Inheritance of Decay of Fresh-cut Lettuce in a Recombinant Inbred Line Population from ‘Salinas 88’ × ‘La Brillante’

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ABSTRACT. Fresh-cut lettuce (*Lactuca sativa*) packaged as salad mixes are increasingly popular to consumers but are highly perishable. Cultivars bred with extended shelf life could increase overall production efficiency by reducing the frequency of product replacement in the marketplace. Understanding the inheritance of shelf life is needed to develop efficient breeding strategies for this trait. A population of 95 recombinant inbred lines (RILs) from slow-decaying ‘Salinas 88’ × rapidly decaying ‘La Brillante’ was grown in four field experiments. Cut lettuce was evaluated for decay in modified atmosphere (MA) packages flushed with N₂ or air (control). Correlations between field experiments ranged from 0.47 to 0.84 ($P < 0.01$). Three quantitative trait loci (QTL) for decay of cut lettuce were detected on linkage groups (LGs) 1, 4, and 9 with ‘Salinas 88’ alleles associated with slower decay. The QTL on LG 4 (*qSL4*) was a major determinant of decay explaining 40% to 74% of the total phenotypic variance of the trait. The greatest effect of this QTL was observed between 29 and 50 days after harvest. QTL × environment interactions contributed less than 14% to the total variation. RILs with the ‘Salinas 88’ allele of *qSL4* had slower decay when packaged in air compared with N₂, whereas no difference between air and N₂ packaging was detected with the ‘La Brillante’ allele. A subset of RILs with either the ‘Salinas 88’ or ‘La Brillante’ allele of *qSL4* was grown in two field experiments and evaluated for decay of whole heads. Genetic variation among RILs for whole-head decay was found but could not be attributed to *qSL4*. Decay of cut lettuce in ‘Salinas 88’ × ‘La Brillante’ is a highly heritable trait conditioned by a few QTL and phenotypic selection is likely to be effective. However, shelf life evaluations are time-consuming, destructive, and require large amounts of field-grown lettuce. Therefore, *qSL4* is a good QTL to develop molecular markers for marker-assisted selection. The mechanism of decay controlled by *qSL4* is unknown but appears to be specific to cut lettuce and may have allele specific interactions with packaging atmospheric compositions.

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Lettuce is a perishable leafy vegetable crop marketed as whole heads or as cut leaves packaged as ready-to-eat salads. Postharvest quality and shelf life are important characteristics for consumers and lettuce shipping companies. Cultivars bred with extended shelf life that reduce postharvest losses could increase production efficiency by reducing the frequency of product replacement in the marketplace. This is often difficult to achieve because there are few reports on the genetics of postharvest quality, and postharvest quality assessments are often difficult to conduct on the large populations used in plant breeding. Furthermore, new lettuce cultivars with improved quality, yield, or pest resistance are continually being bred to improve the sustainability and marketability of commercially

produced crops (Lebeda et al., 2007). Regardless of the trait or traits improved on by breeding, the resulting new cultivar must always possess shelf life and shipping qualities that are at least equivalent to currently used cultivars to be adopted by growers, packers, and processors. The challenge of breeding for shelf life often deters breeders from using exotic or unadapted germplasm, because this material often has unpredictable postharvest quality. As a result, breeders often choose elite, closely related germplasm with known acceptable shelf life to develop their breeding populations. This contributes to a narrow genetic base for the crop, and pedigree and molecular marker analysis indicates that modern lettuce cultivars are closely related (Mikel, 2007, 2013; Simko, 2009; Simko and Hu, 2008). Establishing the genetics of postharvest traits may facilitate the use of exotic germplasm in breeding.

The wounding involved in processing lettuce into packaged cut lettuce shortens shelf life by inducing increased respiration and phenolic metabolism that results in leaf edge browning or pinking (Bolin and Huxsoll, 1991; Kim et al., 2005; Smyth et al., 1998). Discoloration occurring on whole heads that are induced by CO₂ and ethylene is also related to increased phenolic metabolism (Ke and Saltveit, 1988, 1989). In commercially prepared packages of cut lettuce, low O₂, high CO₂ MA packaging in conjunction with low temperatures are used to retard discoloration and extend shelf life of cut lettuce (Kim et al., 2005; Smyth et al., 1998). Modified atmospheres can be achieved passively (passive MA) by sealing packages of cut lettuce in air and allowing the atmosphere conditions to change as a result of respiration and package film gas permeation or achieved actively (active MA) by flushing the package with N₂ before sealing the bags closed (Kim et al., 2005).

Lettuce has genetic variation for decay or shelf life (tissue darkening, water-logging, and complete breakdown) under MA conditions. Zhang et al. (2007) reported environmentally specific QTL for shelf life on LGs 1, 2, 5, 6, and 8 in a RIL population derived from an interspecific cross. Rapid decay was discovered in a few accessions that are important sources of genes for disease resistance, such as the verticillium wilt (caused by *Verticillium dahliae*)-resistant cultivar La Brillante, Batavia, and Latin-type cultivars resistant to bacterial leaf spot (caused by *Xanthomonas campestris* pv. *vitians*), and a number of *Tombusvirus*-resistant romaine accessions (Bull et al., 2007; Hayes et al., 2011a, 2013; Hayes and Liu, 2008; Simko et al., 2012). The cultivar La Brillante has demonstrated twice the rate of decay as the commercially used iceberg cultivar Salinas 88 (Hayes and Liu, 2008). The rapid decay phenotype was repeatable in controlled atmosphere (CA) chambers maintained with O₂ treatments of 0.2% to 5% (Hayes and Liu, 2008). If cultivars with rapid decay under MA conditions are used as parents in a breeding program, selection for slow decay will be required to develop new cultivars with acceptable shelf life. It is not known if the rapid decay phenotype of cut lettuce observed in 'La Brillante' and other cultivars is also expressed in whole heads.

Efficient phenotypic or marker-assisted selections strategies to breed for slow decay can be designed based on an understanding of trait inheritance. In a diploid ($2n = 2x = 18$) autogamous vegetable crop like lettuce, genetic analysis of traits with continuous distributions are frequently conducted using a RIL population of randomly selected near homozygous lines derived from a cross between inbred parents with divergent phenotypes. Phenotypic data for each RIL collected across several environments can be used to calculate the

amount of variation attributable to genotype (G), genotype $\times$ environment (GE), and environmental (E) effects. The trait heritability $\{H^2$ [the ratio of G to total phenotypic (G + GE + E) variation]\} can then be calculated and used to determine the effectiveness of phenotypic selection schemes (Holland et al., 2003). Genetic linkage maps of molecular markers constructed for the RIL population can be used in conjunction with phenotypic data to determine the position of QTL and the magnitude of QTL effects (Collard et al., 2005). Knowledge of the QTL is a starting point to develop molecular markers for marker-assisted selection or map-based cloning of the genes underlying QTL. In this article, we report on the H², G, GE, and QTLs for decay of cut lettuce in the cross 'Salinas 88' $\times$ 'La Brillante'. We further describe a large effect QTL that is specific to cut lettuce decay and interacts with MA packaging method.

Materials and Methods

POPULATION AND GENETIC MAP DEVELOPMENT. A population of 95 RILs from the cross 'Salinas 88' $\times$ 'La Brillante' (S88 $\times$ LB) was inbred to the F₇ generation using single-seed descent (Hayes et al., 2011b). All field experiments were planted using F₈ seed lots of each RIL produced from massing seed from $\approx$ 20 field-grown F₇ plants.

Molecular marker genotyping was conducted using the Illumina Golden Gate[®] single nucleotide polymorphism (SNP) assay on a single F₆ plant randomly sampled from each RIL. One hundred four SNP markers were polymorphic and previously reported in the S88 $\times$ LB RIL population (Hayes et al., 2011b). Ninety recombinant inbred lines were genotyped using the amplified fragment length polymorphism (AFLP[®]) procedure (Vos et al., 1995; Vuylsteke et al., 2007) using 13 *EcoRI*–*MseI* primer combinations (Supplemental Table 1) generating 220 polymorphic markers. Gel images were electronically scanned and markers scored using proprietary technology developed at Keygene N.V. (Wageningen, The Netherlands). When possible, relative band intensities of individual AFLP markers were used to attribute homozygous and heterozygous scores to individual AFLP markers. A molecular linkage map with SNP and AFLP markers was constructed using the software MapDisto 1.7.5 (Lorieu, 2012) and a logarithm of odds (LOD) threshold of three. The S88 $\times$ LB linkage map was aligned to an ultra-high-density, transcript-based, genetic map derived from an interspecific cross between *L. sativa* cultivar Salinas and the *L. serriola* accession UC96US23 (Sal $\times$ UC96) (Truco et al., 2013) using the software MapChart Version 2.2 (Voorrips, 2002). The numbering and orientation of the S88 $\times$ LB LGs were kept the same as in the reference map. When multiple S88 $\times$ LB LGs were associated with a single Sal $\times$ UC96 LG, letters after the LG number were used to identify separate groups (e.g., LG 2a and LG 2b). The relative length of the S88 $\times$ LB linkage map was calculated by dividing the length of each LG by the length of the corresponding LG from the reference map. Segregation distortion was detected using χ^2 tests for each marker. Markers with deviations from 1:1 segregation at $P < 0.05$ were considered to have distorted segregation. Groups of markers with segregation distortion were defined according to Truco et al. (2007) as regions with three or more markers with distorted segregation and with no more than three markers within the group not having distorted segregation.

Two contrasting subpopulations of RILs were created to test the effect of a major QTL [*qSL4* (described in the “Results” section)] on cut lettuce decay when processed under N₂ and air packaging conditions and on whole-head decay. These were created by randomly selecting 14 RILs with the ‘Salinas 88’ allele and 18 RILs with ‘La Brillante’ allele of an AFLP marker located 1.1 cM from the QTL. The subpopulations were designated S-SL4 and L-SL4 for possessing the ‘Salinas 88’ and ‘La Brillante’ QTL alleles, respectively.

FIELD EXPERIMENTS AND EVALUATION METHODS. Up to 90 RILs, ‘Salinas 88’, and ‘La Brillante’ were grown in five field experiments located in Arizona and California with harvest dates between March and October (Supplemental Table 2). Lettuce grown in experiments AZ’10, CL’10, and Sal’10 were used in cut lettuce processing, experiment Sal’11-air was used for cut lettuce processing and for assessing decay of whole heads, and experiment Sal’11-wh was used only for evaluation of whole-head decay. In all field experiments, lettuce was planted and grown to maturity following a standard agricultural configuration of raised beds ≈ 1 m wide $\times$ 25 cm high with two parallel seed lines separated by 28 cm. In AZ’10, Sal’10, Sal’11-air, and Sal’11-wh, RILs and parents were seeded to a 6.1-m-long plot of a single seed line and were assigned to plots using a randomized complete block design with three replications. Field experiment CL’10 used unreplicated plots that were 6.1 m long using both seeds lines of a bed. In all experiments, plots were thinned at ≈ 4 weeks after seeding to achieve spacing of 28 cm between plants within a seed line. All trials were maintained using standard cultural practices appropriate for each region (Ryder, 1999) and RILs and parents were harvested at the market maturity of ‘Salinas 88’ for use in postharvest evaluations.

For cut lettuce processing, three heads from each plot were harvested from AZ’10, Sal’10, and Sal’11-air, whereas nine heads were harvested from the single plot of each RIL and parents in CL’10. Lettuce from CL’10, Sal’10, and Sal’11-air were placed in 4 °C forced-air cooling for 1 to 2 d before processing. Lettuce from AZ’10 was vacuum-cooled in Yuma, AZ, on the day of harvest, shipped by commercial refrigerated truck to the U.S. Agriculture Research Station in Salinas, CA, and then stored at 4 °C until processing. All the lettuce heads within a RIL or parent were bulked within each field experiment and processed into cut lettuce using the methods of Hayes and Liu (2008). The cores were removed, and the leaves were cut into 2.5-cm² lettuce pieces with an Easy LettuceKutter (NEMCO, Hicksville, OH), washed in 0.0016 mol·L⁻¹ NaOCl for 2 min, and dried with a food processing centrifuge (FP-35; Bock Engineered Products, Toledo, OH) at 2 g_n for 5 min. The lettuce pieces were thoroughly mixed and 340 g of tissue was placed in transparent 22.8 $\times$ 30.5-cm bags made from 63.5- μ m-thick polyethylene coextruded film with an O₂ transmission rating of 0.94 nmol·s⁻¹·m⁻²·Pa⁻¹ (as determined by the manufacturer; Printpack, Atlanta, GA). Each RIL or parent produced up to nine bags of cut lettuce. The bags of cut lettuce made from AZ’10, CL’10, and Sal’10 were triple-flushed with N₂ before heat-sealing the bag closed. The bags made from Sal’11-air were sealed without a N₂ flush and contained air at the time of packaging. Additional heads from RILs in the S-SL4 and L-SL4 subpopulations were harvested from Sal’11-air and processed using a N₂ flush. This processing was done concurrently with the entire RIL population and resulted in the packaging of S-SL4 and L-SL4 cut lettuce with N₂ or air flush using lettuce

grown in the same plots of Sal’11-air. Bags of cut lettuce were held at 4 °C after processing and during evaluation. Each bag of cut lettuce was visually evaluated for decay on a 0 through 10 scale that corresponds to the estimated percentage of decayed tissue divided by 10 (Supplemental Fig. 1). Decay was recognized as damaged leaf blade tissue that was water-soaked and limp with a dull to dark or black color. Midrib tissue and heart leaves were water-soaked with a translucent to dark brown color. Pink coloration on cut surfaces was occasionally observed, but this defect was distinct from the tissue decay. Because this discoloration was assumed to result from bags that were improperly sealed, it was not evaluated. No other types of decay were observed. Evaluations were conducted weekly, starting 29 d after harvest (DAH) when decay was readily apparent for CL’10 and AZ’10 and 8 DAH for Sal’10 and Sal’11-air. In all experiments, evaluations were continued until all bags had reached a decay of 10; these data were then used to determine the time to 100% decay (T100D) in days for each bag. Higher T100D indicates longer shelf life. Fifty-two bags from 19 RILs representing 7% of all bags in experiments Sal’11-air had not decayed 100% by 120 DAH. Regardless, the experiment was ended and these bags were assigned the T100D values of 120 for data analysis.

Whole-head decay of RILs in the S-SL4 and L-SL4 subpopulations were evaluated using three replicate sets of cartons (cardboard boxes containing lettuce heads). One set of cartons was harvested from Sal’11-air and two sets were harvested from Sal’11-wh. Each carton contained 20 to 24 lettuce heads of a single RIL, harvested and pooled from all field plots of each RIL. Cartons from Sal’11-air and one set from Sal’11-wh were vacuum-cooled, shipped by commercial refrigerated trucks from Salinas, CA, to Beltsville, MD, and then stored at 5 °C until completion of the experiment. The second set of cartons from Sal’11-wh was retained in Salinas for evaluation; this set was not vacuum-cooled and was held at 4 °C beginning immediately after harvest and during evaluation. Cartons of whole heads were evaluated for decay beginning on the day they arrived in Beltsville, MD (6 d for Sal’11-air and 3 d for Sal’11-wh) or 1 DAH for those cartons kept in Salinas, CA. Each carton was evaluated at 4- to 7-d intervals for 4 to 6 weeks from then on. Each evaluation was conducted by at least three trained judges using a 9-point hedonic scale with 9 = like extremely, 8 = like strongly, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike strongly, and 1 = dislike extremely (Luo, 2007; Meilgaard et al., 1991).

DATA ANALYSIS OF CUT LETTUCE. The parent means, population mean, variance, Quartile 1, median, and Quartile 3 decay and T100D were calculated for each field experiment. No further analysis was attempted using data from the last day of evaluation for AZ’10 (113 DAH), CL’10 (120 DAH), and Sal’10 (92 DAH) because all of the decay values were 10. Mean decay at each evaluation time point and T100D were calculated for each RIL and used to calculate correlations between field experiments. Further analysis of phenotypic T100D data was conducted in Proc Mixed of SAS (Version 9.2; SAS Institute, Cary, NC) to determine if transgressive segregation was present in the population. A model was fit with field experiments and entries (RILs and parents) and the interaction as fixed effects. Least square means and 95% confidence intervals were calculated to detect significant difference between RILs and their parents. Entries with non-overlapping confidence intervals

were considered to be significantly different. Separate error variances were calculated for each RIL or parent using the GROUP = entries option. Heritability estimates and their ses were calculated on a per-bag (equivalent to per-plot) and RIL-mean basis using restricted maximum likelihood estimates of variance components from a completely randomized design (each bag treated as a separate replicate) following the methods of Holland et al. (2003). Data from ‘Salinas 88’ and ‘La Brillante’ were excluded from this analysis. Heritability for decay was calculated for each time point within each field experiment separately using the equation:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_E^2}{er}} \quad [1]$$

Heritability for T100D was calculated from a single analysis combining data from all field experiments using the equation:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{GE}^2}{e} + \frac{\sigma_E^2}{er}} \quad [2]$$

σ^2 are the REML variance component estimates of G, GE, and E effects and e and r are the number of environments and replications. e and r are set to 1 when calculating H^2 on a per-bag basis. The harmonic mean of e and r was calculated as suggested by Holland et al. (2003) and used to calculate H^2 on a RIL-mean basis.

QTL were initially mapped using composite interval mapping (CIM) in the software QGene Version 4.3.10 (Joehanes and Nelson, 2008). Analysis of decay for each time point and T100D was conducted separately for each environment using RIL means calculated from individual bag data. Cofactors were selected using the automatic forward cofactor selection option in the software. The scan interval was set at 2 cM. The genome-wide significance thresholds for LOD scores were generated for each trait using permutation analysis based on 1000 permutations, which resulted in LOD critical values of ≈ 4.2 for $P < 0.05$ and 5.9 for $P < 0.01$. Thresholds calculated through permutation take into consideration the actual distribution of traits in the mapping population (Churchill and Doerge, 1994). The proportion of phenotypic variation explained (R^2) and additive effects (A) were calculated by QGene. Confidence intervals for QTL locations were calculated as 1-LOD and 2-LOD intervals. Mixed model CIM using decay evaluated between 29 through 57 DAH and T100D data were conducted in QTLnetwork Version 2.1 (Yang et al., 2008). A single analysis was conducted using data from all environments to map QTL and estimate A and additive $\times$ environment (AE) effects using a Bayesian method (Yang et al., 2008). QTL $\times$ QTL interactions were not analyzed in this analysis as a result of the insufficient number of RILs tested in these experiments. Otherwise, the default settings of QTLnetwork Version 2.1 were used and the R^2 explained by each effect was estimated by the software as the QTL H^2 . Positive and negative values of A and AE from the CIM and mixed model CIM analysis represent an increase and decrease, respectively, in the trait value as a result of the ‘Salinas 88’ allele.

DATA ANALYSIS OF S-SL4 AND L-SL4 SUBPOPULATIONS. The mean T100D were calculated for the N_2 and air treatments of

the S-SL4 and L-SL4 subpopulations and significant differences between means were determined using t tests assuming unequal variances. The whole-head decay ratings from all evaluators at each time point were averaged for each carton and then used to calculate the relative area under the decay progress curve (rAUDPC) (Fry, 1978). Correlations between sets of cartons were calculated to determine the dependence of RIL rAUDPC across experiments. The rAUDPC was analyzed in Proc Mixed of SAS by treating each set of cartons as separate experiments with an unreplicated carton of each RIL within each set. RILs from both subpopulations were pooled into a single analysis using RIL as a fixed effect and set as a random effect. To determine if S88 $\times$ LB RILs segregate for whole-head decay, the RIL effect was tested for significance using the RIL $\times$ set interaction as the estimate of experimental error. Least square means and 99% confidence intervals were calculated for each RIL to detect significant differences among RIL means. A contrast was performed between S-SL4 and L-SL4 subpopulations to determine the effect of the *qSL4* on whole-head rAUDPC.

Results

PHENOTYPIC DISTRIBUTION, CORRELATION, AND H^2 OF CUT LETTUCE DECAY. The population of RILs from S88 $\times$ LB exhibited variation for fresh-cut lettuce decay and for T100D (Table 1). RILs exhibited limited amounts of decay at 8 to 15 DAH with Quartile 3 and mean decay less than three (Table 1). This was followed by variable amounts of decay 22 to 57 DAH (Table 1). The population variance of decay was greatest in all field experiments between these time points. From 64 DAH to the end of evaluation, Quartile 1 decay was nine in AZ’10 and 10 for CL’10 and Sal’10, which indicates extensive decay during these time points (Table 1). Consequently, decay variance was lower in these time points (Table 1). Experiment Sal’11-air did not exhibit a first quartile decay value of 10 until 78 DAH and exhibited variation for decay for a longer period of time (Table 1). Decay progressed the fastest in Sal’10, which had a T100D population mean of 39, lower than experiments Sal’11-air (55.6), AZ’10 (56.1), and CL’10 (53.7) (Table 1).

In all experiments, ‘La Brillante’ had higher decay means than ‘Salinas 88’ and lower mean T100D than ‘Salinas 88’, demonstrating slower decay in ‘Salinas 88’ (Table 1). Mean decay and T100D of the RIL population were generally intermediate between ‘Salinas 88’ and ‘La Brillante’ (Table 1). No individual RIL with T100D lower than ‘La Brillante’ and no RIL with T100D repeatedly significantly greater than ‘Salinas 88’ were observed (Supplemental Table 3).

The phenotypic variation in this population was observed when grown in diverse locations and seasons and when using active or passive MA packaging (Supplemental Table 2). Regardless, the amount of decay at weekly evaluation time points and T100D for RILs were repeatable across experiments, because nearly all correlations between experiments were significant (Table 2). Estimates of H^2 for decay were dependent on evaluation time point but were generally high, indicating that large portions of phenotypic variation can be attributed to genetic effects (Supplemental Table 4). The maximum H^2 values on a per-bag basis occurred at 43 DAH for AZ’10 ($H^2 = 0.51$, $SE = 0.05$), CL’10 ($H^2 = 0.84$, $SE = 0.02$), and Sal’11-air ($H^2 = 0.81$, $SE = 0.03$), and at 29 DAH for Sal’10

Table 1. Descriptive statistics for fresh-cut lettuce decay assessed between 8 and 120 d after harvest and for time to 100% decay (T100D) for a ‘Salinas 88’ × ‘La Brillante’ recombinant inbred line population grown in four field experiments located in Salinas, CA, and Yuma, AZ, in 2010 and 2011 and placed in modified atmosphere packages filled with N₂ or air after processing.

Statistics ^y	Cut lettuce decay (0–10 scale) ^z																	T100D (d)
	Days after harvest																	
	8	15	22	29	36	43	50	57	64	71	78	85	92	99	106	113	120	
Field experiment Sal’10 ^x																		
La Brillante mean	1.0	6.5	9.3	9.8	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	ND ^w	ND	ND	ND	26.8
Salinas 88 mean	0.0	0.0	0.6	1.2	2.4	3.8	5.0	6.0	8.2	8.2	9.2	9.6	10.0	ND	ND	ND	ND	72.8
Population mean	0.2	2.2	4.7	7.1	8.3	8.9	9.5	9.7	9.9	9.9	10.0	10.0	10.0	ND	ND	ND	ND	39.0
Variance	0.3	4.2	11.1	12.8	8.6	5.3	1.8	1.0	0.4	0.2	0.0	0.0	0.0	ND	ND	ND	ND	195.6
Quartile 1	0.0	0.0	1.0	3.0	7.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	ND	ND	ND	ND	29.0
Median	0.0	2.0	5.0	9.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	ND	ND	ND	ND	36.0
Quartile 3	0.0	3.0	8.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	ND	ND	ND	ND	43.0
Field experiment Sal’11-air																		
La Brillante mean	0.1	1.9	5.9	9.3	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	31.9
Salinas 88 mean	0.0	0.0	0.3	1.2	2.2	3.0	4.8	5.5	6.7	9.7	10.0	10.0	10.0	10.0	10.0	10.0	10.0	71.2
Population mean	0.0	0.7	2.9	5.3	6.8	7.2	7.6	7.8	8.1	8.3	8.9	9.1	9.3	9.6	9.7	9.7	9.8	55.6
Variance	0.0	1.3	8.1	13.2	16.4	15.2	13.4	12.0	10.7	9.3	5.3	4.5	3.1	1.8	1.6	1.3	1.0	877.6
Quartile 1	0.0	0.0	0.0	1.0	2.0	3.0	4.0	5.0	5.0	8.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	36.0
Median	0.0	0.0	2.0	6.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	36.0
Quartile 3	0.0	1.0	5.0	9.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	78.0
Field experiment AZ’10																		
La Brillante mean	ND	ND	ND	9.5	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	ND	26.8
Salinas 88 mean	ND	ND	ND	2.2	2.7	3.6	5.3	7.1	7.1	8.9	9.8	9.8	9.8	9.8	10.0	10.0	ND	76.3
Population mean	ND	ND	ND	3.5	5.5	7.2	8.0	8.7	9.1	9.6	9.8	9.9	9.9	10.0	10.0	10.0	ND	56.1
Variance	ND	ND	ND	6.6	10.6	9.7	7.6	5.3	3.7	1.3	0.5	0.4	0.3	0.1	0.0	0.0	ND	345.5
Quartile 1	ND	ND	ND	1.3	2.0	4.0	6.0	8.3	9.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	ND	29.0
Median	ND	ND	ND	3.0	5.0	9.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	ND	50.0
Quartile 3	ND	ND	ND	5.0	9.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	ND	71.0
Field experiment CL’10																		
La Brillante mean	ND	ND	ND	4.1	9.9	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	35.8
Salinas 88 mean	ND	ND	ND	0.0	0.0	0.6	1.0	2.8	3.6	5.0	7.4	8.7	9.1	9.9	10.0	10.0	10.0	97.2
Population mean	ND	ND	ND	2.8	5.7	7.0	7.7	8.4	9.1	9.4	9.6	9.8	9.9	9.9	10.0	10.0	10.0	53.7
Variance	ND	ND	ND	8.6	16.6	14.2	10.5	7.4	4.0	2.5	1.4	0.6	0.2	0.1	0.0	0.0	0.0	412.6
Quartile 1	ND	ND	ND	1.0	2.0	4.0	6.0	9.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	43.0
Median	ND	ND	ND	2.0	7.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	43.0
Quartile 3	ND	ND	ND	6.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	78.0

^zDecay rated on a 0 (no decay) through 10 (100% decay) scale. Larger T100D indicates longer shelf life.

^yStatistics calculated using data of individual bags of fresh-cut lettuce. Up to nine bags were assessed per cultivar or recombinant inbred line per experiment.

^xAZ’10 was a field experiment in Yuma, AZ, in 2010; CL’10, Sal’10, and Sal’11-air were field experiments in Salinas, CA, in 2010 and 2011. Modified atmosphere packages from AZ’10, CL’10, and Sal’10 were flushed with N₂ before sealing; packages from Sal’11-air were sealed with air. See Supplemental Table 2 for a detailed description of environments.

^wND = no data.

(H² = 0.75, SE = 0.03) (Supplemental Table 4). Heritability estimates from single-location experiments are expected to be biased upward, because the estimates of G variance contain GE effects. Heritability for T100D calculated by combining data from all environments, which excludes GE variation from the estimate of G, were 0.48 (SE = 0.05) on a per-bag basis and 0.87 (SE = 0.02) on an RIL-mean basis.

S88 × LB MOLECULAR MARKER LINKAGE MAP AND QTL FOR DECAY. A linkage map for S88 × LB comprised of 95 SNP markers and 205 AFLP markers was assembled into 14 groups spanning 904 cM (Table 3) and aligned to the Sal × UC96 reference map (Supplemental Fig. 2). Nine SNP and 15 AFLP markers could not be placed into a LG. The 14 S88 × LB LGs ranged in length from 13.1 to 194.4 cM (Table 3). The S88 × LB map has 57% of the length of the Sal × UC96 reference map

(Table 3). The length of individual LGs relative to the reference map ranges from 9% to 83% (Table 3). These RILs are expected to inherit the ‘Salinas 88’ and ‘La Brillante’ marker alleles in a 1:1 ratio. Thirty-six markers on LGs 3, 4, 8a, and 9b in the S88 × LB map were in groups with a significant deviation from 1:1 segregation (χ^2 , 1 df > 3.7; $P < 0.05$), indicating segregation distortion in these regions (Supplemental Table 5). The intervals exhibiting distortion ranged from 8.3 to 62.6 cM in length. The ‘Salinas 88’ alleles were more frequent in the intervals on LGs 3, 8a, and 9, whereas the ‘La Brillante’ allele was more frequent on LG 4.

Quantitative trait loci for decay of cut lettuce were consistently detected at the same or similar genomic locations on LG 1 in AZ’10 and on LGs 4 and 9a for all experiments using CIM (Fig. 1). These QTL were subsequently named *shelf life 1*

Table 2. Correlation of fresh-cut lettuce decay for ‘Salinas 88’ × ‘La Brillante’ recombinant inbred line means among experiments for four field experiments conducted in Yuma, AZ, and Salinas, CA, in 2010 and 2011.

Shelf life variable	Correlation coefficients between field experiments ^z					
	AZ’10, CL’10	AZ’10, Sal’10	AZ’10, Sal’11-air	CL’10, Sal’10	CL’10, Sal’11-air	Sal’10, Sal’11-air
Decay (0–10 scale) ^y						
8 DAH	ND ^x	ND	ND	ND	ND	0.30**
15 DAH	ND	ND	ND	ND	ND	0.80**
22 DAH	ND	ND	ND	ND	ND	0.77**
29 DAH	0.56**	0.47**	0.47**	0.56**	0.64**	0.84**
36 DAH	0.64**	0.55**	0.58**	0.58**	0.72**	0.82**
43 DAH	0.61**	0.48**	0.57**	0.50**	0.76**	0.70**
50 DAH	0.58**	0.44**	0.53**	0.39**	0.71**	0.55**
57 DAH	0.53**	0.43**	0.47**	0.35**	0.62**	0.54**
64 DAH	0.48**	0.47**	0.41**	0.38**	0.55**	0.45**
71 DAH	0.54**	0.48**	0.36**	0.41**	0.51**	0.34**
78 DAH	0.56**	0.57**	0.25*	0.48**	0.43**	0.22*
85 DAH	0.60**	0.61**	0.28**	0.59**	0.47**	0.24*
92 DAH	0.53**	ND	0.17	ND	0.28**	ND
99 DAH	0.41**	ND	0.20	ND	0.27*	ND
106 DAH	0.32**	ND	0.22*	ND	0.10	ND
113 DAH	ND	ND	ND	ND	–0.04	ND
T100D (d)	0.69**	0.61**	0.61**	0.62**	0.71**	0.80**

*, **Significantly different from zero at $P < 0.05$ and $P < 0.01$, respectively.

^zAZ’10 was a field experiment in Yuma, AZ, in 2010; CL’10, Sal’10, and Sal’11-air were field experiments in Salinas, CA, in 2010 and 2011. Modified atmosphere packages from AZ’10, CL’10, and Sal’10 were flushed with N₂ before sealing; packages from Sal’11-air were sealed with air. See Supplemental Table 2 for detailed descriptions of environments.

^yDecay rated on a 0 (no decay) through 10 (100% decay) scale every week from 8 d after harvest (DAH) to up to 120 DAH. T100D = time to 100% decay measured in days.

^xND = no data.

Table 3. The number of markers, linkage group length, and average distance between markers for each linkage group in a genetic map of the lettuce population ‘Salinas 88’ × ‘La Brillante’ and the percentage of map length compared with an ultra-high-density reference map.

Linkage group ^z	Markers (no.)	Linkage group length (cM)	Avg distance between markers (cM)	Reference map length (cM) ^y	Percentage of reference map length (%) ^x
1	40	106.4	2.7	144	74
2a and 2b	38, 8 ^w	90.1, 28.5	2.4, 3.6	152	78
3a and 3b	34, 7	53.7, 32.2	1.6, 2.3	174	49
4	49	194.4	4.0	234	83
5	46	163.8	3.6	234	70
6	6	13.1	2.6	143	9
7a and 7b	17, 3	53.1, 6.9	3.1, 4.6	142	42
8a and 8b	27, 5	92.8, 5.4	3.4, 1.1	204	43
9a and 9b	6, 14	29.1, 34.2	3.4, 2.4	159	40
Total	300	904.0	3.0	1585	57

^zFor linkage groups 2, 3, 7, 8, and 9, the linkage group is broken into two sections, labeled a and b. The sections were determined to belong to the same chromosome by alignment to a reference genetic map (Truco et al., 2013).

^yLength of a ultra-high-density genetic map of lettuce reported in Truco et al. (2013).

^xLength of ‘Salinas 88’ × ‘La Brillante’ map expressed as a percentage of reference map length.

^wGenetic map data are shown for each section within a linkage group. Within these linkage groups, the first value is for the a section and the second value is for the b section.

(*qSL1*), *shelf life 4* (*qSL4*), and *shelf life 9* (*qSL9*), respectively. *qSL1* was significant only in AZ’10 and was found at a position of 40 cM between SNP marker CLRY2600 and AFLP marker E44/M48–318.17 (Fig. 1). *qSL4* was located at a position of 150 to 154 cM near AFLP markers E45/M49–143.55 and E35/M60–495.30 (Fig. 1). *qSL9* was found at a position of 1 cM near AFLP marker E35/M59–208 (Fig. 1).

Slower decay was inherited from ‘Salinas 88’ for all QTL. Negative A and AE values for decay measured on the 0 through

10 decay scale and positive A and AE values for T100D indicate improved shelf life inherited from ‘Salinas 88’. In AZ’10, *qSL1*, *qSL4*, and *qSL9* had similar A and R^2 between 29 through 43 DAH (Fig. 2). *qSL1* and *qSL4* were significant beginning at 29 DAH and continued to be significant until 71 DAH (Fig. 2). In field experiments CL’10, Sal’10, and Sal’11-air, *qSL4* was the most important determinant of decay. *qSL4* was significant beginning at 15 DAH for Sal’10 and Sal’11-air and at 29 DAH for CL’10 (Fig. 2). The A and R^2 values for *qSL4*

Fig. 1. The genomic position of quantitative trait loci (QTL) *shelf life 1* (*qSL1*), *shelf life 4* (*qSL4*), and *shelf life 9* (*qSL9*) on linkage groups 1, 4, and 9a, respectively, in a lettuce population from 'Salinas 88' × 'La Brillante'. QTL are shown as 1-LOD (thick line) and 2-LOD (thin line) support intervals. QTL labels indicate the QTL name followed by an abbreviation for the experiment environment and trait variable name. Field experiment abbreviations indicate field location and production year: AZ'10 = Yuma, AZ, in 2010 (shown in green); CL'10 = Carr Lake, Salinas, CA, in 2010 (shown in red); Sal'10 = USDA, Salinas, CA, in 2010 (shown in blue); and Sal'11-air = USDA, Salinas, CA, in 2011 (shown in black). Cut lettuce was processed and packaged into bags flushed with N₂ (AZ'10, CL'10, and Sal'10) or air (Sal'11-air) and evaluated for decay 8 to 120 d after harvest (DAH) and for time to 100% decay (T100D). Molecular markers are shown as alphanumeric marker codes on the left side of linkage group diagrams; the centimorgans position is shown on the right side. LOD = logarithm of odds.

Fig. 2. Additive effect and proportion of phenotypic variation explained (R^2) for quantitative trait loci (QTL) shelf life 1 ($qSL1$), shelf life 4 ($qSL4$), and shelf life 9 ($qSL9$) in a lettuce population from 'Salinas 88' × 'La Brillante'. Additive effect and R^2 were estimated for QTL for decay of cut lettuce 8 to 120 d after harvest using composite interval mapping. Negative additive effects indicate a reduction in decay resulting from the 'Salinas 88' allele. Significance at $P < 0.05$ was determined using permutation analysis based on 1000 permutations. Field experiment abbreviations indicate field location and production year: AZ'10 = Yuma, AZ, in 2010; CL'10 = Carr Lake, Salinas, CA, in 2010; Sal'10 = USDA, Salinas, CA, in 2010; and Sal'11-air = USDA, Salinas, CA, in 2011. See Supplemental Table 2 for detailed description of environments. Cut lettuce was processed and packaged into bags flushed with N_2 (AZ'10, CL'10, and Sal'10) or air (Sal'11-air).

continued to increase with time, reaching maximum values 43 DAH in CL'10 ($A = -2.8$, $R^2 = 0.47$), 29 DAH in Sal'10 ($A = -3.0$, $R^2 = 0.69$), and 43 DAH in Sal'11-air ($A = -3.5$, $R^2 = 0.74$) (Fig. 2). $qSL9$ was found only during early evaluation time points, 29 DAH in CL'10, 15 DAH in Sal'10, and between 15 to 29 DAH in Sal'11-air. Within these evaluation time points, the A of $qSL9$ ranged from -0.42 to -1.2 and R^2 values ranged

from 0.21 to 0.39, which were similar to $qSL4$. QTL could not be detected after 71 DAH for AZ'10, CL'10, and Sal'10. $qSL4$ was significant in Sal'11-air as late as 120 DAH. Analysis of QTL AE interactions for decay between 29 and 57 DAH and T100D was conducted using mixed-model-based CIM. This analysis identified $qSL1$, $qSL4$, and $qSL9$ in the same genomic location and with about the same A as in the initial CIM analysis as well as QTL AE interactions (Table 4). Although this finding indicates that the magnitude of QTL effects is environmentally dependent, most AE interactions were small with R^2 less than 0.05. The largest AE effects, with R^2 ranging from 0.09 to 0.14, were found for $qSL4$ in the Sal'11-air experiment for T100D and decay evaluated between 43 and 57 DAH.

QSL4 INTERACTION WITH N_2 OR AIR-FLUSHED FRESH-CUT LETTUCE PACKAGES AND EFFECT ON SHELF LIFE OF WHOLE HEADS. The S-SL4 and L-SL4 subpopulations differing for their $qSL4$ allele were created by randomly selecting 14 RILs with the 'Salinas 88' allele and 18 RILs with 'La Brillante' allele, respectively, of AFLP marker E45/M48-162.87. The subpopulation L-SL4 had a significantly lower T100D mean compared S-SL4 regardless of whether cut lettuce was packaged with a N_2 flush or in air (Table 5), further demonstrating the rapid decay conferred by the 'La Brillante' $qSL4$ allele. The effect of packaging with N_2 instead of air flush on cut lettuce decay was dependent on the QTL allele. The S-SL4 subpopulation had a significantly greater T100D for cut lettuce packaged with an air flush compared with those packaged with a N_2 flush (Table 5). No differences in T100D were observed between N_2 and air for the L-SL4 subpopulation (Table 5). Although the RILs in the subpopulations were selected at random, they did not

have equivalent frequencies of the 'Salinas 88' and 'La Brillante' alleles at $qSL9$ (predicted based on the AFLP marker E35/M59-208.30) as a result of segregation distortion on LG 9a. Specifically, only two of 14 RILs in S-SL4 possessed the 'La Brillante' allele of $qSL9$. To determine if the biased frequency of $qSL9$ affected the comparison of the subpopulations, T100D means of S-SL4 and L-SL4 RILs that were fixed

Table 4. The linkage group (LG), centimorgan interval, additive effect (A), additive $\times$ environment interactions, and proportion of phenotypic variation explained (R^2) for fresh-cut lettuce decay quantitative trait loci (QTL) in a ‘Salinas 88’ $\times$ ‘La Brillante’ recombinant inbred line population assessed in four field experiments located in Salinas, CA, and Yuma, AZ, in 2010 and 2011 and placed in modified atmosphere packages filled with N₂ or air after processing.

Variable	QTL	QTL position		A ^y	R ²	Additive × environment interactions ^x			
		LG no.	Interval (cM)			AZ'10 ^w	CL'10	Sal'10	Sal'11-air
Decay (0–10) ^z									
29 DAH	<i>qSL1</i>	1	37.2–43.3	–0.5	0.003				0.45 (0.02)
	<i>qSL4</i>	4	149.5–152.5	–1.9	0.400	1.17 (0.06)	0.54 (0.01)	–0.95 (0.03)	–0.72 (0.03)
	<i>qSL9</i>	9a	0–3	–0.8	0.100				
36 DAH	<i>qSL1</i>	1	43.3–46.3	–0.4	0.002	–0.52 (0.02)			
	<i>qSL4</i>	4	150.5–152.5	–2.5	0.460	0.95 (0.03)			–1.1 (0.04)
43 DAH	<i>qSL1</i>	1	43.3–46.3	–0.3	0.001	–0.51 (0.02)			
	<i>qSL4</i>	4	149.5–152.5	–2.3	0.460	0.81 (0.02)		0.87 (0.03)	–1.20 (0.05)
50 DAH	<i>qSL4</i>	4	150.7–152.5	–1.8	0.400	0.70 (0.02)		1.24 (0.07)	–1.48 (0.10)
57 DAH	<i>qSL4</i>	4	150.5–152.5	–1.4	0.330	0.66 (0.03)		1.09 (0.07)	–1.56 (0.14)
T100D (d)	<i>qSL4</i>	4	149.5–152.5	14.5	0.440	–5.30 (0.03)		–4.75 (0.02)	10.50 (0.09)

^zDecay rated on a 0 (no decay) through 10 (100% decay) scale every week starting 8 d after harvest (DAH). T100D = time to 100% decay. Larger T100D indicates longer shelf life. QTL analysis was conducted in QTLNetwork 2.1 (Yang et al., 2008). Effects shown are all significant at $P < 0.01$.

^yAdditive and additive $\times$ environment effects expressed as deviations from the populations mean. Negative estimates indicate decreased trait value for the ‘Salinas 88’ allele.

^xOnly significant additive $\times$ environment interactions are shown. The proportion of phenotypic variation explained by the interaction is shown in parenthesis adjacent to the additive $\times$ environment interactions.

^wAZ’10 was a field experiment in Yuma, AZ, in 2010. CL’10, Sal’10, and Sal’11-air were field experiments in Salinas, CA, in 2010 and 2011. Modified atmosphere packages from AZ’10, CL’10, and Sal’10 were flushed with N₂ before sealing; packages from Sal’11-air were sealed with air. See Supplemental Table 2 for a detailed description of environments.

Table 5. Shelf life of cut lettuce packaged in air or N₂ and of whole heads in subpopulations of ‘Salinas 88’ $\times$ ‘La Brillante’ recombinant inbred lines possessing the ‘Salinas 88’ (S-SL4) or ‘La Brillante’ (L-SL4) alleles of quantitative trait loci *shelf life 4* (*qSL4*) and grown in Salinas, CA, field experiments in 2011.

Subpopulation ^z	RILs (no.)	Time to 100% decay of cut lettuce (d)		Whole-head rAUDPC ^x
		Packaged in air ^y	Packaged in N ₂	
L-SL4	18	34 ^w	33.9 ^w	3.2
S-SL4	14	89.8 ^y	63.3	3.0 NS

^zRecombinant inbred lines (RILs) randomly sampled with the ‘La Brillante’ or ‘Salinas 88’ allele of marker E45/M48-162.87 located less than 2 cM from *qSL4*.

^yCut lettuce package in air or N₂. Data are from a single experiment.

^xRelative area under the decay progress curve (rAUDPC) calculated using the 0 (poor quality) to 9 (good quality) ratings every 4 to 6 d for up to 28 d after harvest. Note that although time to 100% decay for cut lettuce indicates slower decay, for whole heads higher rAUDPC values indicate a faster decay. Means are from three experiments.

^wTime to 100% decay of L-SL4 significantly less than S-SL4 at $P < 0.01$.

^yTime to 100% decay of air and N₂ gas flush significantly different at $P < 0.01$ within S-SL4.

NS rAUDPC means of S-SL4 and L-SL4 nonsignificantly different.

for the ‘Salinas 88’ allele of *qSL9* were compared using *t* tests. The difference in T100D means for S-SL4 in air (90.9) and N₂ (62.4) were still significantly different at $P < 0.001$, whereas L-SL4 means in air (37.1) and N₂ (36.8) were similar. This indicates that the effect of the unbalanced allele frequencies at *qSL9* is nonexistent or too small to be detected.

Whole-head decay of RILs in the S-SL4 and L-SL4 subpopulations were evaluated to determine the effect of *qSL4* on this trait. Differences between RILs were observed. The major symptoms of deterioration included leaf wilting, tissue darkening and discoloration, loss of green color as well as signs of decay caused by spoilage bacteria and fungi (Supplemental Fig. 3). The mean rAUDPC of RIL whole heads evaluated over 4 to 6 weeks in three replicate evaluations located in Beltsville, MD, or Salinas, CA, using lettuce grown in Sal’11-air and Sal’11-wh were significantly different [$P < 0.001$ (Supplemental Table 6)]; correlations of RIL rAUDPC between replicate evaluations

ranged between 0.43 and 0.48 and were significant ($P < 0.02$). These results indicate genetic variation and segregation for whole-head decay. The genetic variation for whole-head decay in this population cannot be attributed to *qSL4*, because the mean decay of the S-SL4 and L-SL4 subpopulations was not significantly different (Table 5).

Discussion

This research identified three QTL controlling decay of cut lettuce with *qSL4* being a major determinant of decay. Slow decay was inherited from ‘Salinas 88’ for all QTL found. *qSL1* and *qSL9* appear to have minor effects, are important only during the early storage periods, or were specific to a single experiment. As a result of the large amount of phenotypic variation attributable to a single QTL and the high H^2 for cut lettuce shelf life, rapid genetic gain for slow decay is likely in

germplasm with similar pedigrees as S88 × LB using the evaluation methods described here. Other butterhead, romaine, and Latin-type accessions with rapid decay phenotypes indistinguishable from ‘La Brillante’ have been reported (Hayes and Liu, 2008; Simko et al., 2012), although the relationship between the genes conferring rapid decay in ‘La Brillante’ and these cultivars is unknown. *qSL4* had no effect on whole-head shelf life, which is not unexpected because the symptoms and the underlining failure mechanisms of fresh-cut lettuce and whole-head decay are substantially different (Supplemental Figs. 1 and 3). The decline in quality scores of whole heads is primarily associated with the dehydration during cross-country transportation and during initial storage and development of bacteria and fungal decay during extended storage. On the other hand, the symptoms of cut lettuce decay in this population were characterized by extensive water-logging and tissue darkening, which is often associated with physiological disorders caused by anaerobic respiration under low O₂ and high CO₂ conditions (Kim et al., 2005; Smyth et al., 1998). Water-logging and other symptoms in baby leaf (packaged whole seedling leaves) lettuce were described by Wagstaff et al. (2007), which they attributed to physical damage and senescence. The cultivar La Brillante exhibited uniform amounts of decay in CA chambers containing 0.2%, 1%, or 5% O₂ with the remainder of the atmosphere comprised of N₂ (Hayes and Liu, 2008). This may indicate that ‘La Brillante’, and cultivars possessing the same rapid decay QTL as ‘La Brillante’, exhibit damage from anaerobic respiration in environments having less than 5% O₂, whereas cultivars like Salinas 88 do not. Alternatively, the cause of decay may be unrelated to CO₂ and O₂ package composition.

The genetics of cut lettuce decay in S88 × LB appears to be less complicated than other cut lettuce defects, where multiple QTL were found across the lettuce genome (Atkinson et al., 2013; Zhang et al., 2007). However, comparisons between reports on the genetics of shelf life in lettuce are difficult because each study used different populations, processing methods, and evaluated different defects affecting cut lettuce quality or shelf life. Atkinson et al. (2013) identified seven QTL for browning or pinking of cut surfaces of lettuce using an intraspecific population of lettuce. Browning and pinking defects require O₂, and MA is typically used to reduce O₂ and retard browning and pinking in commercially prepared salads (Smyth et al., 1998). Our studies with S88 × LB used MA packaging and pinking was only rarely observed. Over 100 QTL were identified for shelf life (described as “breakdown, bruising, or damage”) and several leaf developmental and biophysical traits hypothesized to be related to shelf life in the *L. sativa* × *L. serriola* population (Zhang et al., 2007). The shelf life QTL found in the Zhang et al. (2007) study were specific to production locations and typically accounted for less than 30% of the phenotypic variation. Zhang et al. (2007) found no shelf life QTL on LGs 4 or 9. The shelf life QTL found in the S88 × LB population on LG 4 appears to be novel from any previously reported as a result of its large effect and reproducibility in different production locations, seasons, years, and salad packaging methods.

The genome coverage of the S88 × LB genetic map relative to Sal × UC96 high-density map was LG-dependent. Gaps in the S88 × LB genetic map may be the result of monomorphic regions between the two parents. Sampling additional molecular markers from the Sal × UC96 map would likely identify

markers polymorphic between ‘Salinas 88’ and ‘La Brillante’ and could be used to increase the coverage of the S88 × LB map. Additional QTL in S88 × LB may exist in genomic regions where there is no genetic map for this cross. Regardless, the map coverage was sufficient to identify *qSL4*, a major QTL for decay accounting for 30% to 70% of phenotypic variation depending on the experiment (Fig. 2; Table 4). Given the large amount of variation attributable to *qSL4*, it seems unlikely that additional large-effect QTL are present in this cross. Segregation distortion was found on LGs 3, 4, 8a, and 9b. Segregation distortion was previously reported on these LGs in intraspecific or interspecific crosses and can result from several causes including inadvertent selection during inbreeding (Truco et al., 2007).

Interactions of QTL with environments were detected and the most pronounced interactions were found for *qSL1* and *qSL4* in the AZ’10 and Sal’11-air experiments (Table 4). Despite the existence of interactions, their small effect and the high correlation between experiments for decay indicate that breeders can select with confidence using evaluations in a limited number of environments. The AZ’10 field experiment was the only location where *qSL1* was detected. This experiment was distinct in location and time of year and had a high incidence of tipburn, a calcium-related leaf necrosis that appears immediately before harvest maturity (Jenni and Hayes, 2010). It seems plausible that these factors influenced shelf life evaluations and the detection of *qSL1*. The significant AE effect for *qSL4* in Sal’11-air indicates that the effect of the ‘Salinas 88’ allele was larger in this experiment compared with the other experiments. This is likely the result of packaging the cut lettuce from Sal’11-air in air rather than in N₂ as done in the other experiments. The effect of air packaging is evident from the experiment using the subpopulations S-SL4 and L-SL4. Decay in L-SL4 was unaffected by the use of N₂ or air, whereas the use of N₂ hastened decay of S-SL4. Consequently, the difference between the ‘Salinas 88’ and ‘La Brillante’ allele of *qSL4* under air packaging was larger compared with experiments using N₂ flushed packages.

Salad processors are most interested in delaying decay until ≈21 to 29 DAH and *qSL9* and *qSL4* are of similar importance preceding 29 DAH. Between 29 and 50 DAH, *qSL4* is the most important determinant of decay, because the largest R² and A values of any QTL were observed for *qSL4* during these time periods. The highest H² estimates and correlations of RIL means between experiments were observed between 29 and 57 DAH. This time period corresponds to ‘La Brillante’ having 100% decay. The results indicate that continuing decay evaluations until ‘La Brillante’ has reached 100% decay may increase selection effectiveness against cut lettuce decay when using phenotypic selection. Although phenotypic selection is likely to be effective for breeding lettuce with slow decay, the assay used in this research is destructive and requires field-grown lettuce. Therefore, the assay is not suitable for single plant selections typically conducted in early segregating generations. Therefore, *qSL4* is a suitable QTL to develop molecular markers for marker-assisted selection that could be used in early generations.

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Decay rating	
0
3
7
10

Supplemental Fig. 1. Decay rating scale for fresh-cut lettuce. The rating scale for each sample corresponds to the estimated percentage of decayed tissue divided by 10.

Supplemental Fig. 2. (Continued on next page).

Supplemental Fig. 2. (Continued on next page).

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Supplemental Fig. 2. Genetic map of lettuce from the cross ‘Salinas 88’ × ‘La Brillante’ (S88 × LB) showing 95 single nucleotide polymorphism and 205 amplified fragment length polymorphism markers. The S88 × LB map is shown in alignment to an ultra-high-density, transcript-based, genetic map derived from an interspecific cross between *Lactuca sativa* cultivar Salinas and the *Lactuca serriola* accession UC96US23 (Sal × UC96) (Truco et al., 2013). LG = linkage group. The upper segment in the map is the a portion (i.e., LG2a); the lower segment is the b portion (i.e., LG2b). Molecular markers codes shown on the Sal × UC96 map are for markers mapped in both populations. A black line on the Sal × UC96 linkage group bars indicates the position of markers mapped only in Sal × UC96. Scale in centimorgans shown at left of page are accurate only within linkage group segments. Distance in centimorgans between a and b linkage group segments is not known.

Supplemental Fig. 3. Appearance of whole heads of two ‘Salinas 88’ × ‘La Brillante’ lettuce recombinant inbred lines (RH08-0111 and RH08-0128) 3 and 23 d after harvest, cooling, and storage at 5 °C. Lettuce was harvested from a Salinas, CA, field experiment, vacuumed cooled in Salinas, CA, and then shipped to Beltsville, MD, by refrigerated truck. Lettuce was harvested and pooled from three replicated plots to fill each carton.

Supplemental Table 1. Primer combinations used to generate amplified fragment length polymorphic (AFLP) markers and the number of markers polymorphic between ‘Salinas 88’ and ‘La Brillante’ generated for each primer combination.

Primer combination	Polymorphic AFLP markers (no.)
E33/M59	22
E35/M48	22
E35/M49	10
E35/M59	24
E35/M60	19
E38/M54	13
E44/M48	26
E44/M49	14
E45/M48	20
E45/M49	13
E49/M58	11
E51/M49	13
E54/M48	13
Total	220

Supplemental Table 2. Salinas, CA, and Yuma, AZ, field experiments having up to 90 recombinant inbred lines (RILs) of ‘Salinas 88’ × ‘La Brillante’ to evaluate shelf life of whole heads and of cut lettuce in modified atmosphere (MA) packages.

Field experiment abbreviation	RILs tested (no.)	Field experiment location	Field experimental design ^z	Harvest date	MA package gas flush	Time after harvest to first evaluation (d)	
						Cut lettuce	Whole head ^y
AZ’10	90	Yuma, AZ	RCBD	31 Mar. 2010	N ₂	29	NA
CL’10	90	Carr Lake, Salinas, CA	Unrep.	24 June 2010	N ₂	29	NA
Sal’10	86	USDA, Salinas, CA	RCBD	18 Oct. 2010	N ₂	8	NA
Sal’11-air	90	USDA, Salinas, CA	RCBD	31 May 2011	Air or N ₂	8	6
Sal’11-wh	36	USDA, Salinas, CA	RCBD	24 Oct. 2011	NA	NA	1 or 3

^zRCBD = randomized complete block design; Unrep = unreplicated.

^yTwo experiments assessing whole-head decay were conducted using lettuce from Sal’11-wh. An experiment with evaluations in Salinas, CA, beginning on Day 1 and an experiment in Beltsville, MD, with evaluations beginning on Day 3; NA = not applicable.

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#AZ'10 was a field experiment in Yuma, AZ, in 2010; CL'10, Sal'10, and Sal'11-air were field experiments in Salinas, CA, in 2010 and 2011. Modified atmosphere packages from AZ'10, CL'10, and Sal'10 were flushed with N₂ before sealing; packages from Sal'10-air were sealed with air. See Supplemental Table 2 for detailed descriptions of environments. VNT = not tested.

^yN^T = not tested.

Supplemental Table 4. Per-bag and RIL-mean heritability (H^2) and SES for cut lettuce decay assessed between 8 and 120 d after harvest (DAH) and for time to 100% decay (T100D) for a ‘Salinas 88’ × ‘La Brillante’ recombinant inbred line population grown in four field experiments located in Salinas, CA, and Yuma, AZ, in 2010 and 2011 and placed in packages filled with N_2 or air after processing.

Variable	AZ'10 ^z		CL'10		Sal'10		Sal'11-air	
	Per-bag H^2 (SE) ^y	RIL-mean H^2 (SE)	Per-bag H^2 (SE)	RIL-mean H^2 (SE)	Per-bag H^2 (SE)	RIL-mean H^2 (SE)	Per-bag H^2 (SE)	RIL-mean H^2 (SE)
Decay (0–10 scale) ^x	ND ^w	ND	ND	ND	ND	ND	ND	ND
8 DAH	ND	ND	ND	ND	0.25 (0.05)	0.66 (0.06)	0.09 (0.03)	0.44 (0.09)
15 DAH	ND	ND	ND	ND	0.55 (0.05)	0.87 (0.02)	0.44 (0.03)	0.86 (0.02)
22 DAH	ND	ND	ND	ND	0.66 (0.04)	0.92 (0.01)	0.64 (0.04)	0.93 (0.01)
29 DAH	0.37 (0.05)	0.76 (0.04)	0.83 (0.02)	0.97 (0.01)	0.75 (0.03)	0.95 (0.01)	0.74 (0.03)	0.96 (0.01)
36 DAH	0.47 (0.05)	0.82 (0.03)	0.82 (0.02)	0.97 (0.01)	0.67 (0.04)	0.92 (0.01)	0.81 (0.03)	0.97 (0.01)
43 DAH	0.51 (0.05)	0.85 (0.03)	0.84 (0.02)	0.97 (0.01)	0.66 (0.04)	0.92 (0.01)	0.81 (0.03)	0.97 (0.01)
50 DAH	0.46 (0.05)	0.82 (0.03)	0.83 (0.02)	0.97 (0.01)	0.49 (0.05)	0.85 (0.02)	0.79 (0.03)	0.97 (0.01)
57 DAH	0.48 (0.05)	0.83 (0.03)	0.81 (0.03)	0.97 (0.01)	0.36 (0.05)	0.77 (0.04)	0.75 (0.03)	0.96 (0.01)
64 DAH	0.48 (0.05)	0.83 (0.03)	0.78 (0.03)	0.96 (0.01)	0.24 (0.05)	0.66 (0.06)	0.72 (0.03)	0.95 (0.01)
71 DAH	0.46 (0.05)	0.82 (0.03)	0.77 (0.03)	0.96 (0.01)	0.23 (0.05)	0.64 (0.06)	0.69 (0.04)	0.95 (0.01)
78 DAH	0.41 (0.05)	0.79 (0.04)	0.74 (0.03)	0.94 (0.01)	0.14 (0.04)	0.48 (0.09)	0.61 (0.04)	0.92 (0.01)
85 DAH	0.40 (0.05)	0.78 (0.04)	0.61 (0.04)	0.90 (0.02)	0 (0)	0 (0)	0.57 (0.04)	0.91 (0.01)
92 DAH	0.38 (0.05)	0.77 (0.04)	0.53 (0.05)	0.87 (0.02)	ND	ND	0.50 (0.05)	0.89 (0.02)
99 DAH	0.36 (0.05)	0.75 (0.04)	0.41 (0.05)	0.81 (0.03)	ND	ND	0.39 (0.05)	0.83 (0.03)
106 DAH	0.27 (0.05)	0.66 (0.06)	0.24 (0.05)	0.65 (0.06)	ND	ND	0.37 (0.05)	0.83 (0.03)
113 DAH	0.003 (0.06)	0.02 (0.30)	0.01 (0.02)	0.01 (0.11)	ND	ND	0.35 (0.05)	0.81 (0.03)
120 DAH	ND	ND	ND	ND	ND	ND	0.31 (0.04)	0.78 (0.04)
T100D (d)	0.56 (0.05)	0.88 (0.02)	0.87 (0.02)	0.98 (0.01)	0.72 (0.04)	0.94 (0.01)	0.72 (0.04)	0.95 (0.01)

^zAZ'10 was a field experiment in Yuma, AZ, in 2010; CL'10, Sal'10, and Sal'11-air were field experiments in Salinas, CA, in 2010 and 2011. Modified atmosphere packages from AZ'10, CL'10, and Sal'10 were flushed with N_2 before sealing; packages from Sal'10-air were sealed with air. See Supplemental Table 2 for detailed descriptions of environments.

^y SES of heritability estimates shown in parentheses.

^xDecay rated on a 0 (no decay) through 10 (100% decay) scale every week from 8 DAH to up to 120 DAH. Larger T100D indicates longer shelf life.

^wND = no data.

Supplemental Table 5. Linkage groups (LG) with intervals of markers showing segregation distortion within a 904-cM genetic map with 14 LG made from 90 ‘Salinas 88’ × ‘La Brillante’ recombinant inbred lines of lettuce.

Linkage group	Markers on entire LG (no.)	Markers in interval		Length of interval with distortion (cM)	Direction of distortion ^y
		with segregation distortion (no.) ^z	Markers with segregation distortion (no.)		
3	34	15	13	17.7	Salinas 88
4	49	15	10	62.6	La Brillante
8a	27	10	7	20.5	Salinas 88
9b	14	6	6	8.3	Salinas 88

^zSegregation distortion detected using χ^2 as loci with deviations from 1:1 segregation. A group of skewed markers was defined according to Truco et al. (2007) as a group of at least three linked markers with distorted segregation with gaps of non-distorted markers of three or less.

^yDirection of distortion indicates the allele that is more frequent in the population.

Supplemental Table 6. Mean relative area under the decay progress curve (rAUDPC) of lettuce heads for 32 ‘Salinas 88’ × ‘La Brillante’ recombinant inbred lines (RILs) evaluated in three experiments using lettuce grown in two Salinas, CA, field experiments in 2011 and stored at 4 °C or 5 °C for up to 28 d after harvest.^z

RILs	rAUDPC ^y		
	Mean ^x	99% confidence interval	
		Lower bound	Upper bound
RH08-0150	2.46	1.85	3.06
RH08-0138	2.56	1.96	3.17
RH08-0185	2.58	1.97	3.18
RH08-0177	2.60	1.99	3.20
RH08-0119	2.64	1.86	3.42
RH08-0134	2.66	2.05	3.27
RH08-0164	2.75	2.14	3.36
RH08-0157	2.75	2.14	3.36
RH08-0186	2.81	2.09	3.53
RH08-0183	2.84	2.23	3.44
RH08-0145	2.85	2.24	3.45
RH08-0127	2.87	2.26	3.47
RH08-0181	2.92	2.31	3.53
RH08-0179	2.92	2.32	3.53
RH08-0139	2.94	2.33	3.54
RH08-0125	2.97	2.36	3.58
RH08-0176	2.97	2.36	3.58
RH08-0171	3.05	2.45	3.66
RH08-0123	3.19	2.58	3.80
RH08-0151	3.33	2.72	3.94
RH08-0193	3.33	2.72	3.94
RH08-0184	3.39	2.78	3.99
RH08-0144	3.43	2.82	4.03
RH08-0149	3.45	2.84	4.05
RH08-0172	3.55	2.95	4.16
RH08-0142	3.58	2.97	4.18
RH08-0152	3.61	3.00	4.22
RH08-0200	3.77	3.17	4.38
RH08-0165	3.90	3.30	4.51
RH08-0170	4.24	3.63	4.85
RH08-0128	4.35	3.75	4.96
RH08-0166	4.54	3.94	5.15

^zIn a single experiment conducted in Salinas, CA, lettuce was held at 4 °C. In two experiments lettuce was vacuum-cooled, shipped by refrigerated truck to Beltsville, MD, and held at 5 °C.

^yrAUDPC calculated using the 0 (poor quality) to 9 (good quality) ratings every 4 to 6 d for up to 28 d after harvest.

^xMeans are from three experiments evaluating one carton of lettuce per experiment. Lettuce was harvested from three replicate plots to fill each carton.