J. Amer. Soc. Hort. Sci. 149(4):206–216. 2024. https://doi.org/10.21273/JASHS05367-24

Screening Subtropical Lettuce Accessions for Heat Tolerance by Incorporating Accelerated Shelf Life Testing

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Keywords. breeding, genetics, *Lactuca sativa*, postharvest disorders, subtropical climate

Abstract. **Lettuce (*Lactuca sativa* L.) is grown worldwide, from temperate to subtropical climates. Spring season production in humid, subtropical regions, such as southern Florida, is characterized by rising ambient temperatures that can stress lettuce to prematurely bolt and lose shelf life. The objectives of this research were 1) to identify genetic variability in heat tolerance and shelf life among lettuce types and accessions grown under humid, subtropical conditions, and 2) to understand the genotype × environment (G × E) interaction to estimate shelf life of these lettuce accessions. Five lettuce types (romaine, crisphead, butterhead, leaf, and Latin) were grown under commercial conditions in the Everglades Agricultural Area near Belle Glade, FL, USA, for five field experiments over two seasons. Lettuce heads were evaluated at harvest, and subsets were transported to a local commercial grower/shipper for vacuum-cooling and storage at 15 °C according to previously determined protocols for accelerated shelf life testing. Visual appearance ratings were made across harvests and storage time points to segregate lettuce accessions with an estimated marketable shelf life >14 days. The breeding lines tested in this research had head weight and marketability comparable to commercial cultivars. Notably, the crisphead accessions 50113, 60157, 60159, and H1098 had the highest estimated and actual shelf life of more than 21 days, with no presence of bolting or tipburn. Meanwhile, romaine, butterhead, leaf, and Latin types had accessions with estimated and actual shelf life ranging from 14 to 28 days and no presence of bolting or tipburn. A G × E analysis indicated that this interaction is significant; therefore, breeders should consider analyzing G × E when developing new cultivars with good horticultural characteristics, longer shelf life, and most importantly, adaptation to warmer humid, subtropical conditions.**

Lettuce (*Lactuca sativa* L.) is among the most consumed vegetables in the United States. In southern Florida, the crop is produced primarily in the Everglades Agricultural Area (EAA) during winter and spring seasons under humid, subtropical conditions (Sandoya and Lu 2020). Lettuce is highly perishable, with ~2 to 3 weeks of shelf life when held at the recommended storage conditions of 1 °C and 95% to 100% relative humidity (RH) (Saltveit 2016).

Limited shelf life restricts market access; thus, breeding for cultivars with longer shelf life and improved quality is a priority for lettuce breeding programs (Damerum et al. 2020; Hayes and Liu 2008; Hunter et al. 2017). Shelf life of lettuce may be shortened in subtropical environments such as in the EAA. Other environmental factors including excess water or shading during growth result in lower harvest yield (fresh weight) and an increased respiration rate that translates into poor shelf life in leaf lettuce (Lee et al. 2017). Leaf senescence is the main cause of reduced shelf life, first appearing as yellowing and wilting or moisture loss (Belisle et al. 2021), and eventually becoming darkened, waterlogged, and decayed (Hayes et al. 2014).

Received for publication 15 Apr 2024. Accepted for publication 3 Jun 2024. Published online 5 Aug 2024.
This research was partially funded through a subrecipient grant awarded by the US Department of Agriculture's (USDA) Agricultural Marketing Service through the Florida Department of Agriculture and Consumer Services (Award No. 266097) and the Hatch projects (FLA-ERC-006174 and FLA-HOS-005972). The contents do not necessarily reflect the views or policies of the USDA nor does mention of trade names, commercial productions, services, or organization imply endorsement by the US government.
We thank the Horticultural Sciences Department at the University of Florida for funding provided to C. Belisle and the Florida Vegetable Exchange for assistance with this project. We thank Jesse Murray, Lis Natali Rodrigues Porto, Amanda Carroll and Heriberto Trevino for help in planting and harvesting, Roth Farms and TKM Bengard Farms for cooling and storage, and Adrian Berry for assistance in experimental planning and setup.
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The shelf life of lettuce can also be limited by harvest maturity (Chiesa et al. 2003), including the genetics of the accession and the macro-(commercial fields) and micro-(processing facilities) environmental conditions encountered from harvest through the supply chain (Hunter et al. 2017; Sthapit Kandel et al. 2020). Previous research indicates that the shelf life of lettuce accessions varied across low-O_2 modified atmosphere (MA) environments (Hayes and Liu 2008), suggesting that macro and micro environmental factors affect the expression of this trait. This phenomenon is termed genotype × environment (G × E) interaction (Juenger et al. 2005; Maloof 2003) and can limit improvement of lettuce accessions. The expression of major and minor genomic regions in the lettuce genome controlling shelf life of lettuce is different across multiple environments (Sthapit Kandel et al. 2020), indicating that the G × E interaction influences this trait.

Shelf life in whole-head (Belisle et al. 2021) might be different from processed lettuce (Sthapit Kandel et al. 2020) but further research is needed to understand differences in shelf life between whole-head and processed lettuce. Evaluations of shelf life can be conducted in real time, where the food product is stored under ideal conditions and monitored for quality changes or by using accelerated shelf life testing (ASLT), also referred to as an accelerated aging test (Labuza and Schmidl 1985). This latter method is based on the principle that food products can be stored in environmental conditions that accelerate the rate of quality changes, thereby compressing the time necessary to estimate shelf life (Calligaris et al. 2019). ASLT has been applied to assess the quality and shelf life of perishable crops, including seeds (Carlos and Silva 2021; Coelho et al. 2022), fresh-cut lettuce (Derossi et al. 2016), and other food products (Calligaris et al. 2019). Shelf life of lettuce heads was compressed to 3 d when stored at 15 °C and to 9 d when stored at 10 °C, permitting more rapid separation of accessions than when stored at recommended temperatures (0 to 1 °C) (Belisle et al. 2021).

Lettuce accessions with improved shelf life are needed for growers and shippers in humid, subtropical growing areas such as the EAA. Such accessions should also be tolerant to increasing ambient temperatures as climate change advances. Therefore, lettuce with longer shelf life should be tolerant to the increasing temperatures during the growing season (Sandoya et al. 2024). Although heat tolerance on lettuce has been identified in butterhead, crisphead, leaf, and romaine for the low dessert in the western United States (Lafta et al. 2017, 2021; Sandoya et al. 2024), subtropical germplasm remained untested for heat tolerance. The first objective of this research was to identify genetic variation in heat tolerance and shelf life among lettuce types and accessions grown under humid, subtropical conditions. The second objective was to understand the G × E interaction of shelf life of lettuce accessions adapted to subtropical warmer growing conditions.

Materials and Methods

Plant material. Romaine, crisphead, butterhead, leaf, and Latin accessions were used in these experiments (Supplemental Table 1). The accessions were composed of common commercial cultivars grown in the EAA, elite breeding lines from the University of Florida, Institute of Food and Agriculture Sciences (UF/IFAS) Lettuce Breeding Program, and commercial cultivars not adapted to the subtropical Florida environment. The crisphead cultivar Salinas was used as a control for this study. ‘Salinas 88’, derived from ‘Salinas’, has an established long shelf life (Hayes and Liu 2008; Sthapit Kandel et al. 2020).

Experiment description. In season 1, 18 accessions (17 romaine and 1 crisphead lettuce) were planted on 13 Nov 2019 and 10 Dec 2019 and named as S1H1 and S1H2 (season 1, harvest 1 or 2) (Table 1). In season 2, 43 accessions (17 romaine, 10 crisphead, 5 butterhead, 8 leaf, and 3 Latin lettuce) were planted on 8 Oct 2020, 16 Dec 2020, and 3 Mar 2021 and named using the nomenclature for season 1, as S2H1, S2H2, and S2H3 (Table 1). All lettuce accessions were planted in commercial fields in the EAA near Belle Glade, FL, USA, using a randomized complete block design (RCBD) with three replications, and were grown using recommended local commercial practices (Sandoya et al. 2022). Lettuce seeds were planted in 6-m-long, double-row plots, with 0.20 m spacing between rows; plants were thinned to 0.43-m in-row spacing at the three true-leaf stage. At maturity, 10 randomly selected heads were harvested per plot (n = 3) between 8:00 and 10:00 AM on 15 Jan 2020 and 17 Feb 2020 in season 1 for romaine lettuce only in S1H1 and S1H2 and in season 2 on 9 Dec 2020 for leaf, butterhead, Latin, and romaine lettuce (crisphead lettuce was not harvested due to warm temperature damage) in S2H1; 12 Feb 2021 for romaine, leaf, butterhead, and Latin; and 19 Feb 2021 for crisphead in S2H2 and 22 Apr 2021 and 28 Apr 2021 for crisphead in S2H3 (Table 1).

Table 1. Experiment planting and harvest dates for different lettuce types grown in the Everglades Agricultural Area (EAA) in Belle Glade, FL, USA during two seasons, season 1 (2019–20) and 2 (2020–21), to study the genetic variation of estimated and actual shelf life and horticultural characteristics on lettuce. Minimum, median, and maximum temperatures are presented for two experiments in season 1 and three experiments in season 2. Growing degree days were retrieved from the Agroclimate project at the University of Florida.

				Harvest date			Temp °C[iv]		
Code[i]	Season and harvest[ii]	Accessions (no.)	Planting date	Leaf, romaine, butterhead, Latin	Crisphead	Growing degree days[iii]	Minimum	Median	Maximum
ND[v]	S1H1	18	13 Nov 2019	15 Jan 2020	ND	1,805	14	19	26
ND	S1H2	18	10 Dec 2019	17 Feb 2020	17 Feb 2020	1,971	14	20	26
E1	S2H1	43	8 Oct 2020	9 Dec 2020	ND	2,173	18	23	29
E2	S2H2	43	16 Dec 2020	12 Feb 2021	19 Feb 2021	1,630	12	18	25
E3	S2H3	43	3 Mar 2021	22 Apr 2021	28 Apr 2021	1,760	15	21	28

[i] Code used to graph environments in a genotype + genotype × environment (GGE) biplot.
[ii] Season (S) and Harvest (H).
[iii] Growing Degree Days obtained from Agroclimate Project (University of Florida 2023).
[iv] Data retrieved from the Florida Automated Weather Network station located at the Everglades Research and Education Center.
[v] ND = no data.

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Horticultural traits recorded at harvest. At harvest, lettuce accessions were evaluated for key horticultural traits. In season 1, these traits were evaluated only in one experiment. For each plot, head weight was recorded on a per-plot basis and averaged (n = 10 heads). Head marketability was based on the number of plants within a plot that had uniform head size, freedom from disorders (including tipburn, bolting) and from damage by pests and disease. Bolting incidence (number of plants presenting stem elongation) was recorded on a per-plot basis at harvest, regardless of the developmental stage. The incidence of tipburn disorder was recorded by transversally slicing 10 random heads per plot. Data were recorded in experiments conducted in both seasons, but results are only shown for season 2.

Lettuce quality during storage. Following commercial grading procedures, the outer leaves of crisphead types not folded onto the head were discarded. For romaine, butterhead, leaf, and Latin types, leaves not folded onto the head were discarded if damaged. Whole heads of each lettuce type were then packed in commercial waxed, corrugated cartons (60 cm length × 39 cm width × 33 cm height, 24 heads/carton). Samples were commercially vacuum cooled within 2 h of harvest to an internal core temperature of ∼5 °C, a commercial handling temperature. In season 1, the cooled lettuce heads were placed in coolers (114 L) on top of collapsed waxed, corrugated fiberboard pieces that were placed over 7 to 15 cm of ice to maintain temperature and prevent freeze damage during the ∼4-h transport to the Postharvest Horticultural Laboratory in Gainesville, FL, USA. Upon arrival, lettuce was held overnight at 1 °C (to minimize quality changes) and evaluations began the following day, noted as "day 0". In season 2, the storage test was set up at a commercial packinghouse in Belle Glade, FL, USA, in which following vacuum-cooling, samples were stored in cold rooms at either 5 °C or 15 °C. For both seasons 1 and 2, whole lettuce heads (n = 3 heads/accession, one head per replicate) were stored individually in breathable, high-density polyethylene bags [Narrow Profile Produce Bags (35.6 × 45.7 cm) Model No. S-19156, Uline®, Pleasant Prairie, WI, USA] at 5 or 15 °C. Bags were intentionally folded but unsealed to maintain 95% to 98% RH without inducing a modified atmosphere.

Storage evaluations took place following the ASLT protocol developed by Belisle et al. (2021) and conversions from Cantwell and Suslow (2002) after 1, 3, and 5 d of storage (season 1) or 1, 3, 5, 7, and 9 d of storage (season 2) at 15 °C. For ASLT validation, storage evaluations also took place after 7, 14, 21, and 28 d of storage at 5 °C in season 2. In all storage evaluations, whole heads were subjectively evaluated for overall appearance including leaf and midrib turgidity, yellowing, and deterioration, by rating the visible leaves, which included outer leaves and distal ends of middle and inner leaves. The overall visual appearance was rated using a 9-point, hedonic rating scale, where 1 = extremely poor, not usable; 3 = poor, excessive defects, not salable; 5 = fair, slightly to moderately objectionable defects, lower limit of salability; 7 = good, minor defects, not objectionable; and 9 = excellent, essentially free from defects (Kader et al. 1973). Detailed descriptors for each subjective rating are presented in Belisle et al. (2021).

For the purposes of this study, accessions were considered commercially viable when the estimated shelf life was >14 d, as determined by an overall appearance rating of ≥5, the "fair" threshold rating. These estimates were calculated by multiplying the number of days in which an accession reached a subjective rating of visual deterioration of ≥5 at 15 °C by a factor of 4. This 4-fold factor was previously shown to be proportional to the deterioration rate (Belisle et al. 2021) and is equivalent to a 2-fold increase in respiration that occurs with each 5 °C increase in storage temperature (Cantwell and Suslow 2002). For validation of the ASLT protocol, the actual shelf life was determined by the number of days in which an accession reached the shelf life threshold rating of ≥5 at 5 °C.

Statistical analysis. The estimated shelf life and the horticultural traits were analyzed following an RCBD to obtain an analysis of variance (ANOVA) using PROC GLIMMIX in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). In the model, the random statement consisted of experiment for analysis of bolting and marketability, replicates (experiment) for tipburn, and residual for head weight. Accession, experiment, and their interaction (accession × experiment) were considered fixed statements. Confidence intervals were calculated at the 95% probability level to compare the least square means.

Overall appearance rating was subjectively rated with an ordinal scale, then analyzed with a nonparametric statistical method, using the ranked means (Brunner et al. 2002; Shah and Madden 2004). Shelf life was evaluated during storage over a period of time points and the data were analyzed as repeated measures. The ANOVA (ANOVA-F) was calculated using PROC MIXED using SAS software (SAS version 9.4; SAS Institute Inc.) following Shah and Madden (2004). The fixed statements were replicate, accession, experiment, and the interaction accession × experiment., while "r" and "corr" covariance structure were used as the repeated statements. Relative marginal effects (RMEs) and their confidence intervals (95%) were calculated for each accession throughout storage using the macros LD_CI and F1_LD_F1 (Shah and Madden 2004). Linear contrasts were calculated between each accession and the control 'Salinas' (US Department of Agriculture–Agriculture Research Service in Salinas, CA, USA).

G × E analysis. The shelf life data at 15 °C of season 2 were subjected to genotype and genotype × environment (GGE) biplot analysis as proposed by Yan et al. (2000), in which the most stable shelf life values on lettuce accessions were identified across the three harvests (environments). The G × E analysis was done in R Statistical Software (v4.2.2; R Core Team 2021) using the 'gge' package (Wright et al. 2023). Once analysis was performed, the "which-won-where" and "means vs. stability" biplots were constructed using the "GGEBiplots" package (Dumble et al. 2022) for visualization of results (Yan and Kang 2003; Yan et al. 2007; Yang and Zhu 2005). In a which-won-where biplot, environments (harvests) that had similar performance were grouped as single mega-environments; similarly, the biplot method was used to identify the accessions with similar performance across the environments (harvests). In which-won-where biplots, the visual representation appears as a polygon graphed with the most responsive accessions located at the farthest location from the origin; these accessions are used to draw the sides of a polygon. Lines perpendicular to the polygon sides divide the biplot into sectors called mega-environments that may include one or more environments (harvests). In the stability biplot, accessions are graphed using the average-environment coordination (AEC) view based on their mean performance and stability across environments (harvests). The single-arrowed line in the graph refers to the AEC abscissa and points to the highest trait value (visual rating) across environments (harvests). The AEC ordinate (double-arrowed line) points to the variability of the trait toward both directions. The list of accessions, experiments

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(harvests), and their respective codes are found in Table 1 for genotypes noted as "G" and for environments noted as "E."

Results

Horticultural traits at harvest. Horticultural traits were recorded in only one experiment for season 1 and for the group of romaine accessions tested (ANOVA not shown). Significant differences were found among romaine accessions for head weight ($P < 0.0001$), bolting ($P < 0.001$), marketability ($P < 0.001$), and tipburn ($P = 0.003$) (Supplemental Table 2). In three experiments during season 2, there were significant differences ($P < 0.001$) among accessions for all lettuce types for head weight, bolting, tipburn, and marketability, depending upon the lettuce type (Supplemental Table 2). For head weight, there were significant differences among butterhead ($P = 0.019$) and leaf ($P = 0.001$) accessions, but no differences among romaine ($P = 0.115$) or crisphead ($P = 0.727$) accessions (Supplemental Table 2). Warmer temperatures in season 2 (Table 1) prevented crisphead lettuce from forming heads (head formation data not shown); therefore, head weight was not recorded for this morphological type in this specific experiment, S2H1. Minimum, median, and maximum temperatures were the warmest among the three experiments conducted in season 2 (Table 1). For bolting, significant differences were observed among romaine ($P < 0.001$), butterhead ($P = 0.026$), and leaf ($P = 0.001$) accessions. Significant differences for tipburn were only found among crisphead ($P = 0.001$) accessions, whereas romaine ($P < 0.001$), butterhead ($P = 0.010$), and leaf ($P < 0.001$) accessions showed significant differences for marketability (Supplemental Table 2).

Regarding elite accessions and commercial cultivars, head weight for romaine accessions 60182 and 50100 (UF/IFAS Lettuce Breeding Program) were statistically similar to commercial cultivars Okeechobee, Manatee, and Hialeah in only one experiment in season 1 (Table 2). Only one commercial cultivar (Holbrook) had statistically higher head weight that the accessions previously described in season 1 (Table 2). Similarly, 60183 and 70096 (UF/IFAS Lettuce Breeding Program) were statistically similar to commercial cultivars grown in the region (Floricos 83, Terrapin, Manatee, Holbrook, and Tall Guzmaine) in season 2 (Table 3). The same was observed in crisphead and butterhead types, where crisphead accession H1078 had statistically similar head weight to 'Chosen' and to the nonadapted cultivar, Salinas. Butterhead breeding lines B1190 and 70202 had higher head weight than 'Palmetto' (Table 3).

Incidence of bolting was observed in the beginning (fall harvest, H1) and the end (spring harvest, H3) of season 2. On average, leaf lettuce accessions presented the highest incidence (17%) among all other types tested, whereas bolting was minor in the other morphological types: romaine (4%), butterhead and Latin (1%), and was nonexistent in all crisphead accessions (Table 3). There were specific accessions with no bolting, namely romaine lines 60183, C1145, and butterhead line 70882. Cultivars with no bolting were as follows: for romaine, Floricos 83, Manatee, Tall Guzmaine; for butterhead, La Brillante and Palmetto; and for leaf lettuce, North Star (Table 3). The physiological disorder, tipburn, was only present in one lettuce accession, the crisphead 'Salinas' (6%) (Table 3). Marketability of accessions in season 2 experiments varied from being unmarketable to accessions (including breeding lines) that had >70% marketability, similar to that for the commercial cultivars (Table 3).

Harvest time also affected head weight and other characteristics across experiments. The highest head weight was registered in S2H3, followed by S2H2 and S2H1, respectively. Lettuce accessions in S2H1 had a higher bolting incidence (14%) than

Table 2. Horticultural traits at harvest for head weight (g), bolting (%), tipburn (%), and marketability (%) of lettuce heads grown in the Everglades Agricultural Area (EAA), near Belle Glade, FL, USA. Least square means with 95% confidence intervals (CIs) were measured as an average of one experiment (10 heads × 1 experiment × 3 replicates, n = 30) conducted during season 1 (2019–20). Lettuce accessions are grouped by type and overall values are provided for romaine lettuce tested in this study. Italic formatting differentiates type from the cultivar name.

Type and accession	Head wt		Bolting		Tipburn		Marketability	
	g	CI	%	CI	%	CI	%	CI
Romaine	*654.6*	*[538.6, 668.1]*	*40.7*	*[25.7, 55.8]*	*4.6*	*[−6.7, 16.0]*	*59.3*	*[44.2, 74.3]*
43007[i]	—	—	—	—	—	—	—	—
50098	560.0	[494.4, 625.6]	90.7	[75.6, 105.7]	38.9	[27.5, 50.2]	9.3	[0.0, 24.4]
50100	683.3	[617.7, 748.9]	80.1	[65.1, 95.2]	2.1	[0.0, 13.4]	19.9	[4.8, 34.9]
60182	740.0	[674.4, 805.6]	28.0	[13.0, 43.1]	2.3	[0.0, 13.6]	72.0	[56.9, 87.1]
60183	513.3	[447.7, 578.9]	2.3	[0.0, 17.4]	0.0	[0.0, 11.3]	97.7	[82.6, 112.8]
60184	596.7	[531.1, 662.3]	26.8	[11.8, 41.9]	0.0	[0.0, 11.3]	73.2	[58.1, 88.3]
70096	536.7	[471.1, 602.3]	76.9	[61.8, 91.9]	2.9	[0.0, 14.2]	23.1	[8.1, 38.2]
C1145	580.0	[514.4, 645.6]	3.6	[0.0, 18.7]	0.0	[0.0, 11.3]	96.4	[81.3, 111.5]
'Floricos 83'	576.7	[511.1, 642.3]	29.7	[14.6, 44.7]	0.0	[0.0, 11.3]	70.3	[55.3, 85.4]
'Green Towers'	483.3	[417.7, 548.9]	32.6	[17.6, 47.7]	0.0	[0.0, 11.3]	67.4	[52.3, 82.4]
'Hialeah'	713.3	[647.7, 778.9]	70.6	[55.5, 85.6]	8.8	[0.0, 20.2]	29.4	[14.4, 44.5]
'Holbrook'	946.7	[881.1, 1012.3]	0.0	[0.0, 15.1]	0.0	[0.0, 11.3]	100.0	[84.9, 115.1]
'Manatee'	766.7	[701.1, 832.3]	0.0	[0.0, 15.1]	0.0	[0.0, 11.3]	100.0	[84.9, 115.1]
'Okeechobee'	776.7	[711.1, 842.3]	42.5	[27.4, 57.5]	18.9	[7.5, 30.2]	57.5	[42.5, 72.6]
'PIC'	560.0	[494.4, 625.6]	49.7	[34.7, 64.8]	0.0	[0.0, 11.3]	50.3	[35.2, 65.3]
'Tall Guzmaine'	786.7	[721.1, 852.3]	47.7	[32.7, 62.8]	0.0	[0.0, 11.3]	52.3	[37.2, 67.3]
'Terrapin'	653.3	[587.7, 718.9]	70.7	[55.6, 85.7]	0.0	[0.0, 11.3]	29.3	[14.3, 44.4]
'Salinas'[ii]	603.3	[538.6, 668.1]	0	[0.0, 15.1]	0.0	[0.0, 11.3]	100.0	[84.9, 115.1]

[i] Lettuce accession not planted in this experiment.
[ii] Cultivar Salinas is a crisphead type and was excluded from the statistical analysis for head weight.

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Table 3. Horticultural traits at harvest for head weight (g), bolting (%), tipburn (%), and marketability (%) of lettuce heads grown in the Everglades Agricultural Area (EAA), near Belle Glade, FL, USA. Least square means with 95% confidence intervals (CIs) were measured as an average of three experiments (10 heads × 3 experiments × 3 replicates, n = 90) conducted during season 2 (2020–21). Lettuce accessions are grouped by type and overall values are provided for romaine, crisphead, butterhead, leaf, and Latin lettuces tested in this study. Italic formatting differentiates type from the cultivar name.

	Head wt		Bolting		Tipburn		Marketability	
Type and accession	g	CI	%	CI	%	CI	%	CI
Romaine	*402.9*	*[337.9, 467.9]*	*4.4*	*[1.8, 10.2]*	*0.0*	*[0.0, 0.4]*	*79.0*	*[57.4, 100.7]*
43007	332.4	[268.1, 396.8]	3.6	[0.0, 10.0]	0.0	[0.0, 0.4]	66.0	[42.5, 89.5]
50098	324.2	[259.8, 388.5]	23.3	[16.9, 29.6]	0.0	[0.0, 0.4]	81.1	[57.6, 104.6]
50100	385.8	[321.4, 450.2]	6.7	[0.3, 13.0]	0.0	[0.0, 0.4]	75.4	[54.9, 95.9]
60182	404.6	[340.2, 468.9]	1.6	[0.0, 7.9]	0.0	[0.0, 0.4]	79.7	[59.2, 100.2]
60183	438.9	[374.5, 503.3]	0.0	[0.0, 6.3]	0.0	[0.0, 0.4]	90.6	[67.1, 114.1]
60184	354.8	[285.2, 424.5]	1.6	[0.0, 8.4]	0.0	[0.0, 0.4]	80.4	[58.2, 102.7]
70096	421.9	[357.5, 486.2]	1.7	[0.0, 8.1]	0.0	[0.0, 0.4]	78.3	[57.8, 98.8]
C1145	392.8	[328.4, 457.2]	0.0	[0.0, 6.3]	0.0	[0.0, 0.4]	80.9	[60.4, 101.4]
'Floricos 83'	489.2	[424.8, 553.6]	0.0	[0.0, 6.3]	0.0	[0.0, 0.4]	87.2	[66.7, 107.7]
'Green Towers'	407.7	[343.3, 472.0]	3.9	[0.0, 10.3]	0.0	[0.0, 0.4]	77.5	[54.1, 101.0]
'Hialeah'	406.8	[342.4, 471.2]	1.2	[0.0, 7.5]	0.0	[0.0, 0.4]	66.5	[46.1, 87.0]
'Holbrook'	417.3	[353.0, 481.7]	0.7	[0.0, 7.1]	0.0	[0.0, 0.4]	72.8	[52.3, 93.3]
'Manatee'	445.1	[380.7, 509.5]	0.0	[0.0, 6.3]	0.0	[0.0, 0.4]	88.6	[68.1, 109.1]
'Okeechobee'	362.9	[298.5, 427.3]	17.8	[11.4, 24.1]	0.0	[0.0, 0.4]	73.0	[52.5, 93.5]
'PIC'	381.9	[317.5, 446.3]	3.8	[0.0, 10.2]	0.0	[0.0, 0.4]	73.6	[50.1, 97.1]
'Tall Guzmaine'	411.6	[347.2, 475.9]	0.0	[0.0, 6.3]	0.0	[0.0, 0.4]	87.8	[64.4, 111.3]
'Terrapin'	471.7	[402.0, 541.4]	8.2	[1.4, 15.1]	0.0	[0.0, 0.4]	83.4	[62.9, 103.9]
Crisphead	*433.4*	*[326.6, 540.2]*	*0.0*	*[0.0, 9.8]*	*0.7*	*[0.6, 1.2]*	*68.4*	*[47.0, 89.8]*
'Salinas'	493.8	[384.4, 603.3]	0.0	[0.0, 13.3]	6.67	[5.6, 7.7]	66.4	[45.9, 86.8]
50113	446.3	[336.9, 555.8]	0.0	[0.0, 13.3]	0.0	[0.0, 0.4]	53.6	[33.1, 74.1]
60155	416.1	[351.7, 480.5]	0.0	[0.0, 6.3]	0.0	[0.0, 0.4]	71.7	[48.2, 95.2]
60157	375.6	[248.0, 503.2]	0.0	[0.0, 13.3]	0.0	[0.0, 0.4]	69.9	[46.4, 93.4]
60159	433.7	[324.2, 543.1]	0.0	[0.0, 13.3]	0.0	[0.0, 0.4]	63.1	[42.6, 83.6]
60167	395.7	[286.2, 505.2]	0.0	[0.0, 13.3]	0.0	[0.0, 0.4]	65.4	[44.9, 85.9]
'Chosen'	451.2	[341.7, 560.6]	0.0	[0.0, 13.3]	0.0	[0.0, 0.4]	76.3	[52.8, 99.8]
H1078	466.7	[357.2, 576.1]	0.0	[0.0, 13.3]	0.0	[0.0, 0.4]	78.7	[58.2, 99.2]
H1098	430.3	[320.9, 539.8]	0.0	[0.0, 13.3]	0.0	[0.0, 0.4]	64.7	[44.2, 85.2]
H2020	424.5	[315.0, 534.0]	0.0	[0.0, 13.3]	0.0	[0.0, 0.4]	74.4	[53.9, 94.9]
Butterhead	*276.5*	*[212.2, 340.9]*	*0.9*	*[0.0, 7.3]*	*0.0*	*[0.0, 0.4]*	*50.4*	*[29.9, 70.9]*
70202	302.8	[238.5, 367.2]	2.6	[0.0, 8.9]	0.0	[0.0, 0.4]	60.9	[40.4, 81.4]
70882	250.2	[185.8, 314.6]	0.0	[0.0, 6.3]	0.0	[0.0, 0.4]	33.5	[13.0, 54.0]
B1190	332.1	[267.7, 396.5]	2.1	[0.0, 8.5]	0.0	[0.0, 0.4]	71.7	[51.2, 92.2]
'La Brillante'	213.3	[148.9, 277.7]	0.0	[0.0, 6.3]	0.0	[0.0, 0.4]	27.1	[6.6, 47.6]
'Palmetto'	284.2	[219.9, 348.6]	0.0	[0.0, 6.3]	0.0	[0.0, 0.4]	58.8	[38.3, 79.3]
Leaf	*274.9*	*[209.9, 340.0]*	*17.1*	*[11.6, 23.5]*	*0.0*	*[0.0, 0.4]*	*66.7*	*[49.4, 88.4]*
'Cordoba'	204.0	[139.6, 268.4]	7.1	[0.8, 13.5]	0.0	[0.0, 0.4]	77.6	[57.1, 98.1]
'Galactic'	159.1	[94.7, 223.5]	14.4	[8.1, 20.8]	0.0	[0.0, 0.4]	77.1	[56.6, 97.6]
'North Star'	283.7	[214.1, 353.4]	0.0	[0.0, 6.8]	0.0	[0.0, 0.4]	79.7	[56.2, 103.2]
PI 358001-1	242.3	[178.0, 306.7]	39.4	[33.1, 45.7]	0.0	[0.0, 0.4]	0.0	[0.0, 20.3]
'Red Rage'	271.0	[206.6, 335.4]	35.7	[29.4, 42.1]	0.0	[0.0, 0.4]	77.4	[56.9, 97.9]
'RSX743'	383.3	[318.9, 447.6]	20.5	[14.1, 26.8]	0.0	[0.0, 0.4]	78.1	[57.6, 98.6]
'Tehama'	312.2	[247.9, 376.6]	7.2	[0.9, 13.5]	0.0	[0.0, 0.4]	73.3	[52.8, 93.8]
'Two Star'	343.9	[279.6, 408.3]	12.5	[6.1, 18.8]	0.0	[0.0, 0.4]	73.8	[50.3, 97.3]
Latin	*274.3*	*[203.6, 345.1]*	*0.9*	*[0.0, 7.9]*	*0.0*	*[0.0, 0.4]*	*72.4*	*[48.7, 96.2]*
45060	263.3	[199.0, 327.7]	1.0	[0.0, 7.3]	0.0	[0.0, 0.4]	58.8	[38.3, 79.3]
C1146	278.0	[213.6, 342.4]	1.3	[0.0, 7.6]	0.0	[0.0, 0.4]	75.1	[54.6, 95.6]
C1148	281.6	[198.1, 365.2]	0.4	[0.0, 8.7]	0.0	[0.0, 0.4]	83.4	[53.2, 113.7]

S2H3 (4%); there was no bolting in S2H2. However, a similar percentage of marketability was found across the three harvest times, S2H3 being slightly higher (individual experiment by experiment data not shown).

Estimated and actual shelf life. In season 1, significant differences were observed for estimated shelf life among accessions (n = 18) ($P < 0.001$) during storage at 15 °C, and the interaction of accession × experiment ($P < 0.001$) (Supplemental

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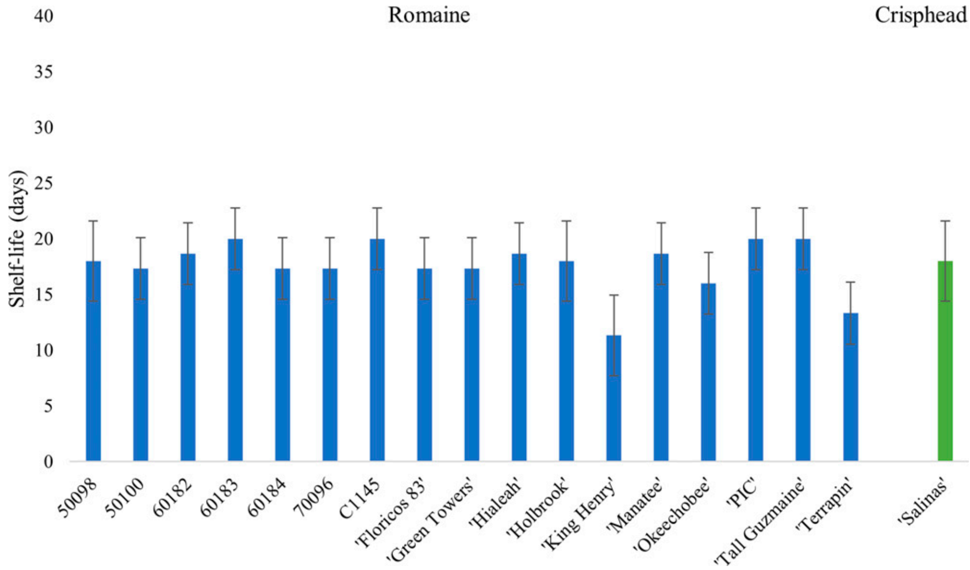

Fig. 1. Estimated shelf life (days) with 95% confidence intervals (CIs; error bars) for 17 romaine lettuce accessions and one crisphead lettuce (control cultivar Salinas) stored at 15 °C in two experiments during season 1 (2019–20) in the Everglades Agricultural Area (EAA) near Belle Glade, FL, USA. Shelf life was calculated as the number of days (multiplied by 4-fold factor) lettuce had an average visual appearance rating of ≥5, which indicated the final day at which lettuce was free of major defects following procedures from Belisle et al. (2021). Shelf life values were measured as an average of two experiments, three heads or replicates each (3 heads × 2 experiments, n = 6) conducted during season 1 (2019–20).

Table 3). The crisphead 'Salinas' maintained a marketable shelf life (≥5 on the rating scale) for 18 d (Fig. 1). One romaine breeding line, 50098, and the cultivar Holbrook had the same shelf life as Salinas. However, two additional breeding lines (60183 and C1145) and two cultivars (PIC and Tall Guzmaine) were estimated to have longer (20 d) shelf life, whereas King Henry maintained acceptable shelf life for only 11 d (Fig. 1). In season 2, a more extensive screening was conducted for visual appearance during storage at 15 °C to estimate shelf life. Significant variation for estimated shelf life was found among accessions (n = 43) ($P < 0.001$), and the accession × experiment interaction ($P < 0.001$), whereas no differences were observed among the three experiments (Supplemental Table 3).

Within romaine accessions, cultivar Holbrook had the longest shelf life (≥5 on the rating scale) estimated to be 28 d, similar to the control Salinas (34 d) (Fig. 2). The breeding line 43007 and

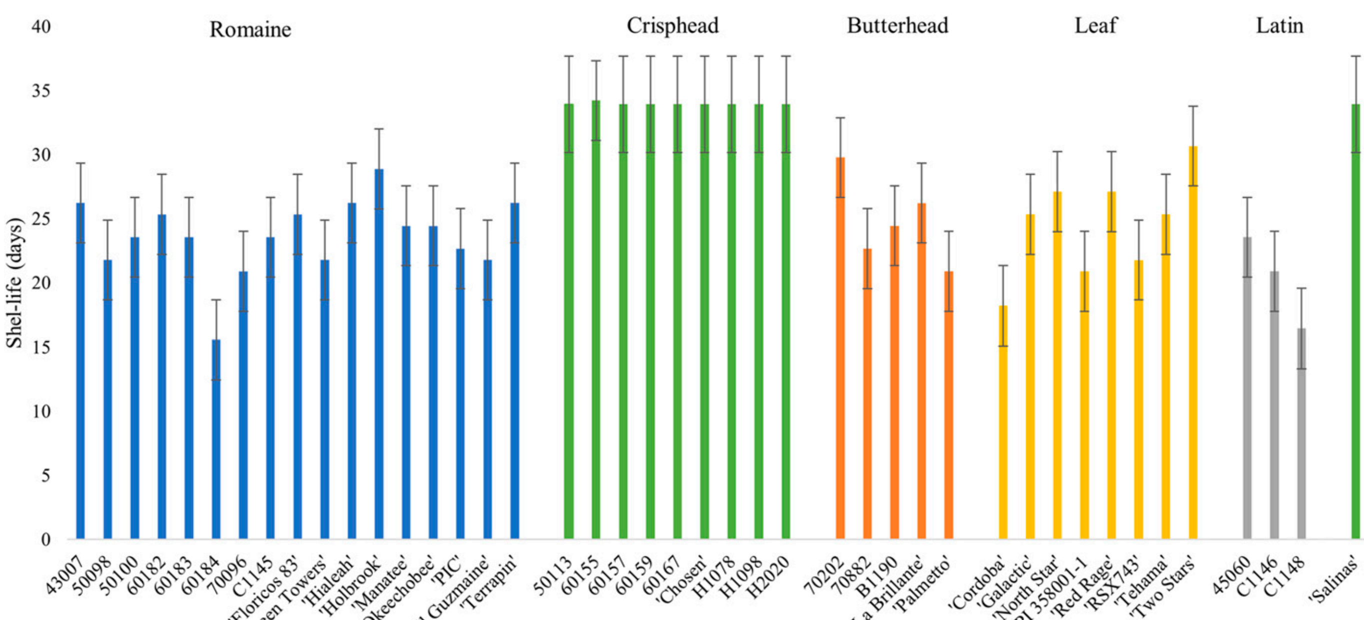

Fig. 2. Estimated shelf life (days) with 95% confidence intervals (CIs; error bars) for lettuce accessions stored at 15 °C in romaine, crisphead, butterhead (except for cultivar La Brillante, which is a Batavia type of lettuce), leaf, and Latin types and control cultivar Salinas in three experiments conducted during season 2 (2020–21) in the Everglades Agricultural Area (EAA) near Belle Glade, FL, USA. Shelf life was calculated as the number of days (multiplied by 4-fold factor) lettuce had an average visual appearance rating of ≥5, which indicated the final day at which lettuce was free of major defects following procedures from Belisle et al. (2021). Shelf life values were measured as an average of three experiments, three heads, or replicates each (3 heads × 3 experiments, n = 9) conducted during season 2 (2020–21).

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cultivars Hialeah and Terrapin also maintained acceptable shelf life, estimated to be 26 d. Oppositely, breeding line 60184 had the shortest shelf life within romaine lettuce, estimated to be 16 d (Fig. 2). Crisphead lettuce accessions maintained the longest shelf life at 15 °C among all types tested. All crisphead accessions, breeding lines and cultivars, had 34 d of estimated shelf life including the control 'Salinas' (Fig. 2). Shorter shelf life was detected in other morphological types. Within butterhead type, breeding line 70202 had the longest estimated shelf life of 30 d, which was statistically similar to Salinas, while cultivar Palmetto had only 21 d of shelf life (Fig. 2). Considering leaf type, cultivar Two Star had a long shelf life of 31 d similar to Salinas, and cultivar Cordoba had the shortest with only 18 d of shelf life. Even shorter shelf life was detected for the three Latin lettuce breeding lines tested in these experiments; while breeding line 45060 had 24 d of shelf life, C1148 only had 16 d of shelf life (Fig. 2). Examples of lettuce deterioration during evaluation at 15 °C are presented in Fig. 3.

In season 2, the 43 accessions were also stored at 5 °C to obtain actual shelf life under cooler storage conditions. Significant variation was observed for actual shelf life at 5 °C across the 43 accessions ($P < 0.001$) (Supplemental Table 3) and for the interaction of accession × experiment ($P < 0.001$) (Supplemental Table 3). There were slight differences on which accessions were similar to 'Salinas' (Fig. 4). Specific accessions within a morphological type had longer shelf life (>20 d). Specifically, 'Green Towers', 'Hialeah', 'PIC', and 'Tall Guzmaine' within romaine, all crisphead breeding lines and cultivars, and PI 358001-1 were similar to 'Salinas'. The preceding accessions deteriorated a little faster than the control except for all the crisphead accessions (Fig. 4).

Levels of deterioration across accessions during storage. Lettuce deteriorated at different rates during storage at 15 °C in both season 1 (Supplemental Table 4) and in season 2 (Supplemental Table 5). Similarly, lettuce deteriorated differently during storage at 5 °C in season 2 (Supplemental Table 5).

After 1 d of storage at 15 °C, significant linear contrasts in 50100 ($P = 0.027$), C1145 ($P = 0.034$), 'Hialeah' ($P = 0.035$), and 'King Henry' ($P < 0.001$) indicated the development of minor defects in these breeding lines and cultivars as demonstrated by their lowest median values and consequently lower RMEs as compared with the control 'Salinas' (Supplemental Tables 4 and 5). After 3 d, the number of significant linear contrasts between the evaluated accessions and the control cultivar (Salinas) increased to nine (50100, C1145, 'Floricos 83', 'Hialeah', 'King Henry', 'Manatee', 'Okeechobee', 'Tall Guzmaine', and 'Terrapin'), indicating that these accessions deteriorated faster than 'Salinas' (Supplemental Tables 4 and 5). After 5 d at 15 °C, only two (50098 and 'Holbrook') of the 17 accessions remained equivalent to 'Salinas' (Supplemental Tables 4 and 5).

In season 2, after 1 d of storage at 15 °C, no significant ($P = 1.000$) linear contrasts were identified, indicating that all 42 accessions maintained similar visual ratings as indicated by their median values and RMEs (Supplemental Tables 6 and 7). On day 3, 60184 ($P = 0.001$), 70096 ($P = 0.004$), 'Okeechobee' ($P < 0.001$), and 'Two Star' ($P = 0.001$) deteriorated significantly faster than the control 'Salinas' (Supplemental Table 7). A higher number of accessions deteriorated at a faster rate than 'Salinas' by day 5 of storage; such differences were visible for 'Terrapin', 45060, B1190, C1146, 'La Brillante', 'Cordoba', PI 358001-1, and 'RSX743', which had lower median values and RMEs than 'Salinas' (Supplemental Tables 6 and 7). By day 7, 25 of the accessions deteriorated significantly faster than 'Salinas' (Supplemental Table 6); only crisphead lettuce accessions maintained similar median values and RMEs as the control (Supplemental Table 7). By day 9, 28 of the accessions deteriorated faster than 'Salinas' (Supplemental Table 6); although,

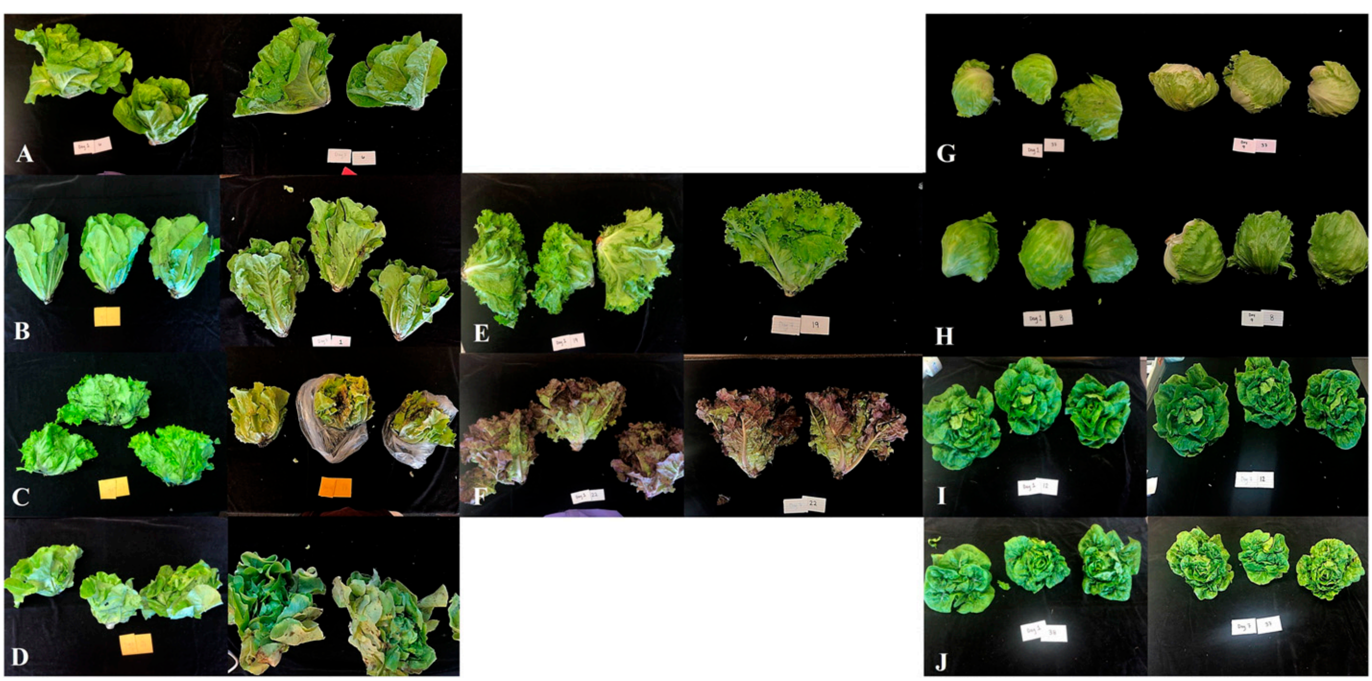

Fig. 3. Paired images of lettuce accessions from five morphological types after 1 d (left) and 5 d (right) of storage at 5 °C. (**A**) Romaine cultivar Floricos 83 and (**B**) breeding line 60184, (**C**) Batavia cultivar La Brillante and (**D**) butterhead breeding line B1190, (**E**) leaf lettuce cultivars Two Star and (**F**) Cordoba, (**G**) crisphead breeding line 60155 and (**H**) cultivar Salinas, and (**I**) Latin lettuce breeding lines 45060 and (**J**) C1148.

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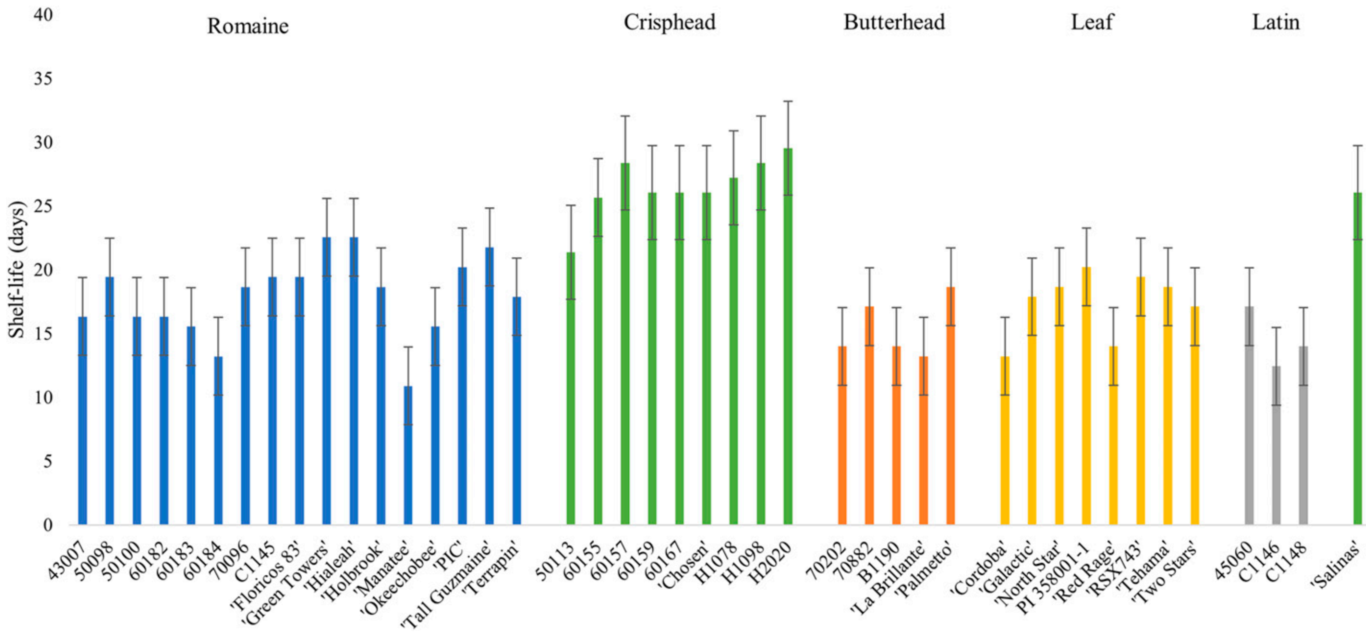

Fig. 4. Actual shelf life (days) with 95% confidence intervals (CIs; error bars) for lettuce accessions stored at 5 °C in romaine, crisphead, butterhead (except for cultivar La Brillante, which is a Batavia type of lettuce), leaf, and Latin types and control cultivar Salinas in three experiments conducted during season 2 (2020–21) in the Everglades Agricultural Area (EAA) near Belle Glade, FL, USA. Shelf life was calculated as the number of days lettuce had an average visual appearance rating of ≥5, which indicated the final day at which lettuce was free of major defects following procedures from Belisle et al. (2021). Shelf life values were measured as an average of three experiments, three heads or replicates each (3 heads × 3 experiments, n = 9) conducted during season 2 (2020–21).

several accessions, mainly in the crisphead type, maintained similar quality as 'Salinas' (Supplemental Tables 6 and 7).

Lettuce accessions also deteriorated at a different rate when stored at a lower storage temperature of 5 °C. As previously stated, in season 2, evaluations were conducted over a longer storage period and started after 7 d. No significant contrasts (P = 0.089) were detected between 'Salinas' and the tested accessions on day 7 (Supplemental Table 8). On day 14, 17 linear contrasts with 'Salinas' were significant (between P = 0.034 and $P < 0.001$) (Supplemental Table 8); these 17 accessions deteriorated at a faster rate than 'Salinas', as indicated by their lower median and RMEs (Supplemental Tables 6 and 8). Similarly, 26 linear contrasts were slightly significant on day 21 when compared with Salinas (Supplemental Table 8); these accessions deteriorated faster than the control cultivar 'Salinas' (Supplemental Tables 6 and 8). Thirty-six linear contrasts between these accessions and cultivar Salinas were slightly significant on day 28 (Supplemental Tables 6 and 8); the crisphead breeding lines 60157, H1078, H1098, and H2020 had a significantly higher median and RME values than control 'Salinas', while the rest of accessions deteriorated faster than the control cultivar or had similar rates of deterioration (Supplemental Tables 6 and 8).

G × E interaction for estimated shelf life. The GGE biplot analysis allowed the effects of genotype and G × E interaction to be dissected on the estimated shelf life of lettuce accessions at 15 °C. In addition, principal component (PC) scores derived from singular value decomposition allowed the construction of which-won-where biplots, and consequently, the identification of superior accessions at specific environments (Yan et al. 2000).

In this research, the first two PCs (PC1 and PC2) together accounted for 85.7% of the total variability for estimated shelf life at 15 °C (Fig. 5A and B). For estimated shelf life, the three environments tested (harvests 1, 2, and 3) were grouped into two mega-environments (Fig. 5A). The first one comprised environments 1 and 3 (S2H1 and S2H3), while the second mega-environment consisted of environment 2 (S2H2) alone. In the first mega-environments, the winning accessions included all the crisphead cultivars and breeding lines tested: 50113 (G19), 60155 (G20), 60157 (G21), 60159 (G22), 60167 (G23), 'Chosen' (G24), H1078 (G25), H1098 (G26), and H2020 (G27) (Fig. 5A). Only two accessions fell within the second mega-environment; the leaf cultivar Two Star (G40) was the winner accession followed by the romaine cultivar Holbrook (G12) (Fig. 5A).

The stability analysis view of the GGE biplot revealed that crisphead breeding lines and cultivars were the accessions with the closest distance to the "ideal accession," based on their estimated shelf life values and stability (Fig. 5B). Despite the lower values of estimated shelf life compared with crisphead accessions, 'Two Star' (G40) and 70202 (G28) were placed furthest from the coordinate origin compared to other accessions, with 70202 being the most stable accession among all (Fig. 5B). Moreover, estimated shelf life was slightly correlated among experiments. Significant correlations were detected between S2H1 and S2H2 (r = 0.61, $P < 0.001$; n = 102), and S2H1 and S2H3 (r = 0.76, $P < 0.001$; n = 120). A less significant correlation was detected between S2H2 and S2H3 (r = 0.38, P = 0.035; n = 129). In addition, estimated (storage at 15 °C) and actual (storage at 5 °C) shelf life were highly correlated within experiments in season 2. Significant correlations among estimated and actual shelf life were identified for S2H1 (r = 0.31, P = 0.002;

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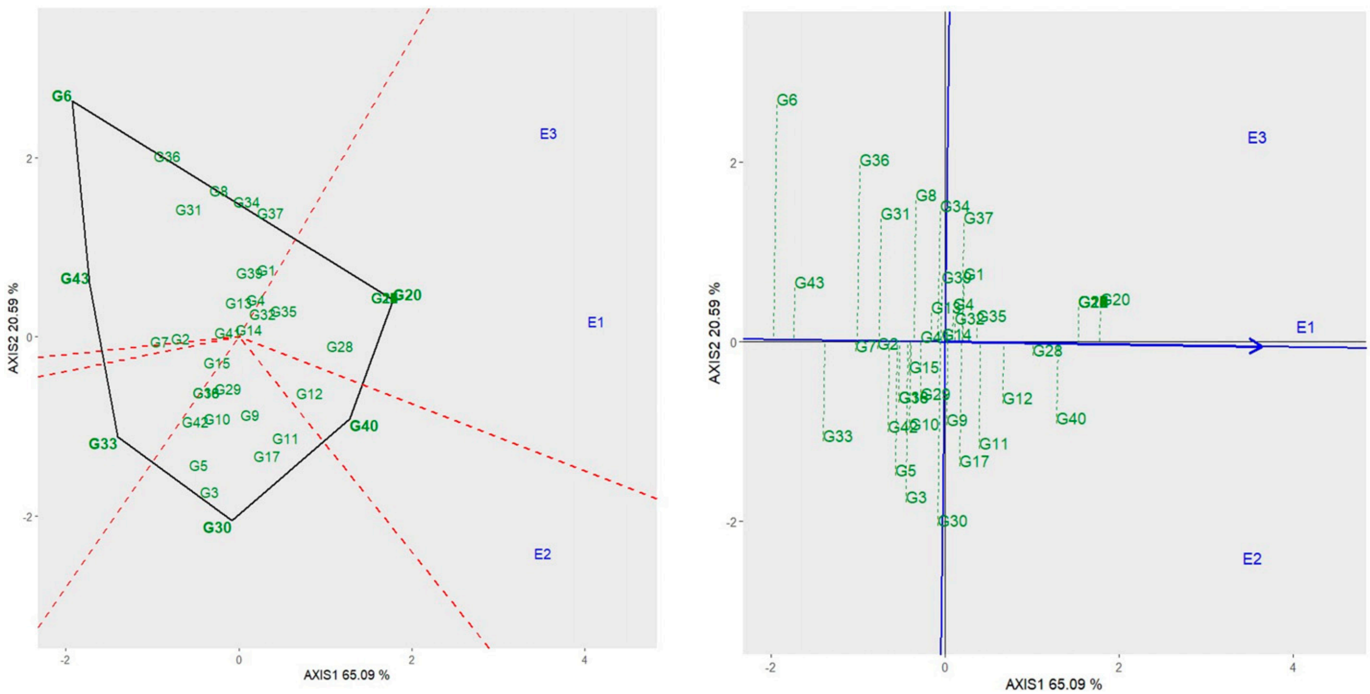

Fig. 5. Which-won-where (**A**) and stability analysis (**B**) of the genotype and genotype × environment (GGE) biplot analysis of estimated shelf life for lettuce accessions stored at 15 °C in romaine, crisphead, butterhead (except for cultivar La Brillante, which is a Batavia type of lettuce), leaf, and Latin types and control cultivar Salinas in three experiments conducted during season 2 (2020–21) in the Everglades Agricultural Area (EAA) near Belle Glade, FL, USA. The values for genotypes are represented as G1 to G43 (refers to genotype 1 to genotype 43) and the values for environments are represented as E1, E2, and E3 (S2H1, S2H2, and S2H3, respectively) as listed in Table 1. Red dotted lines drawn on the which-won-where GGE biplot (**A**) group environments into mega-environments where estimated shelf life performed similarly, and the accessions with the most extreme position within each mega-environment have the highest trait value. All genotypes positioned closest to the x-axis (**B**) are considered the most stable across the different experiments.

n = 102), for S2H2 (r = 0.21, P = 0.019; n = 129) and for S2H3 (r = 0.54, $P < 0.001$; n = 129).

Discussion

Heat tolerance, along with good postharvest quality characteristics including shelf life, is needed when improving lettuce cultivars for longer sustainability of winter/spring production for the subtropics in the United States. Breeding lines with less bolting, no tipburn, acceptable marketability, and head weight comparable to commercial cultivars were identified in this research. Romaine breeding lines 60183, C1145, all crisphead breeding lines, and the butterhead breeding line 70882 were considered tolerant to bolting in these experiments. Similarly, there was no bolting in cultivars Floricos 83, Manatee, and Tall Guzmaine in romaine; Chosen in crisphead; La Brillante and Palmetto in butterhead; and North Star in leaf lettuce. The lack of bolting in these accessions may be attributed to their tolerance to warmer temperatures (Kreutz et al. 2021; Lafta et al. 2017, 2021). Specifically, lettuce breeding lines 50113, 60157, 60159, and H1098 that had no bolting or tipburn also had a shelf life similar to ‘Salinas’ (34 d) according to the ASLT test employed in this investigation. These accessions may provide an additional advantage as sources of breeding for shelf life while maintaining tolerance to warmer temperatures that are currently affecting the lettuce industry in the subtropics (Kreutz et al. 2021).

Lettuce with longer shelf life was previously reported in accessions adapted to temperate environmental conditions (Hayes and Liu 2008; Tudela et al. 2016). When these accessions are grown under warmer temperatures, shelf life is typically reduced because of the higher respiration rate (Tudela et al. 2013). In this research, subtropical accessions were identified with longer shelf life, primarily in breeding lines and cultivars developed by the UF/IFAS Lettuce Breeding Program. These accessions have been intentionally bred to withstand the shorter daylength and warmer conditions commonly encountered during the fall, winter, and spring growing seasons in the subtropics of Florida. Over time, these breeding efforts have presumably conferred some of these subtropical accessions with longer shelf life, which could explain their better performance observed in this study.

Limited information is available on the pedigree of lettuce accessions with longer shelf life identified in this research (Belisle et al. 2023). The crisphead breeding lines (50113, 60157, 60159, and H1098) that slowly deteriorated in this study have common progenitors (Belisle et al. 2023) but are presumably distinct from other lettuce accessions previously identified with longer shelf life. The newly identified subtropical accessions in the present study are unique and may provide additional genomic regions to generate breeding populations that incorporate desired shelf life and horticultural characteristics.

Although most of the lettuce accessions with longer shelf life were found within the crisphead type, including the control ‘Salinas’, multiple breeding lines and cultivars within other lettuce types exhibited a shelf life of 25 d or more at 5 °C. These accessions exceed the standard shelf life of 14 to 21 d previously described for crisphead lettuce (Saltveit 2016). Romaine breeding

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line 43007 (26 d), butterhead breeding line 70202 (29 d), leaf lettuce cultivars North Star (27 d) and Two Star (31 d) are potential candidates to produce breeding crosses that combine extended shelf life and morphological characteristics within a type.

Published reports expressing shelf life as a time point are limited to MAP studies with highly perishable, fresh-cut lettuce (Firouz et al. 2021; Tudela et al. 2013). For example, fresh-cut lettuce was reported to have a shelf life of >21 d when stored in MAP at 4 °C (Sthapit Kandel et al. 2020). The current study was conducted using whole heads of several lettuce types stored at 5 or 15 °C. In this research, whole-head lettuce accessions deteriorated at different rates across storage times, similar to fresh-cut lettuce stored in modified atmosphere packaging (MAP) (Hayes and Simko 2016; Peng and Simko 2023). Differences found across storage time points within a specific accession may be attributed to the genetic attributes of the accession and the field environmental (planting season) conditions (Hayes and Simko 2016; Peng and Simko 2023) rather than the environmental conditions in the fresh-cut processing facility. Similar conditions for packaging, cooling, and processing were used in these experiments. However, a deeper understanding of the pre- and postharvest environmental factors contributing to the expression of lettuce shelf life should be pursued (Lee et al. 2017).

In this study, the G × E interaction was found to be significant in lettuce stored at 15 °C, corroborating that growing lettuce in different planting seasons influenced the estimated whole-head shelf life. Lettuce planted at the beginning of the two seasons and at the end of season 2 faced warmer (night) temperatures that might have decreased the shelf life of lettuce, especially in S1H1, S2H1, and S2H3. Lettuce is a crop better adapted to maximum daytime temperatures of 25 °C (Hayes 2018); in these three experiments the maximum temperatures were 26, 29 and 28 °C, respectively. This is in agreement with previous findings by Sthapit Kandel et al. (2020) and Simko and Hayes (2018) who reported that shelf life of fresh-cut lettuce in MAP was dependent upon the growing environment. It has been proven that warm nighttime temperatures dramatically decrease the quality of lettuce in terms of yield and marketability (Kreutz et al. 2021), and as seen in this research, warmer temperatures also decreased shelf life. Furthermore, the G × E interaction was also significant at the lower storage temperature (5 °C) because the rate of deterioration in lettuce during postharvest storage is temperature-dependent (Belisle et al. 2021; Ben-Yehoshua 1985; Cantwell and Suslow 2002).

A closer analysis of the G × E interaction in season 2 indicated that the three growing environments (experiments) were grouped into two mega-environments; the first mega-environment consisted of S2H1 and S2H3, and the second mega-environment consisted of only S2H2. The first and third experiments from season 2 (S2H1 and S2H3) were conducted at the beginning and end, respectively, of the lettuce growing season in Florida. During these periods, both average daily and nightly temperatures were higher compared with middle-season (S2H2) conditions. The warmer temperatures observed in S2H1 and S2H3 suggest that these two environments negatively impacted the shelf life of lettuce accessions in a similar manner. Although this initially suggests that breeding strategies for shelf life should consider the planting time (initial, middle, and final periods of the growing season), additional studies are needed to further understand the nature of the G × E across multiple years and environments.

Shelf life is genetically complex and highly influenced by the environment in which lettuce is grown or processed (Simko and Hayes 2018; Sthapit Kandel et al. 2020). For instance, crisphead lettuce was not evaluated for visual ratings in S2H1 because this morphological lettuce type did not form marketable heads, presumably because of the warmer temperatures at the beginning of the lettuce season. Warmer temperatures influence the G × E in head weight and its related traits including marketability for crisphead lettuce indicating that these traits are of a crossover interaction (Lafta et al. 2021) and should be included when evaluating breeding lines in multiple trials.

Accessions with longer shelf life were identified in this study, especially within crisphead lettuce. Longer shelf life accessions can be used as sources of germplasm in lettuce breeding programs, especially the ones adapted to subtropical environments similar to that of Florida. The accessions identified with longer shelf life had no bolting and maintained acceptable yield and marketability, despite being planted in warmer environments, suggesting potential heat tolerance in these accessions. Developing mapping and breeding populations that combine both favorable horticultural characteristics and longer shelf life under warmer growing conditions should be a priority for lettuce genetics studies and breeding purposes, especially due to climate change. The time these experiments were conducted was a key environmental factor that negatively influenced lettuce shelf life and other horticultural traits across two seasons as indicated by the significant G × E interactions. Under these growing conditions, accessions with longer shelf life should have tolerance to warmer temperatures especially in the subtropics. Selection of heat-tolerant germplasm with longer shelf life should still be conducted in multitrials because the G × E for heat-tolerant–related traits proved to be crossover in previous investigations.

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