Characterization and Performance of 16 New Inbred Lines of Lettuce

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Lettuce (*Lactuca sativa* L.) is the most popular, commercially produced, leafy vegetable in the world (FAOSTAT, 2013). Lettuce cultivars are divided into horticultural types based on the shape and size of the head; the shape, size, and texture of the leaves; and stem length (e.g., Simko, 2013). Three prevalent types of lettuce used in U.S. cultivation are crisphead (which includes the subtypes iceberg and Batavia), romaine, and leaf. Crisphead cultivars form a spherical head; the iceberg subtype has a large, dense head, whereas the Batavia subtype has a smaller and less dense head. Romaine cultivars form an elongated head that may or may not close on the top. Leaf-type cultivars are highly variable in terms of leaf color, shape, size, texture, leaf margin, and blistering. Leaf-type lettuces generally have leaves that are shorter than romaine and heads that do not close to cover younger leaves.

The USDA lettuce breeding program in Salinas, CA, was initiated in 1956 (Whitaker, 1974). The program has released a large number of highly popular cultivars, including Autumn Gold, Climax, Merit, Pacific, Salinas, Salinas 88, Sea Green, Tiber, Vanguard, Vanguard 75, Winterset, etc. (COMPOSITdb, 2013; Ryder, 1979a, 1979b, 1981, 1986, 1991; Ryder and Robinson, 1991; Ryder and Waycott, 1998; Thompson and Ryder, 1961). Most of these cultivars belong to the iceberg type, the major type of lettuce produced in the United States through 1980s (for the simplicity of description, the iceberg subtype will be called “type” in the remaining part of the article). The USDA breeding priorities, however, shifted in the 1990s as a result of the increased popularity of romaine and leaf lettuces and also as a result of a larger involvement of private seed companies in lettuce breeding. The USDA breeding program is focused mostly on development of improved iceberg, romaine, and leaf lettuce inbred lines through introgression of desirable traits from wild species, heirloom material, and unadapted germplasm. The new lines are then released into the public domain and used by private or public breeding programs directly for seed increase and sales or for development of new cultivars through additional rounds of selection or mating.

We describe here the development and performance of new iceberg (six), romaine (four), and leaf (six) breeding lines. These inbred lines were evaluated for performance and resistance in field, greenhouse, and laboratory experiments. Detailed descriptions of each line provided here will allow seed companies to assess the suitability of the material for commercial production or for further development in their breeding programs.

Material and Methods

Field experiments. Eight field experiments were conducted in Salinas, CA, in

2012 and 2013 using a randomized complete block design with three replications (Table 1). Seeds were seeded in two rows 35 cm apart on 102-cm wide beds (center-to-center), at least 7 m in length per genotype, and thinned to the final spacing of ≈ 30 cm between plants within a seedline, resulting in 30+ plants per replicate. Crop cultivation was done using standard cultural practices for the area except that fungicide treatment was not applied to allow for assessment of disease resistance. Experiments included the 16 new inbred lines, their parents (or more distant ancestors), and control cultivars. Because of limiting factors such as the availability of field space, amount of seeds, disease progress, or logistics, not all accessions were evaluated for all traits in all experiments. The following accessions of the three horticultural types were evaluated: iceberg breeding lines RH08-0111, SM13-I1, SM13-I2, SM13-I3, SM13-I4, SM13-I5, parents [Calmar, Glacier, Iceberg (Batavia subtype), La Brillante (Batavia subtype), Salinas, Salinas 88] and controls (Silverado, Tiber); romaine breeding lines RH08-0464, SM13-R1, SM13-R2, SM13-R3, parents [Balady Banha (stem type), Darkland, Parris Island Cos, PI 491224] and controls (Green Towers, Hearts Delight, Lobjoits, Triple Threat); and leaf breeding lines SM13-L1, SM13-L2, SM13-L3, SM13-L4, SM13-L5, SM13-L6, parents (Grand Rapids, Lolla Rossa) and controls (Big Red, Big Star, Red Fox, Two Star). At least 12 of the evaluated parents and controls are current cultivars in production, including cvs. Big Red, Big Star, Darkland, Green Towers, Hearts Delight, Lolla Rossa, Parris Island Cos, Red Fox, Salinas (marketed under other names), Silverado, Tiber, and Two Star.

Horticultural traits, yield, and tipburn. Horticultural traits, yield, and tipburn were evaluated at harvest maturity in the 12.5SP and 13.5SP experiments (Table 1). Ten plants per plot that were representative of the accession were harvested and weighed together. An average head weight was determined by dividing the total weight by 10. These 10 heads were cut in half vertically and the number of heads with tipburn symptoms was recorded (as percentage). Five of the heads were used to measure core length, head height (iceberg and romaine), head diameter at the top and bottom (romaine), and head diameter at the midsection (iceberg). The midsection head diameter was calculated from two measurements of radii that were perpendicular to each other. An experienced evaluator visually estimated the number of harvestable heads per plot. The heads were considered harvestable if their size and shape met the commercial standard for iceberg, romaine, or leaf lettuce. The percent of harvestable heads was calculated from the total number of plants per plot. The same evaluator assessed leaf ruffling and margin undulation and savoy, firmness, and closure of iceberg heads. Leaf ruffling and undulation and head savoy and closure were evaluated on a scale from 1 to 4, where higher values

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Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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Table 1. List of experiments, evaluated traits, and measurement units.

Expt.	Location	Planting date	Evaluations and units
12.5FB	Salinas—Field B	2 May 2012	Lettuce drop (%)
12.5FC	Salinas—Field C	5 May 2012	Verticillium wilt (%)
12.5SP	Salinas—Spence	8 May 2012	Head weight (g), head height (cm), head midsection diameter for iceberg (cm), head top diameter for romaine (cm), head bottom diameter for romaine (cm), core length (cm), head closure (1 to 4 scale), tipburn (%), downy mildew (0 to 5 scale), salad decay (AUDPS), whole head decay (cAUDPS)
12.6FA	Salinas—Field A	26 June 2012	Downy mildew (0 to 5 scale), leafminer stings at 30 d after planting (count), leafminer mines at 30 d after planting (count), leafminer stings at harvest maturity (count), salad decay (AUDPS)
12.6SP	Salinas—Spence	26 June 2012	Downy mildew (0 to 5 scale), leafminer stings at 30 d after planting (count), leafminer mines at 30 d after planting (count), leafminer stings at harvest maturity (count), salad decay (AUDPS)
12.8FB	Salinas—Field B	15 Aug. 2012	Downy mildew (0 to 5 scale), lettuce drop (%), leafminer stings at 30 d after planting (count), leafminer mines at 30 d after planting (count), leafminer stings at harvest maturity (count)
13.5SP	Salinas—Spence	13 May 2013	Head weight (g), head height (cm), head midsection diameter for iceberg (cm), head top diameter for romaine (cm), core length (cm), head firmness for iceberg (1 to 5 scale), head savoy (1 to 4 scale), number of harvestable heads (%), tipburn (%), downy mildew (0 to 5 scale), salad decay (AUDPS), whole head decay (cAUDPS), decay index (0 to 1 range)
13.8FB	Salinas—Field B	7 Aug. 2013	Downy mildew (0 to 5 scale), lettuce drop (%)
GH	Greenhouse	2013	Bacterial leaf spot (0 to 3 scale, transformed to 0 to 1 scale through integrated rating where 0 was given to the most resistant accession from all horticultural types and the value of 1 was given to the most susceptible one)
Laboratory	Laboratory	2013	Dieback (detection of resistant [R_1 , R_2] or susceptible [S_1] haplotypes with molecular markers)

AUDPS = area under the disease progress stairs approach; cAUDPS = complementary area under the disease (deterioration in this analysis) progress stairs approach.

indicated more pronounced characteristics. Head firmness was evaluated on a scale from 1 to 5 (1 = no heading, 2 = closed, puffy heads, 3 = heads that slightly yield to hand pressure, 4 = firm heads that do not yield to hand pressure, 5 = very firm, splitting heads).

Resistance to downy mildew. Six field experiments (12.5SP, 12.6FA, 12.6SP, 12.8FB, 13.5SP, and 13.8FB; Table 1) became naturally infected with *Bremia lactucae*. The resulting downy mildew severity was evaluated at harvest maturity using a 0 to 5 rating scale, where 0 = no lesions, 1 = sporadic lesions with less than one lesion per plant on average, 2 = up to two lesions per plant, 3 = three to 10 lesions per plant, 4 = more than 10 lesions per plant, and 5 = severe plant infection and merging of lesions (Simko et al., 2013). Field experiments did not include accessions with resistance based on functional *R*-gene(s), because our breeding program focuses on development of breeding lines with quantitative resistance to the disease (Simko, 2013; Simko et al., 2013).

Resistance to leafminer. Damage from leafminers (*Liriomyza* sp.) resulting from natural infestations was evaluated in experiments 12.6FA, 12.6SP, and 12.8FB (Table 1). The total number of leafminer mines was counted on two randomly selected plants from each plot at the baby leaf stage (≈ 30 d after planting). Leafminer stings were evaluated at the baby leaf stage and at harvest maturity. Stings within the 20-cm² area of the leaf with the highest sting density were counted for each plant per plot (Mou and Liu, 2003) and averaged.

Resistance to lettuce drop. Spring (12.5FB) and fall field experiments (12.8FB and 13.8FB) were conducted to evaluate resistance to lettuce drop caused by *Sclerotinia minor* (Table 1). Plots were infested with sclerotia of *S. minor* in the spring of 2012 and again in the spring of 2013 as described by Hayes et al. (2010). For the 12.5FB experiment, sclerotia were applied immediately before planting, whereas 12.8FB and 13.8FB

experiments assessed disease resistance in soil that was infested with sclerotia produced from the previous crop. The percentage of plants that died from or exhibited lettuce drop symptoms by market maturity of the iceberg cultivars was recorded for each experiment.

Resistance to Verticillium wilt. Iceberg, romaine, and leaf lettuces were evaluated for resistance to *Verticillium dahliae* (race 1) in a *V. dahliae*-infested field (12.5FC) according to methods of Hayes et al. (2007) (Table 1). At market maturity 10 plants from each plot were uprooted. Disease incidence was assessed by cutting taproots longitudinally and recording the number of plants exhibiting any amount of discoloration of root vascular tissues typical of Verticillium wilt.

Postharvest decay of fresh-cut lettuce. Lettuce grown in experiments 12.5SP, 12.6FA, 12.6SP, and 13.5SP was used in evaluation of decay after processing (Table 1). Three heads (or more if heads were small) from each plot were harvested from each of three replications and placed in a 4 °C cold storage room for 1 d before processing. All the lettuce heads within an accession were bulked and processed into cut lettuce using the method of Hayes and Liu (2008). Nine bags per accession were produced, triple-flushed with N₂ gas, sealed, and stored at 4 °C. Each of the 22.8 × 30.5-cm transparent bags contained 340 g of tissue cut into 2.5-cm² pieces. Decay was visually evaluated in weekly intervals on a 0 to 10 scale that corresponds to the estimated percentage of decayed tissue divided by 10 (Simko et al., 2012). The experiment was ended 4 weeks after processing and individual ratings were combined into an overall score by the area under the disease (deterioration in this analysis) progress stairs approach (AUDPS) (Simko et al., 2012; Simko and Piepho, 2012).

Postharvest decay of whole heads. Lettuce heads were harvested from 12.5SP and 13.5SP experiments for evaluation of whole-head shelf life. Romaine and leaf types

were harvested at peak maturity of 'Green Towers', whereas iceberg types were harvested at peak maturity of 'Salinas'. Equal numbers of heads from each replicate were pooled into a single carton, resulting in one carton per breeding line or cultivar. Cartons were cooled to 5 °C and shipped to Maryland through a refrigerated shipping truck provided by Taylor Farms (Salinas, CA). On arrival, the products were immediately transferred to the USDA-ARS Food Quality Laboratory in Beltsville, MD, and stored at 5 °C. Lettuce head quality was evaluated for each accession at Day 0 (arrival quality), Day 7, Day 14, and for romaine and leaf accessions also at Day 17. After this period all the material was considered unacceptable for sale. Product quality was evaluated by at least three trained evaluators. Overall quality was assessed following a modified procedure from Loaiza and Cantwell (1997) using a 9-point hedonic scale where 9 = like extremely; 7 = like moderately; 5 = neither like nor dislike; 3 = dislike moderately; and 1 = dislike extremely (Meilgaard et al., 1991). Scores from weekly evaluations were combined into an overall rating using the AUDPS approach (Simko and Piepho, 2012). The AUDPS scores were then converted into a complementary scale (cAUDPS) by subtracting actual AUDPS scores from the maximum possible AUDPS_{Max} scores (rating of 9 at all days) for the test: cAUDPS = AUDPS_{Max} – AUDPS. This conversion was performed to keep the scale consistent with scales for other traits (e.g., resistance to diseases, tipburn, decays of fresh-cut lettuce, decay index) where higher values are less desirable.

Decay index was evaluated on the final day when the majority of lettuce heads had some amount of decay. Lettuce heads for each accession were cut in half and scores (0 to 4) were assigned based on the decayed areas: 0 = no decay; 1 = decayed area less than 25% of lettuce head; 2 = decayed area ranging from 25% to 50%; 3 = decayed area ranging from 50% to 75%; 4 = decayed

Table 3. Performance of four new romaine breeding lines and eight commercial standards and parental accessions as measured by 19 quality and disease reaction traits.

Trait (unit)	Expt.	Mean ^s	Commercial standards and parental accessions								New romaine lines ^c					
			Balady Banha	Darkland	Green Towers	Hearts Delight	Lobjoits	Parris Island Cos	PI 491224	Triple Threat	RH08-0464	SM13-R1	SM13-R2	SM13-R3		
Head weight (g)	12.5SP	1413	1260 ^w	1437	1319	1407	1550 ^u	1378	1338	—	1513	1337	1549 ^u	1451		
	13.5SP	1012	886	1022	1002	1120	1113	902	872	1013	1172	961	972	1107		
Head height (cm)	12.5SP	29.6	34.8 ^H	27.7	28.9	29.8	29.0	28.7	29.6	—	31.7 ^H	29.3	27.8 ^L	28.5		
	13.5SP	30.9	35.9 ^H	29.9	28.4 ^L	31.2	29.7	30.2	28.4 ^L	—	34.2 ^H	31.5	29.1	30.0		
Head top diameter (cm)	12.5SP	19.5	16.1 ^L	23.4 ^H	18.3	16.1 ^L	21.4	20.1	18.4	—	17.5	20.4	22.6 ^H	19.7		
	13.5SP	29.1	31.2	27.6	30.9	31.2	31.1	27.0	29.6	29.3	32.0	26.9	24.8	28.0		
Head bottom diameter (cm)	12.5SP	11.4	9.1 ^L	11.4	12.0	10.6	13.7 ^H	11.1	12.3	—	10 ^L	10.3	12.1	12.5		
Core length (cm)	12.5SP	7.2	10.6 ^H	5.0 ^L	4.8 ^L	5.0 ^L	9.9 ^H	5.2 ^L	6.4	—	9.4 ^H	9.0 ^H	8.9 ^H	5.0 ^L		
	13.5SP	8.5	10.9 ^H	7.2	6.9 ^L	8.2	8.6	7.0	7.2	7.4	11.6 ^H	9.0	9.8	8.0		
Head closure (1 to 4)	12.5SP	2.6	2.3	1.8	2.7	3.0	3.3 ^H	2.3	2.5	—	3.3 ^H	2.3	2.5	2.7		
Head savoy (1 to 4)	13.5SP	2.8	3.0	3.0	3.0	2.3	3.0	2.7	2.7	3.0	2.0	3.0	3.3 ^H	2.0		
Harvestable heads (%)	13.5SP	42	18 ^L	62 ^H	55	39	20 ^L	68 ^H	26	49	17 ^L	50	50	45		
Tipburn (%)	12.5SP	70	97 ^H	53	50	77	93	67	67	—	100 ^H	50	73	43		
	13.5SP	39	80 ^H	13	10	53	57	33	27	50	83 ^H	7	53	3 ^L		
Downy mildew (0 to 5)	12.5SP	2.7	1.3 ^L	2.7	2.5	3.0	3.7 ^H	2.3	3.9 ^H	3.4	0.8 ^L	3.1	2.9	2.9		
	12.6FA	3.3	2.8	3.5	3.4	3.6	3.3	3.4	2.9	3.7	1.9	3.4	3.8	3.5		
	12.6SP	2.4	—	—	2.5	3.1 ^H	—	2.3	—	2.9	1.0 ^L	—	—	—		
	12.8FB	3.0	2.5	3.0	3.1	3.1	3.0	3.1	3.8 ^H	3.3	2.3 ^L	3.3	3.0	3.0		
	13.5SP	2.9	2.2	2.9	2.8	3.3	2.8	2.9	2.9	3.4	1.4	3.3	2.9	3.4		
	13.8FB	3.8	2.7 ^L	4.5 ^H	4.0	4.0	4.3	4.2	3.9	—	2.3 ^L	4.3	4.2	3.7		
Lettuce drop (%)	12.5FB	58	54	55	73	64	57	56	37	—	73	67	47	58		
	12.8FB	36	20	43	56 ^H	48	—	38	24	40	38	43	19	27		
	13.8FB	31	30	38	40	27	53 ^H	41	21	—	26	28	21	20		
Leafminer stings at Day 30 (count)	12.6FA	11.7	8.7	13.7	10.1	15.2	8.3	6.9 ^L	16.5 ^H	9.7	12.4	12.7	8.6	18.0 ^u		
	12.6SP	9.1	—	—	—	9.8	—	9.7	—	5.6 ^L	11.4	—	—	—		
	12.8FB	10.8	—	—	—	9.5	—	10.9	—	9.8	13.0	—	—	—		
Leafminer mines at Day 30 (count)	12.6FA	2.4	2.3	2.3	2.3	2.3	2.8	2.3	2.8	2.5	2.0	2.3	1.8 ^L	2.5		
	12.6SP	1.9	—	—	—	1.5	—	1.9	—	2.5	1.6	—	—	—		
	12.8FB	2.2	—	—	—	2.2	—	2.0	—	2.3	2.2	—	—	—		
Leafminer stings at harvest (count)	12.6FA	14.0	12.9	14.0	13.4	15.3	13.9	10.2 ^L	18.0 ^H	12.0	15.5	14.3	12.3	16.1 ^u		
	12.6SP	15.7	—	—	—	16.3	—	16.5	—	12.4	17.5	—	—	—		
	12.8FB	11.6	—	—	—	10.3	—	11.0	—	11.7	13.5	—	—	—		
Bacterial leaf spot (0 to 1 ^y)	GH	0.62	1.00 ^H	0.63	0.54	0.55	—	0.55	0.51	—	0.78 ^H	0.57	0.57	0.54		
Salad decay (AUDPS)	12.5SP	49	23 ^L	18 ^L	42	8 ^L	38	11 ^L	157 ^H	—	69 ^H	136 ^H	27	11 ^L		
	12.6FA	85	123	19 ^L	96	58	12 ^L	51	187 ^H	190 ^H	26 ^L	185 ^H	41 ^L	26 ^L		
	12.6SP	79	—	—	—	26 ^L	—	36 ^L	—	207 ^H	45	—	—	—		
	13.5SP	68	34	25 ^L	40	12 ^L	20 ^L	39	161 ^H	152 ^H	93	159 ^H	68	23 ^L		
Whole head decay (cAUDPS)	12.5SP	61	—	39 ^L	54	72 ^H	—	65	40 ^L	—	107 ^H	60	72 ^H	38.5 ^L		
	13.5SP	61	95 ^H	61	69 ^H	52	49 ^L	45 ^L	57	54	73 ^H	66	56	49 ^L		
Decay index (0 to 1)	13.5SP	0.38	0.92 ^H	0.28 ^L	0.39	0.24 ^L	0.25 ^L	0.27 ^L	0.43 ^H	0.41	0.51 ^H	0.27 ^L	0.29 ^L	0.28 ^L		
Dieback (haplotype)	Laboratory	—	—	—	—	—	—	—	—	—	—	—	—	—		

^aPedigrees of romaine breeding lines include cvs. Balady Banha, Darkland, and Parris Island Cos and accessions PI 491214 and PI 491224 (detailed information is in Table 6).^yOriginal scale of 0 to 3 was transformed to 0 to 1 through integrated rating, where 0 was given to the most resistant accession (from all horticultural types) and the value of 1 was given to the most susceptible one.^uMean of all breeding lines, controls, and parents tested in the experiment.^wH and L letters indicate values significantly ($P < 0.05$) higher or lower, respectively, than the overall mean.

AUDPS = area under the disease progress stairs approach; cAUDPS = complementary area under the disease (deterioration in this analysis) progress stairs approach.

Table 4. Performance of six new leaf lettuce breeding lines and six commercial standards and parental accessions as measured by 17 quality and disease reaction traits.

Trait (unit)	Experiment	Mean ^x	Commercial standards and parental accessions						New leaf lettuce lines ^z						
			Big Red	Big Star	Grand Rapids	Lolla Rossa	Red Fox	Two Star	SM13-L1	SM13-L2	SM13-L3	SM13-L4	SM13-L5	SM13-L6	
Head weight (g)	12.5SP	1129	—	—	1141	930 ^w	1146	1161	1249 ^H	1118	1103	1108	1164	1173	
	13.5SP	738	763	760	676	334 ^L	804	807	872 ^H	808	709	754	800	775	
Head height (cm)	12.5SP	20.2	—	—	17.9 ^L	17.8 ^L	24.2 ^H	20.0	26.2 ^H	16.8	17.3 ^L	16.6 ^L	22.0	23.2 ^H	
Core length (cm)	12.5SP	6.8	—	—	5.2	9.3 ^H	6.5	4.0 ^L	15.2 ^H	6.1	4.3 ^L	4.2 ^L	6.9	6.3	
	13.5SP	8.2	8.6	7.1	7.4	8.5	8.1	7.1	11.3 ^H	8.3	7.4	7.9	9.3	7.6	
Head closure (1 to 4)	12.5SP	2.3	—	—	2.8	2.0	1.3	2.3	1.7	2.5	3.3 ^H	3.5 ^H	1.8	1.8	
Head savor (1 to 4)	13.5SP	3.3	3.0	2.3 ^L	3.3	2.3 ^L	3.3	2.7	4.0	4.0	4.0	4.0	3.7	3.3	
Harvestable heads (%)	13.5SP	59	50	71	43	43	78	77	31	67	36	7 ^L	72	78	
Tipburn (%)	12.5SP	36	—	—	97 ^H	0 ^L	0 ^L	33	10	27	70 ^H	97 ^H	10	15	
	13.5SP	37	50	0 ^L	70	10	10	37	10	3 ^L	97 ^H	100 ^H	20	40	
Downy mildew (0 to 5)	12.5SP	1.1	3.3 ^H	—	0.8	0.6	1.4	2.8 ^H	0.9	0.2 ^L	0.2 ^L	0.4	0.2 ^L	1.8 ^H	
	12.6FA	2.0	2.5	—	2.2	1.7	—	3.7 ^H	1.8	1.0	1.9	1.8	0.8	2.7	
	12.6SP	1.4	2.4 ^H	—	0.9	0.5 ^L	1.5	2.8 ^H	1.0	—	1.0	—	0.8 ^L	2.0	
	12.8FB	2.0	—	—	2.0	1.8	2.0	3.3 ^H	1.3 ^L	1.5	1.5	1.5	1.3 ^L	2.8 ^H	
	13.5SP	1.6	2.6 ^H	2.1	1.3	0.9	2.4 ^H	2.6 ^H	1.0	1.2	0.5 ^L	1.2	1.2	1.8	
	13.8FB	2.4	3.3 ^H	3.4 ^H	2.3	1.2 ^L	2.6	3.6 ^H	2.2	1.7 ^L	1.3 ^L	2.0	1.6 ^L	3.3	
Lettuce drop (%)	12.5FB	54	—	—	39	54	69	64	50	31	50	57	71	53	
	12.8FB	20	—	—	24	19	29	38 ^H	4 ^L	11	10	15	7	39 ^H	
	13.8FB	26	22	47	17	25	37	30	13	17	19	19	18	42	
	12.6FA	4.3	8.7 ^H	—	2.5	3.2	—	6.6 ^H	2.4	2.5	3.2	4.5	3.3	5.6	
Leafminer stings at Day 30 (count)	12.6SP	4.5	—	—	4.4	—	—	8.1 ^H	2.2	—	2	—	4.5	5.7	
	12.8FB	4.7	—	—	3.6	3.4	—	—	2.2	—	4.6	—	—	9.5 ^H	
	12.6FA	2.0	2.8	—	2.8	1.8	—	2.0	2.3	2.0	1.8	2.3	1.8	0.8 ^L	
Leafminer stings at harvest (count)	12.6SP	1.7	—	—	2.0	—	—	2.9 ^H	2.1	—	1.3	—	1.4	0.6 ^L	
	12.8FB	1.6	—	—	1.7	1.8	—	—	2.3	—	1.2	—	—	1.0	
	12.6FA	4.7	10.8 ^H	—	4.9	2.8	—	5.9	2.5	3.0	2.6	2.3	3.9	7.9	
Bacterial leaf spot (0 to 1 ^y)	12.6SP	3.9	—	—	3.5	—	—	5.5 ^H	2.4 ^L	—	2.5 ^L	—	3.7	6.0 ^H	
	12.8FB	3.3	—	—	3.3	2.1	—	—	2.0	—	3.1	—	—	5.8 ^H	
	GH	0.45	—	—	0.56	0.54	0.40	—	0.23	0.51	0.39	0.55	0.31	0.54	
Salad decay (AUDPS)	12.5SP	51	—	—	51	87 ^H	79 ^H	10 ^L	44	44	73 ^H	33 ^L	57	35 ^L	
	12.6FA	87	156 ^H	—	49 ^L	123	—	41 ^L	103	58	56	93	125 ^H	63	
	12.6SP	69	125 ^H	—	46	71	78	97 ^H	58	—	46	—	47	51	
	13.5SP	94	90	43 ^L	129 ^H	65	114	64 ^L	114	89	122	117	89	87	
	12.5SP	100	—	—	131 ^H	—	88 ^L	119 ^H	79 ^L	102	110 ^H	138 ^H	86 ^L	51 ^L	
Whole head decay (cAUDPS)	13.5SP	72	50 ^L	47 ^L	94	79	34 ^L	68	70	71	105 ^H	102 ^H	110 ^H	37 ^L	
Decay index (0 to 1)	13.5SP	0.48	0.49	0.26 ^L	0.71 ^H	0.60 ^H	0.31 ^L	0.58	0.36 ^L	0.35 ^L	0.53	0.53	0.69 ^H	0.36	
Dieback (haplotype)	Laboratory	—	—	—	—	S ₁	—	R ₂	S ₁	R ₂	R ₂	R ₂	R ₂	R ₂	

^yPedigrees of leaf lettuce breeding lines include cvs. Grand Rapids, Iceberg, and Lolla Rossa and accession PI 491224 (detailed information is in Table 6).^xOriginal scale of 0 to 3 was transformed to 0 to 1 through integrated rating, where 0 was given to the most resistant accession (from all horticultural types) and the value of 1 was given to the most susceptible one.^wMean of all breeding lines, controls, and parents tested in the experiment.^H and ^L letters indicate values significantly ($P < 0.05$) higher or lower, respectively, than the overall mean.

AUDPS = area under the disease progress stairs approach; cAUDPS = complementary area under the disease (deterioration in this analysis) progress stairs approach.

area greater than 75%. Decay indices were calculated according to the formula: $DI = (0 \times N_0 + 1 \times N_1 + 2 \times N_2 + 3 \times N_3 + 4 \times N_4) \div (4 \times N_T)$, where N_0 to N_4 are the quantities of lettuce heads assigned each respective score and N_T is the total number of lettuce heads examined for the accession.

Resistance to bacterial leaf spot. Evaluations of seedlings for resistance to bacterial leaf spot caused by *Xanthomonas campestris* pv. *vitians* (*Xcv*) were conducted using a greenhouse assay that provides data that are closely correlated with results in field experiments (Bull et al., 2007; Hayes et al., 2008, 2014). Seeds were planted in plug trays (22 cm wide and 65 cm long) with 31 rows of 11 cells that are 20 mm $\times$ 20 mm $\times$ 60 mm representing a final plant density of $\approx$ 2380 plants/m². Plots consisted of a single row of nine plants with the perimeter of each plug tray planted with the susceptible cv. Vista Verde. In the first of three experiments, three *Xcv* strains (BS339, BS340, and BS347) were used as inoculum; however, after determining that strain BS347 was more virulent than the other strains (data not shown), only BS347 was used in the second and third experiments. All of these strains were isolated from lettuce plants in California (Barak and Gilbertson, 2003; Bull and Koike, 2005). *Xcv* suspensions of $\approx 1 \times 10^8$ colony-forming units/mL were prepared according to Bull et al. (2007). The suspensions of each isolate were then mixed in equal proportions or the single isolate was used as prepared. Seedlings were sprayed to runoff with the *Xcv* suspension and incubated under constant leaf wetness for 7 d. Plants were rated for disease severity using a 0 to 3 rating scale (0 = no disease; 1 = few lesions less than 3 mm; 2 = lesions greater than 3 mm; 3 = coalesced lesions). Disease severity values were averaged across all plants within each treatment replicate. Data from multiple experiments were combined into an overall integrated rating using rank-aggregation approach (Simko et al., 2012; Simko and Piepho, 2011). Integrated ratings were then linearly adjusted to a 0 to 1 scale, where the value of 0 was given to the most resistant accession from all horticultural types and the value of 1 was given to the most susceptible one.

Resistance to lettuce dieback. Lettuce dieback is caused by two soilborne viruses from the family *Tombusviridae*: Tomato bushy stunt virus and Lettuce necrotic stunt virus. Resistance to the disease is conferred by *Tvr1*, a single dominant gene (Grube et al., 2005; Simko et al., 2009). Resistance to lettuce dieback was evaluated with molecular makers closely linked to the *Tvr1* gene (Simko et al., 2009). Presence of the resistance alleles in lettuce accessions was tested by high-resolution DNA melting assay according to Simko (2013).

Statistical analyses. Data from each of the three lettuce types were analyzed separately with the exception of the Verticillium wilt experiment that combined all horticultural types. Normality of data distribution was tested with Shapiro-Wilk W test implemented

in JMP 9.0.0 software (SAS Institute, Cary, NC). Traits with normally distributed data were analyzed using analysis of means (ANOM). Traits with non-normally distributed data or ratings performed on ordinal scales were analyzed with ANOM for ranks (JMP 9.0.0 software). The *P* value to identify accessions significantly different from the overall mean was set at 0.05. Integrated ratings from ranked data were calculated with Winsteps 3.65.0 (Winsteps.com, Beaverton, OR) according to Simko and Linacre (2010).

Results and Discussion

Five iceberg lines (SM13-I1 to SM13-I5) formed heads of acceptable quality with weight, height, diameter, core lengths, and firmness similar to the overall mean and control cultivars that represent industry standard (Table 2). The percentage of harvestable heads for these five lines was also acceptable with SM13-I4 having a significantly higher percentage of harvestable heads per area than the overall mean. Tipburn incidence was generally higher in the breeding lines compared with the tipburn-resistant control cvs. Silverado and Tiber; however, SM13-I5 showed no tipburn in 12.5SP. Resistance of SM13-I1 through SM13-I5 to downy mildew was usually better than in the established iceberg cvs. Glacier, Salinas, Salinas 88, Silverado, and Tiber, the favorable alleles probably originating from the highly resistant cv. Iceberg. Resistance to other diseases and pests (lettuce drop, bacterial leaf spot, and leafminer) was similar to the iceberg control cultivars. Decay of fresh-cut lettuce and whole head decay was similar to cv. Salinas with the exception of SM13-I2 and SM13-I3, in which more rapid (but still acceptable) decay was observed on whole heads. RH08-0111 formed smaller and less firm heads than established cultivars or other breeding lines; thus, the percentage of harvestable heads was

significantly smaller for this line. However, RH08-0111 had significantly higher resistance to downy mildew and bacterial leaf spot compared with the overall averages. Alleles for the higher resistance to these diseases in this breeding line likely originate from Batavia cv. La Brillante. All iceberg breeding lines possessed the *R₁*-haplotype alleles indicating a complete resistance to lettuce dieback.

Three romaine breeding lines (SM13-R1 to SM13-R3) had heads of average size and yield (Table 3). SM13-R3 had significantly lower tipburn incidence than the overall mean in one experiment (13.5SP). The remaining breeding line (RH08-0464) formed elongated heads with a long core, low percentage of harvestable heads, and high tipburn incidence. Alleles for these traits were likely inherited from cv. Balady Banha that is not used for commercial production in the United States. However, this breeding line had consistently less downy mildew than other tested romaine accessions (values were significantly different in four of six experiments). This breeding line had high susceptibility to bacterial leaf spot and more rapid decay of whole heads than was the mean of romaine accessions. These traits were likely inherited from cv. Balady Banha. RH08-0464 inherited alleles for susceptibility to dieback from cv. Darkland. The other three breeding lines had average resistance to downy mildew and bacterial leaf spot. SM13-R3 had a high number of leafminer stings but good shelf life as whole heads and fresh-cut lettuce. SM13-R1 had poor shelf life when processed for salad but acceptable shelf life of whole heads. SM13-R2 showed good resistance to leafminers and acceptable postharvest quality for whole heads and fresh-cut lettuce. All three breeding lines had the *R₂*-haplotype alleles inherited from accessions PI 491224 or PI 491214, indicating their complete resistance to dieback.

Table 5. Reactions of new breeding lines, commercial standards and parental accessions to Verticillium wilt race 1.

Accession	Horticultural type	Verticillium wilt incidence (%)
Commercial standards and parental accessions		
Balady Banha	Stem	27
Darkland	Romaine	57
Hearts Delight	Romaine	50
La Brillante	Batavia	0 ^L
Lobjoits	Romaine	47
Lolla Rossa	Leaf	0 ^L
Parris Island Cos	Romaine	54
PI 491224	Romaine	60
Red Fox	Leaf	69
Salinas	Iceberg	83 ^H
Tiber	Iceberg	67
New breeding lines ^a		
RH08-0111	Iceberg	0 ^L
RH08-0464	Romaine	87 ^H
S13-R1	Romaine	57
S13-R2	Romaine	73
S13-R3	Romaine	43
S13-L6	Leaf	0 ^L
Mean		46

^aDetailed pedigree information is in Table 6.

^HH and ^LL letters indicate values significantly (*P* < 0.05) higher or lower, respectively, than the overall mean.

Table 6. Pedigree, description, and potential use of six iceberg, four romaine, and six leaf lettuce new breeding lines.

Line	Pedigree	Description	Advantage ^z	Disadvantage ^z	Potential use
RH08-0111	F ₈ : Salinas 88 × La Brillante	Green, slightly ruffled leaves with undulation	Improved resistance to downy mildew, bacterial leaf spot, and Verticillium wilt race 1	Small, soft heads that do not meet the modern iceberg standard	Salad blend, breeding programs
SM13-I1	F ₆ : [(Salinas × Iceberg) × (Iceberg × Calmar)] × Glacier	Green, slightly ruffled leaves with undulation	Improved resistance to downy mildew	Susceptible to tipburn	Whole head, salad blend
SM13-I2	F ₆ : [(Salinas × Iceberg) × (Iceberg × Calmar)] × Glacier	Green, slightly ruffled leaves with undulation	Improved resistance to downy mildew	Relatively rapid decay of whole heads	Whole head, salad blend
SM13-I3	F ₆ : [(Salinas × Iceberg) × (Iceberg × Calmar)] × Glacier	Green, slightly ruffled leaves with undulation	Improved resistance to downy mildew	Relatively rapid decay of whole heads	Whole head, salad blend
SM13-I4	F ₆ : [(Salinas × Iceberg) × (Iceberg × Calmar)] × Glacier	Green, slightly ruffled leaves with undulation	High percentage of harvestable heads, improved resistance to downy mildew	Susceptible to tipburn	Whole heads, salad blend
SM13-I5	F ₈ : (Iceberg × Salinas) × (Iceberg × Salinas)	Green, scalloped leaves with undulation	Improved resistance to downy mildew	Whole heads, salad blend	Whole heads, salad blend
RH08-0464	F ₆ : Balady Banha × Darkland	Dark green smooth leaves with slight undulation	Improved resistance to downy mildew	Long core; small percentage of harvestable heads; susceptible to tipburn, bacterial leaf spot, Verticillium wilt and dieback, rapid decay of whole heads	Breeding programs
SM13-R1	F ₇ : PI 491224 × Parris Island Cos	Green smooth leaves with undulation	Low tipburn incidence, resistant to dieback	Rapid decay after processing for salad	Whole head
SM13-R2	F ₈ : PI 491224 × Parris Island Cos	Green smooth leaves with undulation	Slightly more resistant to leafminer, slow decays of whole heads, resistant to dieback		Whole head, spring mix, salad blend
SM13-R3	F ₈ : Darkland × PI 491214	Green smooth leaves with undulation	Low tipburn incidence, slow decay of whole heads, slow decay after processing for salad, resistance to dieback	Susceptible to leafminer	Whole head, spring mix, salad blend
SM13-L1	F ₇ : Grand Rapids × Iceberg	Light green ruffled leaves with undulation	Relatively large head, improved resistance to downy mildew, slow decay of whole heads	Long core (early bolting), susceptible to dieback	Whole head, spring mix, salad blend
SM13-L2	F ₇ : Grand Rapids × Iceberg	Light green ruffled leaves with undulation	Low tipburn incidence, improved resistance to downy mildew, slow decay of whole heads, resistant to dieback		Whole head, spring mix, salad blend
SM13-L3	F ₇ : Grand Rapids × Iceberg	Light green ruffled leaves with undulation	Improved resistance to downy mildew, resistant to dieback	Susceptible to tipburn, relatively rapid decay of whole heads	Spring mix, salad blend
SM13-L4	F ₇ : Grand Rapids × Iceberg	Light green ruffled leaves with undulation	Improved resistance to downy mildew, resistant to dieback	Susceptible to tipburn, low percentage of harvestable heads, relatively rapid decay of whole heads	Spring mix, salad blend
SM13-L5	F ₇ : Grand Rapids × Iceberg	Light green ruffled leaves with undulation	Improved resistance to downy mildew, resistant to dieback	Relatively rapid decay of whole heads	Spring mix, salad blend
SM13-L6	F ₇ : Lolla Rossa × PI 491224	Dark green/reddish smooth leaves with undulation	Slow decay of whole heads, resistant to dieback and Verticillium wilt race 1	Less resistant to downy mildew than other five new breeding lines of leaf lettuce	Whole head, spring mix, salad blend

^zAdvantages and disadvantages as compared with commercial standard cultivars for the respective horticultural type.

Breeding lines of leaf lettuce produced heads of acceptable size (Table 4). SM13-L1 had above average head weight accompanied by longer cores, probably as a result of earlier maturity and the initiation of bolting. SM13-L2 had lower than average incidence of tipburn (in 13.5SP), whereas SM13-L4 and SM13-L5 had higher than average tipburn incidence. The low percentage of harvestable heads in SM13-L4 was caused by high tipburn incidence. Five leaf-type breeding lines (SM13-L1 to SM13-L5) had average resistance to lettuce drop, bacterial leaf spot, and leafminers. Postharvest decay after processing for salad was in the acceptable range, although it was significantly higher than the

overall mean in two cases. Two of the lines (SM13-L1 and SM13-L2) showed less rapid decay of whole heads, whereas two lines (SM12-L3 and SM13-L4) had decay more rapid than the average. Results for SM13-L5 were inconsistent. All five lines had average to above average resistance to downy mildew. The resistance alleles in these lines originate from both the Batavia cv. Iceberg and the leaf cv. Grand Rapids (Simko et al., 2013). The remaining breeding line (SM13-L6) was relatively more susceptible to downy mildew and lettuce drop. Interestingly, this line had often a relatively high number of leafminer stings but very few leafminer mines. This may indicate presence of a limiting factor

that prevents leafminer laying eggs or development of larvae. SM13-L6 had acceptable postharvest quality of whole heads and also after processing for salad. All leaf-type breeding lines, with the exception of SM13-L1, had the R_2 -haplotype alleles inherited from cv. Grand Rapids and PI 491224. These alleles are associated with the complete resistance to dieback.

Testing a selected subset of accessions in a field infected with *V. dahliae* race 1 (12.5FC) identified two resistant breeding lines (Table 5). The iceberg breeding line RH08-0111 inherited *Vr1* resistance gene from cv. La Brillante (Hayes et al., 2011), whereas the leaf-type breeding line SM13-L6 inherited

Fig. 1. Phenotypes of 16 released breeding lines from three horticultural types. The top four rows show plants growing in Expt. 12.5SP. The bottom row shows selected lines from the same experiment before shipping to Maryland for evaluations of whole head decay.

resistance from cv. Lolla Rossa. It is unknown if the resistance in cv. Lolla Rossa is also conferred by *Vr1*. Breeding lines not tested for resistance to Verticillium wilt will likely have moderate to high susceptibility to the disease, because all accessions in their pedigree are susceptible to the disease (Hayes et al., 2007; Hayes, unpublished results and present data).

The 16 new breeding lines can be used for different markets or breeding programs based on their performance. The relative performance of inbred lines (as compared with accessions of the same type), their advantages, disadvantages, and recommended use are summarized in Table 6, whereas phenotypes are shown in Figure 1. Five of six iceberg lines (SM13-I1, SM13-I2, SM13-I3, SM13-I4, SM13-I5) are suitable for salad blend and whole head markets. Although SM13-I2 and SM13-I3 exhibited more rapid decay of whole heads than the overall average, their rates of decay are expected to be acceptable for commercial production. RH08-0111 is not acceptable for commercial production of whole heads because of its small, soft heads that do not meet the standard for modern iceberg cultivars. However, the line can be used in iceberg breeding programs as a donor of alleles for resistance to downy mildew, bacterial leaf spot, and Verticillium wilt race 1 (Hayes et al., 2011). Two romaine breeding lines (SM13-R2 and SM13-R3) are suitable for salad blend, spring mix, and whole head production. Romaine breeding line SM13-R1 cannot be used for fresh-cut products, because it decays rapidly after processing, but it is suitable for the whole head market. An important trait of these three breeding lines is their resistance to dieback, because most of the currently grown romaine cultivars are susceptible to this disease (Simko et al., 2009). Romaine breeding line RH08-0464 has improved field resistance to downy mildew inherited from cv. Balady Banha. RH08-0464 can be used in breeding programs but is not suitable for commercial production. All six leaf lettuce breeding lines are acceptable for commercial production of salad blend and spring mix. SM13-L1, SM13-L2, and SM13-L6 could also be used for whole head production. SM13-L1 to SM13-L5 demonstrated very high field resistance to downy mildew with resistance alleles inherited from cvs. Iceberg and Grand Rapids. Resistance to downy mildew in these legacy cultivars appears to be durable as it was reported over 50 years ago in cv. Grand Rapids (Verhoeff, 1960) and over 90 years ago in cv. Iceberg (Milbrath, 1923). However, the lack of recent use of these cultivars in a high-intensity production environment has reduced their exposure to sustained selection for virulent races of the pathogen. Five of the leaf lettuce breeding lines (SM13-L1 being the exception) have alleles for dieback resistance, a disease that may substantially limit lettuce production in California and Arizona (Simko et al., 2009). SM13-L1 is earlier

bolting than other breeding lines. SM13-L6 has resistance to Verticillium wilt race 1.

On average $\approx 99.2\%$ of loci that were heterozygous in F_1 are expected to be homozygous in F_8 . The value decreases to 98.4% in F_7 and 96.9% in F_6 generations. Therefore, there is a possibility that some of the released breeding lines may be still genetically segregating for some of the traits. No segregation is expected, however, for resistance to dieback, because only lines homozygous at *Tvr1* were selected for seed production through marker-assisted selection.

Limited samples of seeds are available for distribution to all interested parties for research or commercial purposes. It is requested that appropriate recognition be made if the breeding lines contribute to research or the development of new germplasm, breeding lines, or cultivars. Written requests should be sent to the first or the second author.

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