association studies (GWAS). Accessions showing high level of resistance to one or more BLS strains include accessions of *Lactuca serriola*, several plant introductions, and cultivars Bunte Forellen, Grenadier, La Brillante, and Tango. Four thousand, six hundred and fifteen (4,615) high-quality, single-nucleotide polymorphism markers were used for GWAS of BLS resistance by mixed linear model analyses. The genetic relationships among the individuals were incorporated into statistical models using principal component analysis and kinship matrix. A significant QTL was detected on Linkage Group 2 (LG2) for isolate BS0347 and for combined results of Florida isolates (Figure 5). A major QTL for resistance to BLS in LG2 has been previously reported in recombinant inbred line (RIL) populations. Molecular markers closely linked to the QTL may be potentially used for marker-assisted selection of BLS-resistant lettuce germplasm and development of new lettuce cultivars with disease resistance.

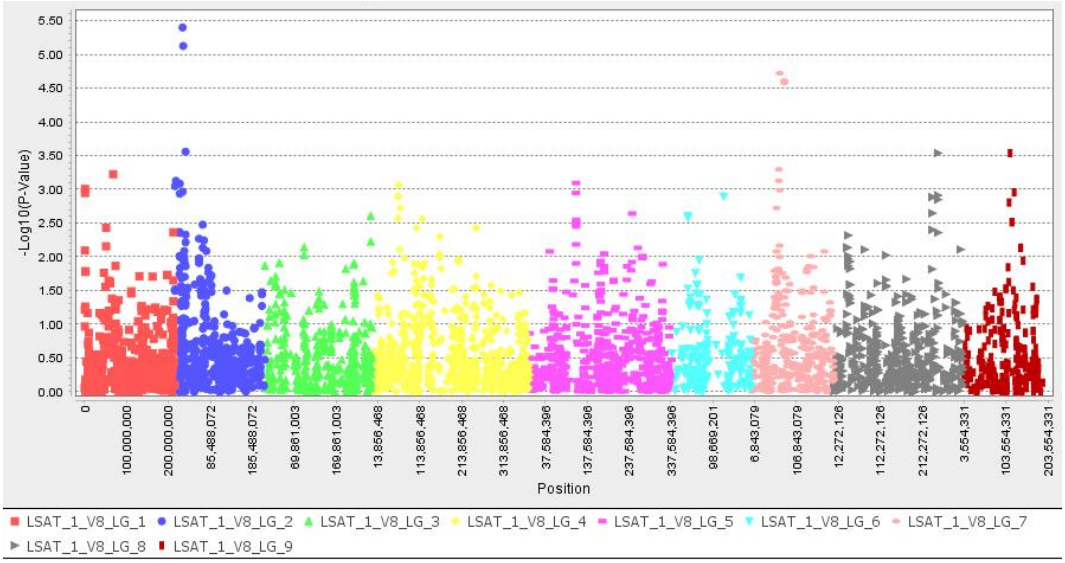

Figure 5. The Manhattan plot from genome-wide association study of 493 lettuce accessions for resistance to three bacterial leaf spot isolates tested in Florida.

LEAFMINER

The predominant species of leafminers in central California is *Liriomyza langei.* They have a wide host range including broccoli, cauliflower, celery, lettuce, melons, spinach, tomato, and many weeds. Damages caused by adult sting and larval mining of leaves reduce photosynthetic capacity and product quality, render lettuce leaves unmarketable, and provide an entrance for disease organisms. Chemical control is not long lasting, and it is well documented that leafminers can develop a high degree of resistance to insecticides. The most economical means to control leafminers is through the use of plant resistance. However, high level of leafminer resistance is currently not available in commercial lettuce cultivars.

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

F_2 to F_6 progeny plants from crosses between leafminer resistant source (PI 169513, Red Grenoble, Merlot, Lolla Rossa, Bibb, and Tom Thumb) and good horticultural types (Salinas, Salinas 88, Tiber, Prizehead, and Lobjoits) were selected in the field for leafminer and multiple resistances, and some of them were backcrossed to restore horticultural traits. Twelve promising F_7 to F_{12} breeding lines of green leaf, red leaf, and romaine lettuces were trialed at Spence Farm in Salinas from June to September 2019 with four replications, along with commercial cultivars and resistant controls. All breeding lines had significantly lower leafminer sting density than commercial cultivars and resistant controls, and the plant weight, core length, and tipburn of the breeding lines were generally similar to or better than commercial cultivars (Tables 3-5). Many of these breeding lines also have the *cor* resistant gene, so they are resistant to corky root disease as well (Tables 3 and 5). Some lettuce breeding lines showed moderate to high levels of resistance to downy mildew. Four green leaf lettuce breeding lines had better downy mildew disease ratings than the resistant controls 'Grand Rapids' and 'Iceberg' (Table 3). These breeding lines will be evaluated in the field again in 2020 to confirm the results. Best performing breeding lines will be publicly released to the seed companies and the lettuce industry.

Table 3. 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 2019.

Genotype[z]	Stings/cm^{2}[,y]	Plant wt. (g)	Core length (cm)	Downy mildew[x]
Waldmann's Green	3.9 A	475.8 A	7.4 A	3.3 B
Grand Rapids	3.1 B	355.7 D	4.8 BC	2.0 D
Iceberg	3.1 B	361.3 CD	5.1 BC	2.5 C
Two Star	3.1 B	449.0 ABC	5.3 BC	4.0 A
Shining Star	2.5 B	466.8 AB	5.9 B	4.0 A
MU18-280-1 (*cor*)	0.3 C	518.5 A	5.4 BC	1.5 E
MU18-247 (*cor*)	0.3 C	368.3 BCD	4.2 C	1.5 E
MU17-352-1 (*cor*)	0.3 C	413.7 ABCD	4.4 C	1.0 F
MU18-283-1 (*cor*)	0.3 C	439.5 ABCD	4.5 BC	1.5 E

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

Table 4. 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 2019.

Genotype	Stings/cm^{2}[,z]	Plant wt. (g)	Core length (cm)	Tipburn[y]
Red Hot	3.7 A	564.0 A	6.2 ABC	0.0 B
Prizehead	3.4 AB	390.3 B	5.6 C	3.1 A
Merlot	3.3 AB	216.5 C	5.7 BC	0.0 B
Big Red	3.3 AB	416.3 B	5.7 BC	0.5 AB
Red Fox	3.0 BC	512.9 A	7.2 A	0.0 B
Lolla Rossa	2.7 C	195.2 C	5.2 C	0.0 B
MU18-250	0.8 D	417.2 B	6.7 AB	0.0 B
MU18-243	0.5 D	374.2 B	6.1 BC	0.0 B

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

Table 5. 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 2019.

Genotype[z]	Stings/cm^2[,y]	Plant wt. (g)	Core length (cm)	Tipburn[x]
Clemente (*cor*)	4.9 A	944.0 A	8.4 A	1.3 AB
Heart's Delight (*cor*)	4.6 AB	849.2 AB	8.0 A	0.6 AB
Green Towers	4.5 AB	901.5 AB	7.2 ABCD	1.4 AB
Parris Island Cos	4.3 AB	642.0 C	6.5 BCD	0.0 B
Valmaine	4.0 B	738.5 BC	6.3 CD	0.2 B
Green Forest	4.0 B	878.7 AB	8.0 A	0.0 B
MU18-259 (*cor*)	0.9 C	744.5 BC	6.4 CD	2.4 A
MU17-303-1 (*cor*)	0.5 C	900.3 AB	7.7 AB	0.0 B
MU18-262 (*cor*)	0.4 C	802.9 ABC	6.5 BCD	0.5 AB
MU18-258 (*cor*)	0.4 C	843.5 AB	7.1 ABCD	1.1 AB
MU17-534-1 (*cor*)	0.3 C	633.7 C	6.1 D	1.0 AB
MU18-254	0.3 C	843.9 AB	7.3 ABC	0.0 B

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

LETTUCE APHID (in collaboration with A. Del Pozo, D. Hasegawa, and Y.B. Liu)

Partial resistance to lettuce aphid biotype Nr:0 from PI 491093 (*L. serriola*) is being introgressed to cultivated lettuce (*L. sativa*). Progress has, however, been hampered by thrips infestations and INSV infection.

Lettuce aphid biotype Nr:1 emerged in Salinas Valley in 2018 and was widespread in 2019. Biotype Nr:1 overcomes all commercially available biotype Nr:0-resistant cultivars. IVT 280, the original source of resistance to biotype Nr:0, is reportedly resistant to Nr:1. Our interest in screening germplasm for resistance to Nr:1 is completely contingent upon establishment of Nr:1 colonies. Toward that end, we have collaborated with UCCE Monterey County Entomologist Alejandro Del Pozo and new ARS, Salinas entomologist Daniel Hasegawa to characterize the current population structure of lettuce aphid in Salinas Valley. Efforts to establish Nr:1 colonies have been unsuccessful to date.

TIPBURN

Tipburn is a physiological disorder characterized by necrotic lesions on leaf margins and is triggered by environmental factors that are not completely understood. In commercial lettuce, even low incidence of tipburn renders the heads unsalable. In addition, tipburn provides entry points for postharvest storage decay organisms that reduce shelf life and culinary quality. We

continue to evaluate breeding lines with romaine check cultivars for tipburn incidence, yield and horticultural quality. Populations from the cross of cultivar Green Towers (susceptible) and Salinas (low incidence of tipburn) have been under pedigree selection and, in 2019, 242 F8 lines from 11 families and check cultivars were planted in two field trials and evaluated for incidence of tipburn and horticultural traits. Plots were 20' long, with two seed lines on 40" beds. Entries were replicated three times. During the growing season, plants showed symptoms of *Impatiens necrotic spot virus* (INSV) infection. Upon incidence, number of plants per plot dead due to INSV were counted weekly until harvest. Prior to harvest, each plot was rated for maturity, head closure, and number of marketable heads. At harvest, 10 heads were collected from each plot and collectively weighed. Five plants were cut in half and internal measurements of height, core length, bottom width and top width were taken. All 10 plants were opened and examined for presence of tipburn (Tables 6 and 7). Selections were made within and between families. Seed was increased in the greenhouse for further evaluation and selected lines were backcrossed to romaine-type to improve horticultural traits. Ten F3 families from an additional four romaine by iceberg crosses are under development and evaluation (Table 8).

Table 6. Tipburn incidence, head height, core length, top width and bottom width, and weight of 242 F8 derived from a cross of Green Towers (low incidence of tipburn, romaine) and Salinas (low incidence of tipburn, iceberg).

Entry	Tipburn	Height	Core	Top	Bottom	Weight
Salinas	2.4	15.6	6.2	10.3	7.2	10.5
Green Towers	5.6	29.4	12.2	24.8	13.9	9.8
RH13-0012	4.7	29.1	11.5	23.3	14.4	10.4
RH13-0013	5.2	30.1	12.1	23.2	14.1	10.8
RH13-0014	6.0	30.5	12.0	23.0	13.5	10.7
RH13-0015	5.4	29.4	10.7	23.3	13.5	11.1
RH13-0016	6.3	30.1	11.2	23.1	13.7	10.6
RH13-0017	3.2	30.8	16.4	25.6	13.3	11.7
RH13-0018	1.9	30.0	14.9	27.9	14.9	12.2
RH13-0019	2.7	30.0	16.7	27.7	14.8	12.6
RH13-0020	1.2	37.5	27.8	22.7	13.5	11.5
RH13-0021	2.5	33.2	13.3	31.1	14.9	12.4
RH13-0022	1.1	33.4	12.9	33.1	16.0	11.3

Table 7. Number infected with *Impatiens necrotic spot virus*, maturity, closure, and number of marketable heads, of 242 F8 derived from a cross of Green Towers (low incidence of tipburn, romaine) and Salinas (low incidence of tipburn, iceberg).

Entry	INSV	Maturity	Closure	Market.
Salinas	1.2	4.5	4.5	15.4
Green Towers	1.6	3.4	2.7	12.8
RH13-0012	2.3	3.1	2.5	12.5
RH13-0013	2.1	2.9	2.8	14.4
RH13-0014	2.1	3.3	2.8	13.8
RH13-0015	2.0	3.3	2.8	14.4
RH13-0016	1.8	3.0	2.6	13.3
RH13-0017	1.1	4.2	3.6	14.1
RH13-0018	0.6	4.2	3.7	11.0
RH13-0019	0.7	4.0	3.7	11.5
RH13-0020	1.1	4.8	3.9	12.1
RH13-0021	1.2	4.0	3.4	11.6
RH13-0022	1.2	3.8	2.9	11.7

Table 8. Tipburn incidence, number infected with *Impatiens necrotic spot virus*, maturity, and head closure of 10 F3 derived from the four crosses: Beacon x King Henry, Gabilan x King Henry, Gabilan x Green Towers, and Lighthouse x Green Towers.

Entry	Pedigree	Tipburn	INSV	Maturity	Closure
Beacon		1.8	0.8	.	4.6
Gabilan		1.8	1.0	.	4.6
Green Towers		5.6	1.6	3.4	2.7
King Henry		8.0	2.2	.	3.0
Lighthouse		1.6	0.9	.	4.6
RHF1-0911	Beacon x King Henry	2.8	1.6	.	1.9
RHF1-0912	Gabilan x King Henry	3.5	1.6	2.0	2.0
RHF1-0913	Gabilan x Green Towers	3.5	1.4	.	2.3
RHF1-0915	Lighthouse x Green Towers	3.6	1.6	2.5	2.5

POSTHARVEST DETERIORATION OF FRESH-CUT LETTUCE (in collaboration with H. Peng, J. Sthapit, and R. Michelmore)

To study the effect of CO_2 on the deterioration rate of fresh-cut lettuce, cultivars Salinas 88 (Sal88) and La Brillante (LaB) were tested with high CO_2 (25%) packaging. CO_2 was found to significantly accelerate the deterioration of both cultivars, though at 15 days after procession (DAP) the difference was not significant for Sal88 (Figure 6A). CO_2 concentration declined in the bags initially supplied with 25% CO_2, while increased in bags with pure N_2 (Figure 6B). O_2 declined in all sample bags after samples were stored for one day (Figure 6C). Changes in electrical conductivity (EC) (Figure 7A) and total dissolved solids (TDS) (Figure 7B) corresponded well with deterioration of samples. pH of Sal88 samples was found generally lower that of LaB

samples at 0 and 6 DAP (Figure 7C). Changes in chlorophyll fluorescence (evaluated on LaB) were in line with observed levels of deterioration (Figure 8).

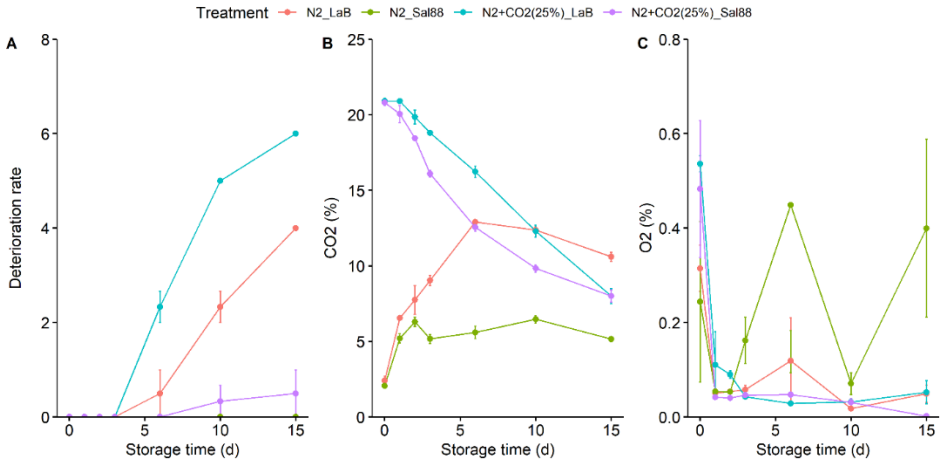

Figure 6. Deterioration levels and atmosphere composition of fresh-cut lettuce treated with CO_2. A: The deterioration rate of fresh-cut lettuce from cultivar Salinas 88 (Sal88) and La Brillante (LaB) during storage. B: CO_2 content in MAP bags during storage. C: O_2 content in MAP bags during storage. Data represented the mean values from at least two replicates; error bars indicate standard error.

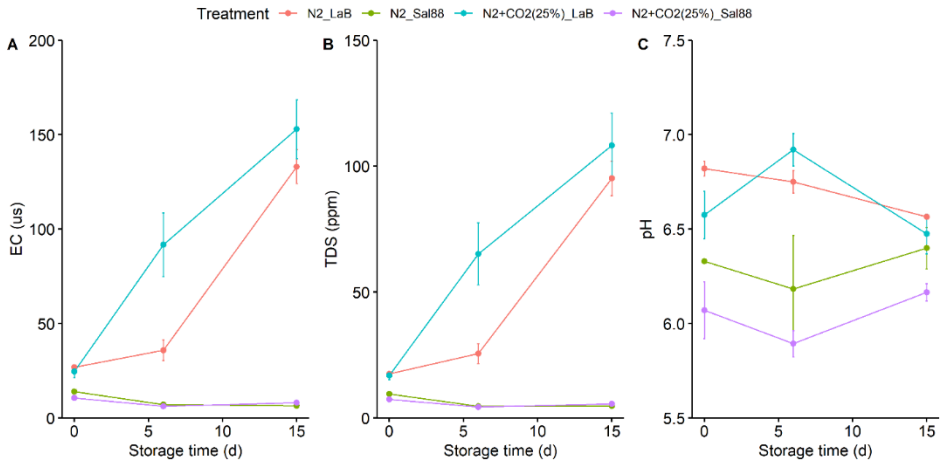

Figure 7. Change of electrical conductivity (EC), total dissolved solids (TDS), and pH in fresh-cut lettuce treated with CO_2. Data represented the mean values from at least two replicates; error bars indicate standard error.

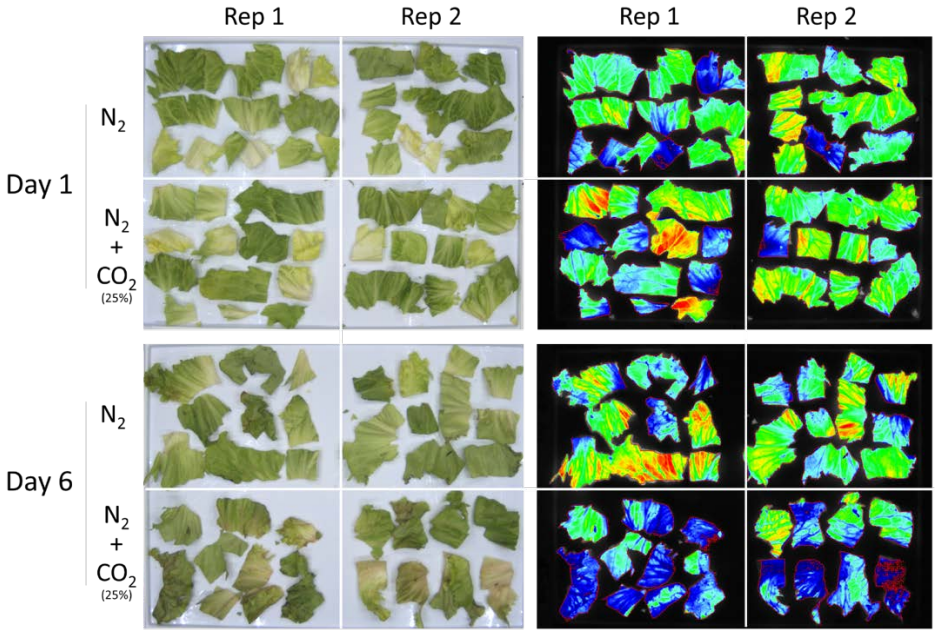

Figure 8. RGB and Fluocam images of fresh-cut La Brillante lettuce treated with CO_2. Blue color indicates decreased chlorophyll fluorescence.

To identify the candidate genes associated with the deterioration rate, fine-mapping was performed with a recombinant population (F_2) from a cross of a deterioration resistant RIL and LaB. The *qSL4* region was narrowed to 5.1 kb through genetic and phenotypic analyses (Figure 9). An alignment analysis of the target region from 12 cultivars revealed genomic differences between cultivars with rapid and slow rates of deterioration.

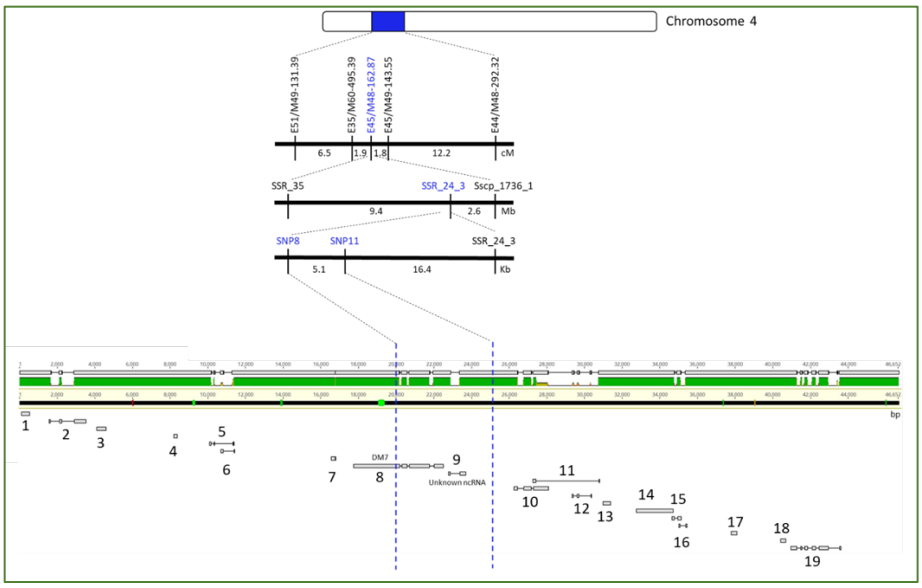

Figure 9. Schematic diagram of fine-mapping of *qSL4*. The candidate region (5.1 kb) was defined by blue dash lines. The extended region (46.6 kb) from lettuce genome (V9) included 19 predicted unique transcripts (1-19). For each transcripts, open boxes and solid lines represented exons and introns, respectively.

POSTHARVEST PINKING AND BROWNING OF FRESH-CUT LETTUCE (in collaboration with Y. Luo)

Discoloration (browning) represents a major challenge that limits the quality and shelf life of packaged lettuce. The lack of effective browning control has resulted in processors relying on modified atmosphere packaging (MAP) to achieve low oxygen atmospheric conditions and maintain the shelf life. However, this induces other problems, especially the development of off-odors and physiological disorders that often occur at extremely low O_2 and high CO_2 during the end of the shelf life. Furthermore, low oxygen conditions coupled with high temperature abuse promote population growth and increased virulence factor gene transcription of the human pathogenic bacteria, *Escherichia coli* O157:H7 (Chua et al., 2008; Sharma et al., 2011). It may be possible to reduce food safety risks and increase quality in fresh-cut salads by using lettuce varieties that tend to have less browning. To find romaine lettuce accessions with low browning potential midribs of 14 accessions (11 cultivars, two breeding lines, and a single plant introduction) were shredded and packaged in perforated bags for five days. Images of processed samples were captured daily and analyzed with computer vision technology to quantify browning intensity via L*a*b* color values and browning index (BI). Enzymatic activity, including phenylalanine lyase (PAL), peroxidase (POD), polyphenol oxidase (PPO), and total phenolic content (TPC) were measured daily. After five days in storage, the accessions in the Tall Guzmaine and Parris Island Cos pedigree groups exhibited the greatest and least browning, respectively (Figure 10). In addition, while the PAL, POD, and TPC increased substantially over time, the PPO of twelve accessions fluctuated with only minor increases. For all accessions, the temporal increase of PAL, POD, and TPC showed significant, positive correlation to browning progression. Comparing between accessions, those that had greater amounts of accumulated

PAL and smaller amounts of POD tended to have a greater amount of browning after five days of storage, despite relatively low correlation coefficients. However, the accumulation of TPC and PPO was not correlated to browning severity after five days of storage. This systematic study provides lettuce growers and breeders with guidance for selecting accessions with limited browning, and it supplies researchers in plant physiology and genetics with more information on the roles of enzymes in the lettuce browning process.

Results of the study were published in Postharvest Biology and Technology. https://doi.org/10.1016/j.postharvbio.2019.110931

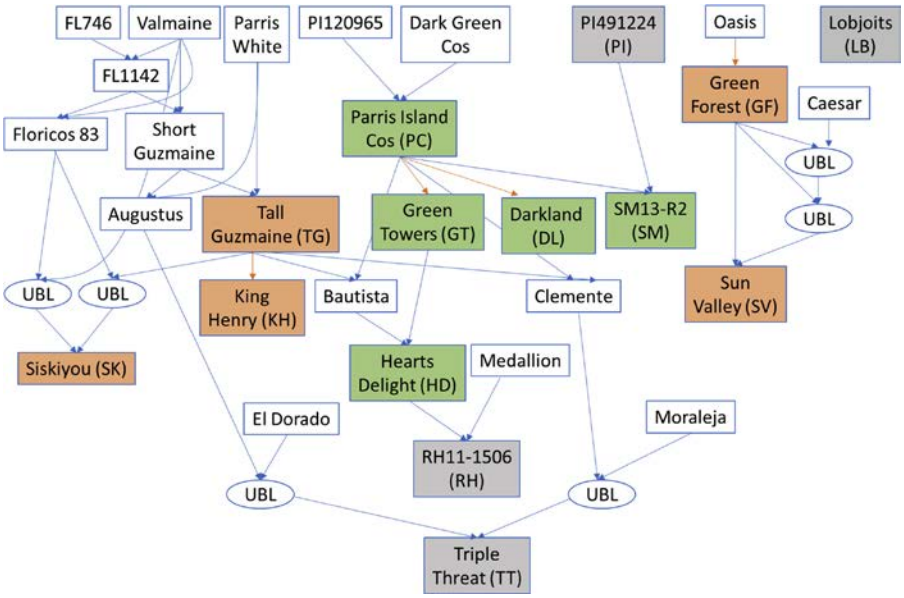

Figure 10. Complex pedigree relationship among accessions evaluated for browning potential of cut romaine lettuce. Blue arrows indicate the direction leading from parental accessions to their descendant. Blue arrows indicate the direction of selection for accessions that were developed by a single plant selection from another accession. The UBL abbreviation indicate known, but unnamed breeding lines or hybrids in the pedigrees. Accessions tested in this study are colored green (least browning), grey (intermediate browning), and orange (greatest browning). Colors are based on values of Browning Index (BI) at Day 5. The accessions highlighted in green and orange represent the ones with the lowest and highest BI, according to analysis of means (ANOM).

GENETIC VARIATION AND RELATIONSHIP AMONG CONTENT OF VITAMINS, PIGMENTS, AND SUGARS IN BABY LEAF LETTUCE

Baby leaf lettuce harvested approximately 30 days after planting is the primary component of spring mix salads, a popular type of packaged salads. Very little is known, however, about the content of vitamins, sugars, and pigments in young lettuce plants. Therefore, plants of 42 accessions harvested at baby leaf stage were analyzed for the contents of vitamin C, ß-

carotene, anthocyanins, chlorophylls, glucose, fructose, and sucrose. Significant differences among accessions were found for content of all seven compounds plus sucrose sweetness equivalency (SSE) and average vitamin load (AVL_{AC}) (Figure 11). Cultivar Floricos was highest in all sugars, SSE and vitamin C; Taiwan was highest in ß-carotene and AVL_{AC}, and Annapolis and Darkland were highest for anthocyanins and chlorophyll contents, respectively (Figure 12). The lowest content of glucose and sucrose was found in iceberg cultivar Salinas, fructose in *L. serriola* accession UC96US23, vitamin C in PI 257288, and β-carotene in cultivar Solar. The lowest relative sweetness (SSE) was calculated for UC96US23, followed by Salinas, while the lowest AVL_{AC} was estimated for PI 257288. There were very strong, positive correlations among contents of the three sugars, and between β-carotene and vitamin C, and β-carotene and anthocyanins. Composition profiles of accessions presented in this study, together with identified associations between compounds, can be used by breeders, growers, and producers to select lettuces with desirable combinations of sugars, pigments, and vitamins. This information can help in development of new cultivars and breeding lines with desirable combination of traits, pleasing taste, and higher vitamin content.

Results of the study were published in Food Science and Nutrition.
https://doi.org/10.1002/fsn3.1196

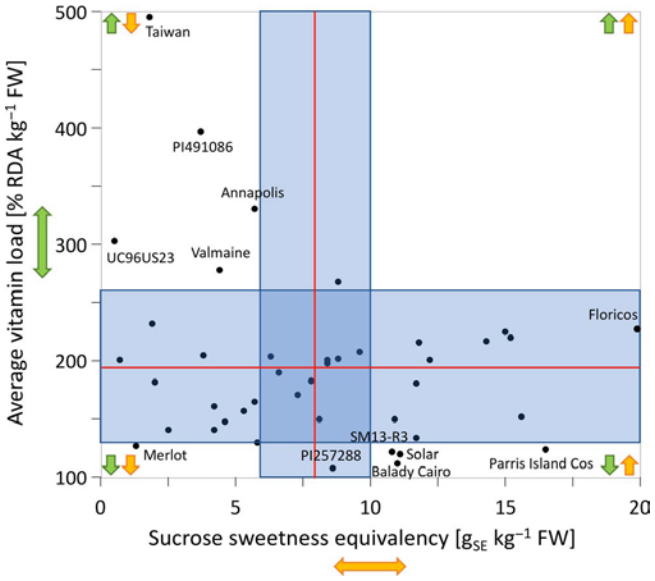

Figure 11. Differences in sucrose sweetness equivalency (SSE) and average vitamin load (AVL_{AC}) among 42 lettuce accessions harvested at baby leaf stage. Analysis of means (ANOM) was performed to identify accessions with quantities significantly different from the overall mean. Values that are outside of light blue areas are significantly different ($p < .05$) from the overall means that are indicated as horizontal and vertical red lines. SSE was calculated from the content of glucose, fructose, and sucrose per kg of FW and multiplied by relative sweetness of sugars. AVL_{AC} value indicates percent of Recommended Daily Allowance (RDA) of vitamin A and vitamin C that is obtained from 1 kg FW of lettuce. Detailed information regarding calculations of SSE and AVL_{AC} is provided in material and methods. Yellow- and green-colored arrows mark

quadrants with high and low relative sweetness and AVL_{AC}, respectively. For clarity of the figure, names are shown only for the accessions whose values are significantly different from the overall mean at both traits or have the lowest or the highest value of the trait.

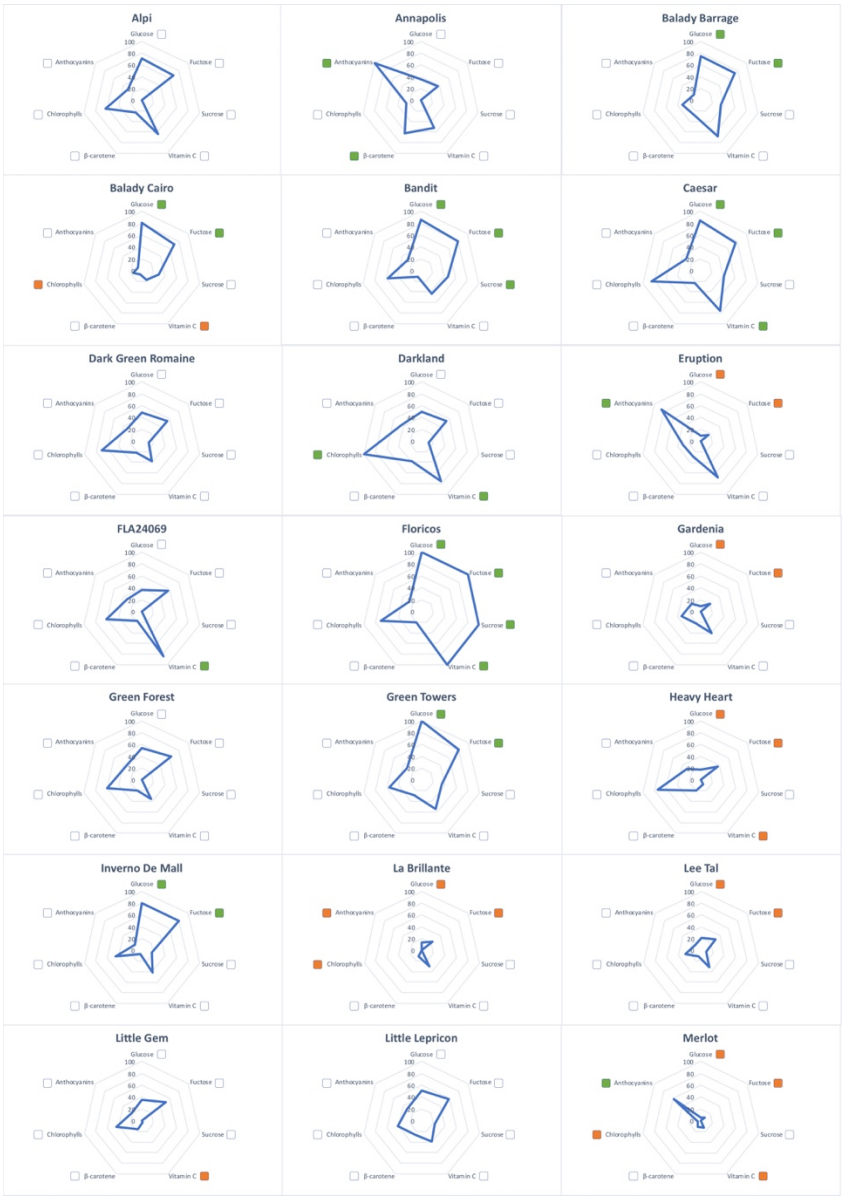

Figure 12. Content of vitamin C, ß-carotene, anthocyanins, chlorophylls, glucose, fructose, and sucrose in tested accession. Data for all accessions can be found at https://doi.org/10.1002/fsn3.1196

GENETIC VARIATION IN RESPONSE TO N, P, OR K DEPRIVATION IN BABY LEAF LETTUCE

Lettuce harvested at baby leaf stage is a popular component of mixed salads in ready to use packages. Little is known, however, about response of baby leaf lettuce to nitrogen (N), phosphorus (P), and potassium (K) fertilization. Eight lettuce accessions were subjected to five fertilization treatments to investigate genetic differences in reaction to N, P and K fertilization. The control treatment provided optimal levels of macronutrients for plant growth, while other treatments deprived plants of either N, P, or K. Potassium deprivation had no obvious effect on plant weight or composition apart from substantially decreased potassium content. Nitrogen and phosphorus deprivations, however, extensively decreased fresh weight and affected plant composition. Phosphorus and nitrogen deprivation considerably increased anthocyanin content in red colored accessions, but anthocyanin was decreased in dark green colored accessions, indicating differences in regulation of anthocyanin biosynthesis. Correlations between fresh weight, chlorophyll, anthocyanin, nitrogen, phosphorus, and potassium content were substantially affected by selection of datasets used for analyses; some relationships were revealed when analyzed separately on individual treatments, while others were more likely to be detected when analyzed on individual accessions (Figure 13). Absolute (Δ_{ABS}) and relative ($2\Delta_{REL}$) parameters described in this study are suitable for detecting over- and underperforming accessions. Δ_{ABS} identifies absolute Lb-fold (logarithm to the base of 2, binary logarithm) change in performance of an accession in a treatment as compared to its performance in control conditions. $2\Delta_{REL}$ parameter shows relative Lb-fold change for an accession as compared to overall mean of Δ_{ABS} values of all accessions tested in control and the treatment conditions.

Results of the study were published in Horticulturae.
https://doi.org/10.3390/horticulturae6010015

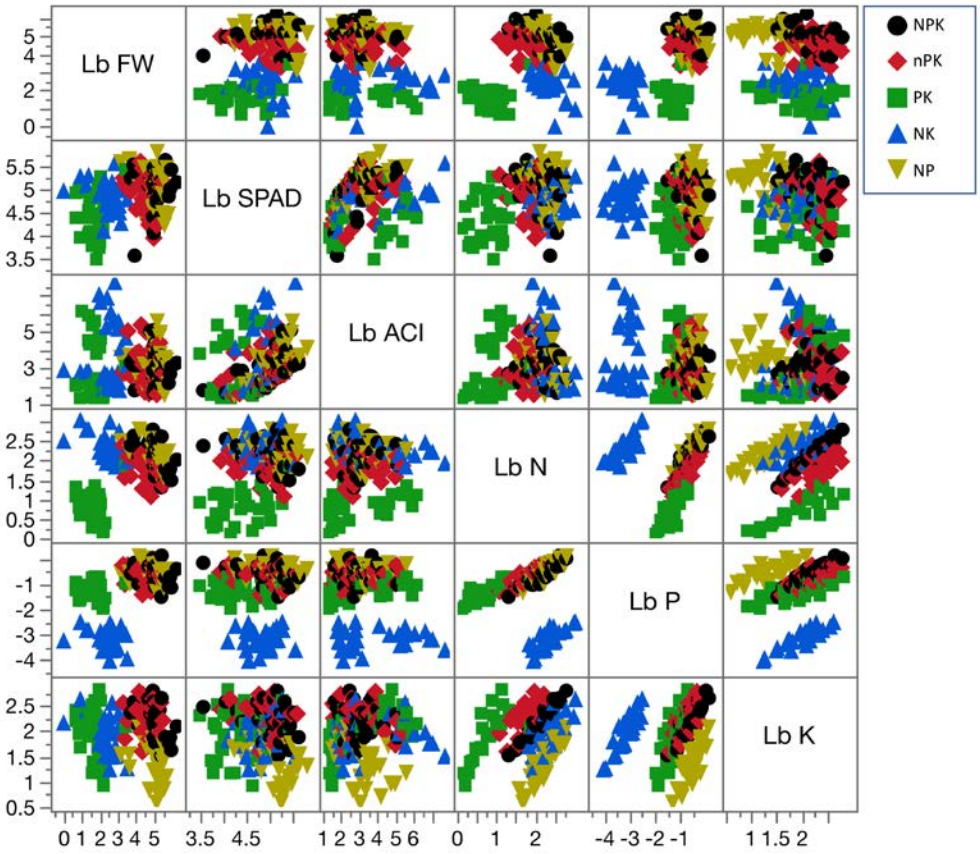

Figure 13. Scatterplot matrix of six traits evaluated in eight accessions in five treatments. Relationships between fresh weight (Lb FW) and content of chlorophyll (Lb SPAD), anthocyanin (Lb ACI), nitrogen (Lb N), phosphorus (Lb P), and potassium (Lb K) are shown in individual windows. Data from five treatments are indicated by icons with different shapes and colors. NPK treatment (control) supplied optimal concentrations of all three macronutrients, nPK treatment supplied only 1/3 of N compared to control, PK treatment did not supply N, NK treatment did not supply P, and NP treatment did not supply K. Note that regressions between traits calculated on data from different fertilizer treatments may have dissimilar slopes or intercepts but similar correlation coefficients.

GENOME-WIDE ASSOCIATION MAPPING (in collaboration with J. Sthapit, H. Peng, and R. Hayes)

Fresh-cut lettuce in packaged salad can have short shelf life and visible deterioration may start within a week after processing. Yield and developmental rate are important aspect for lettuce production. Genetic diversity and genome-wide association studies (GWAS) were performed on 493 accessions with the genotypic data of 4,615 high-quality single-nucleotide polymorphism markers. Population structure (Q), principal component (PC), and phylogenetic analyses displayed genetic relationships associated with lettuce types and geographic distribution (Figure 14). Data for shelf life, yield, developmental rate, and their stability indices were used for statistical analysis and GWAS was performed by general and mixed linear models (MLM). The genetic relationship among the individuals were incorporated into the models using kinship

matrix, PC, and Q. Broad-sense heritability (H^2) across environments was 0.43 for shelf life, 0.36 for yield, and 0.60 for developmental rate. There was negative correlation between yield and developmental rate. Significant marker-trait association (SMTA) was detected for shelf life on chromosome 4. The most significant quantitative trait locus (*qSL4*, *P*=2.23E-17) explained 24% of the total phenotypic variation (R^2). The major QTL for developmental rate was detected on chromosome 7 (*qDEV7*, *P*= 2.43E-16, R^2 = 17%), while additional QTLs with smaller effect were found in all chromosomes. No SMTA was detected for yield. The study identified lettuce accessions with extended and stable shelf life, stable yield, and desirable developmental rate.

Results of the study were published in Theoretical and Applied Genetics.
https://doi.org/10.1007/s00122-020-03568-2

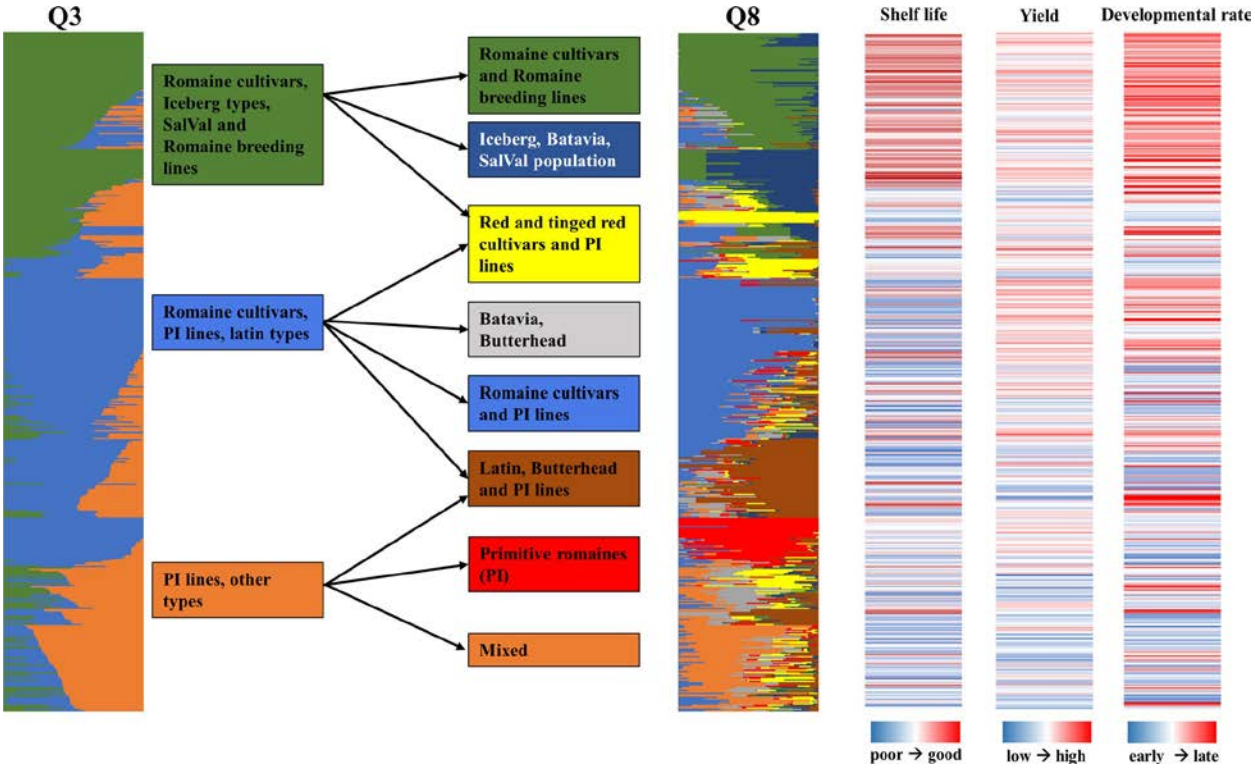

Figure 14. Population structure of 493 lettuce accessions and its relationship to shelf life and yield. Q3 and Q8 show the barplots of the population structure when the number of subpopulations were regarded as 3 and 8, respectively, in STRUCTURE analysis. Description in colored boxes corresponds to lettuce types and groups in Q3 and Q8. Columns shelf life, yield, and developmental rate show the distribution of the levels of shelf life, yield, and developmental rate across the accessions, respectively. Blue lines in Shelf life represent accessions with poor shelf life and red lines represent good shelf life. Blue lines in Yield represent accessions with low yield and red lines represent high yield. Blue lines in Developmental rate represent early-bolting accessions with fast developmental rate and red lines represent slow bolting accessions with slow developmental rate. White lines represent accessions with medium level of the trait

RECENT (2019 – 2020) PUBLICATIONS RELEVANT TO THIS PROJECT

Adhikari, N.D., I. Simko, and B. Mou. 2019. Phenomic and physiological analysis of salinity effects on lettuce. Sensors 19: 4814. http://dx.doi.org/10.3390/s19214814

Adhikari, N.D., R.L. Erikesen, A. Shi, and B. Mou. 2020. Proteomics analysis suggests a higher flux through major metabolic pathways in *Lactuca sativa* cv. Salinas than *Lactuca serriola* accession US96UC2. Proteomics (in press)

Eriksen, R.L., N.D. Adhikari, and B. Mou. 2020. Comparative Photosynthesis Physiology of Cultivated and Wild Lettuce Under Control and Low-Water Stress. Crop Science. https://doi.org/10.1002/csc2.20184

Inderbitzin, P., Christopoulou, M., Lavelle, D., Reyes-Chin-Wo, S., Michelmore, R. W., Subbarao, K. V., & Simko, I. (2019). The LsVe1L allele provides a molecular marker for resistance to Verticillium dahliae race 1 in lettuce. BMC Plant Biology, 19(1), 305.

Kandel, J., H. Peng, R. Hayes, B. Mou, and I. Simko. 2020. Genome-wide association mapping reveals loci for shelf life and developmental rate of lettuce. Theoretical and Applied Genetics 133: 1947-1966. https://doi.org/10.1007/s00122-020-03568-2

Macias-González, M., Truco, M. J., Bertier, L. D., Jenni, S., Simko, I., Hayes, R. J., & Michelmore, R. W. (2019). Genetic architecture of tipburn resistance in lettuce. Theoretical and Applied Genetics, 132(8), 2209-2222.

Mamo, B. E., Hayes, R. J., Truco, M. J., Puri, K. D., Michelmore, R. W., Subbarao, K. V., & Simko, I. (2019). The genetics of resistance to lettuce drop (*Sclerotinia* spp.) in lettuce in a recombinant inbred line population from Reine des Glaces × Eruption. Theoretical and Applied Genetics, 132(8), 2439-2460.

Park, S., A. Shi, and B. Mou. 2020. Genome-wide identification and expression analysis of the CBF/DREB1 gene family in lettuce. Scientific Reports (*in press*)

Sandoya, GV, Maisonneuve, B, Truco, MJ, Bull, CT, Simko, I, Trent, M, Hayes, RJ, and Michelmore, RW (2019) Genetic analyses of resistance to bacterial leaf spot in the heirloom lettuce cultivar Reine des Glaces. *Mol. Breeding* 39:160. https://doi.org/10.1007/s11032-019-1072-6

Simko, I. (2019). Genetic variation and relationship among content of vitamins, pigments, and sugars in baby leaf lettuce. Food Science & Nutrition, 7(10), 3317-3326.

Simko, I. (2020). Genetic variation in response to N, P, or K deprivation in baby leaf lettuce. Horticulturae, 6(1), 15.

Teng, Z., Luo, Y., Bornhorst, E. R., Zhou, B., Simko, I., & Trouth, F. (2019). Identification of romaine lettuce (*Lactuca sativa* var. longifolia) cultivars with reduced browning discoloration for fresh-cut processing. Postharvest Biology and Technology, 156, 110931.