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Heat-tolerant Lettuce Germplasm (*Lactuca sativa* L.) Identified in Romaine and Butterhead Types for Warmer Plantings

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Abstract. **Warmer temperatures during crop production are not desirable for a cool-season crop such as lettuce (*Lactuca sativa* L.). Lettuce is among the top 10 most consumed vegetables in the United States. Production of this vegetable is concentrated mostly in temperate areas of California, and during the wintertime in Arizona and Florida as a result of their mild climatic conditions. Heat-tolerant cultivars are needed for the leafy vegetable industry to continue thriving. However, there is very little information on heat-tolerant germplasms of lettuce that can be used as a source to improve heat tolerance in lettuce. This is particularly important in romaine and butterhead lettuce, which are two morphological types with increasing demand in the market. Therefore, research was conducted to identify germplasm that performs acceptably in warmer regions in the western United States. This investigation also aimed to understand the reaction of varieties to different environments, which could help plant breeders select and evaluate lettuce plants during the breeding process. Twenty-three and 25 accessions of romaine and butterhead lettuce, respectively, were planted in five trials near Holtville, CA, USA: Five Points, CA, USA, under warmer temperatures and Salinas, CA, USA, under cooler temperatures. Romaine genotypes Bambi, Blonde Lente a Monter, Medallion MT, and Red Eye Cos; and butterhead genotypes Butter King and Margarita had no bolting, an acceptable head weight, short cores, and acceptable head height. Head weight and related traits (including core length, height, width, etc.) and heat-related disorders were significantly different across multiple experiments, indicating genetic variation. The major component of the phenotypic variation in these experiments was a result of environmental factors. Therefore, plant breeders may still need to evaluate progeny in multiple trials and multiple locations to select heat-tolerant romaine and butterhead lettuce effectively.**

Cultivated lettuce (*Lactuca sativa* L.) is one of the most consumed vegetables in the United States (US Department of Agriculture, National Agricultural Statistics Service 2018). The United States is the second largest lettuce producer worldwide just after China (Food and Agriculture Organization of the United Nations 2021), with most of the production occurring in California. Most of the lettuce crop is planted in temperate coastal areas in the Salinas and Santa Maria valleys of California, USA, where production can occur all year long (Simko et al. 2014), except during the lettuce-free period in the tricounty area of the Salinas Valley (Monterey, San Benito, and Santa Cruz, CA, USA) because of regulations to interrupt the transmission of the Lettuce mosaic virus (*Potyvirus*). Lettuce production transitions from the coastal areas to the San Joaquin Valley for fall and spring production, and then to the Coachella, Imperial, and Palo Verde valleys in California, and Yuma Valley in Arizona for winter production (Simko et al. 2014). A smaller proportion of lettuce is produced in Florida in the wintertime during the cooler months (December–April/May). Many other states produce lettuce in very limited and small proportions during fall and spring (US Department of Agriculture, National Agricultural Statistics Service 2018) when temperatures are ideal for lettuce production.

Several of the lettuce production areas in the United States are currently prone to warmer temperatures during the growing season (Lafta et al. 2021). Such locations include inland California, southern California, and Arizona. Warmer temperatures have affected early (October) and late (April) plantings in Florida, which in turn have shortened the lettuce season (Kreutz et al. 2021). Warmer weather first affects lettuce establishment because the crop germinates better at temperatures no higher than 29 °C; lettuce is a thermosensitive species incapable of germinating at warmer temperatures (Negm et al. 1972; Thompson et al. 1979). Another challenge that the crop faces is warmer temperatures during the crop cycle. Warmer temperatures during lettuce cultivation increase yield losses because the crop does not reach the desired marketability (Ryder 1999), and lettuce is more prone to physiological disorders such as bolting and tipburn (Jenni and Hayes 2010; Jenni and Yan 2009; Ryder 1999). In addition to temperature, bolting is affected in part by daylength, because lettuce is a species that is sensitive to light hours during the production cycle (Ryder 1999).

Climate change calls for integrated management strategies to adapt to the warming planet. A unique approach to overcome the difficulty of producing lettuce during warmer temperature is to develop heat-tolerant lettuce cultivars using classic breeding approaches. Leaf and iceberg lettuce varieties have been identified to have a tolerance for warmer temperatures when planted in warmer conditions (Holmes et al. 2019; Lafta et al. 2017, 2021). Some of the identified heat-tolerant accessions have less tipburn, bolting, and other physiological damage when cultivated in harmful conditions (Lafta et al. 2017, 2021). Identifying additional heat tolerance in all types of lettuce is needed because breeders will have to improve tolerance in all horticultural types to meet market demands. In addition, consumer preferences could switch to other lettuce types. As seen in the United States, iceberg lettuce was the most planted morphological type until the 1990s, but then the popularity of romaine lettuce in the market changed this situation (Hayes 2018a). Currently, head lettuce (including iceberg and butterhead types) account for 39% of the total area planted; romaine lettuce alone represents 36% and leaf lettuce, 25% (US Department of Agriculture, National Agricultural Statistics Service 2022). Therefore, heat-tolerant lettuce cultivars of all types must be developed.

Heat tolerance was observed in leaf and crisphead lettuce germplasm planted in field locations with warm temperatures (Lafta et al. 2017, 2021). These lettuce accessions produced an acceptable head weight (HW) and had less tipburn and bolting. Plant breeders could benefit from the identification of such characteristics in additional horticultural types, because recovering offspring with desirable characteristics could be somewhat faster than those derived from crosses. For instance, a cross of romaine with another romaine could produce marketable heads in a shorter period. Therefore, the main objective of this research was to identify heat-tolerant germplasm within a selection of romaine and butterhead lettuce planted in warmer environments. Furthermore, this research was designed to understand the genotype × environment (G × E) interaction for romaine and butterhead for HW and heat tolerance-related traits, including bolting and tipburn, under warmer temperatures.

Material and Methods

Germplasm studied. A set of 23 romaine and 25 butterhead accessions were used to identify heat tolerance when planted in five environments in field experiments (Table 1, Supplemental Fig. 1). These genotypes were selected from preliminary evaluations of 3-week-old lettuce plants. Seedlings were exposed to heat stress (day/night temperatures, 43/35 °C) for 7 d and were then evaluated for leaf damage and seedling survival. These accessions were selected because they showed less plant damage. These preliminary experiments were conducted with more than 500 accessions, and were conducted with the goal of narrowing the number of accessions tested further in field conditions. Most of these accessions are registered in the US Department of Agriculture (USDA)-Agricultural Research Service (ARS) Germplasm Resource Information Network (GRIN)-National Plant Germplasm System (NPGS) and were provided with a PI number though the USDA-ARS GRIN-NPGS. For the purpose of our study, these accessions were maintained and increased at the USDA-ARS (Salinas, CA, USA), where the seed for these experiments was produced.

Field experiments and locations. Five field experiments were conducted in three different locations and different planting dates during 2012 and 2013. Two experiments were conducted in the San Joaquin Valley at the University of California (UC), West Side Research and Extension Center, Five Points, CA, USA. Two experiments were planted in the Imperial Valley at the UC Desert Research and Extension Center, Holtville, CA, USA. One experiment was planted at the USDA-ARS, Salinas, CA, USA (Supplemental Fig. 1). The experiments planted in March and May correspond with off-season plantings for southern production areas in California. These experiments were planted in Five Points, CA, USA, on 5 Mar 2013 (P313) and 8 May 2012 (P512); and in Holtville, CA, USA, 7 Mar 2013 (E313) and 9 May 2012 (E512). The June planting in Salinas, CA, USA, corresponds to the summer in-season plantings in the Salinas Valley, and the experiment was planted on 26 Jun 2012 (S612). Further details on several environmental variables of these locations can be found in Table 2. Additional details regarding the management of these field experiments can be found at Lafta et al. (2017).

Each genotype was direct-seeded in a 6-m-long row in a 1-m-wide bed with two rows separated by 35 cm. Plants were hand-thinned to be 30 cm apart in the row 3 weeks after

Table 1. Romaine and butterhead accession PI (and cultivar when available) planted in three locations in Five Points, Holtville, and Salinas, CA, USA, in five different experiments for heat tolerance studies.

PI	Romaine cultivar	Code[i]	PI	Butterhead cultivar	Code
NA[ii]	Bambi	Bam	NA	Amandine	Ama
PI 536849	Barnwood Gem	Bar	NA	Amber	Amb
NA	Black Seeded Bath	Blc	PI 441729	Aurelia	Aur
PI 634670	Blonde Lente a Monter	Blo	NA	Baronet	Bar
PI 557564	Clemente	Cle	PI 536697	Bibb	Bib
PI 602945	Gladiator	Gld	PI 536831	Butter King	Buk
PI 612126	Green Forest	Gre	PI 536804	Buttercrunch[iii]	Buc
NA	Hazera Cos	Haz	NA	Dark Green Boston	Dgb
PI 606778	Hearts Delight	Hrt	NA	Edogawa Salad	Edo
PI 635066	Heavy Heart	Hvy	NA	Envoy	Env
PI 667699	Infantry	Inf	NA	Golden Bibb	Gol
PI 667700	Jericho	Jer	NA	Hamlet	Ham
PI 595620	King Henry	Kin	PI 615067	Indiana Amish	Ind
PI 617959	Little Gem	Lit	NA	Interrex	Int
NA	Medallion MT	Med	PI 596550	Margarita	Mar
PI 342479	68081	P34	PI 667704	Novir	Nov
PI 547105	Red Eye Cos	Rey	NA	Novita	Not
NA	Rubens Red	Rub	NA	Oberto	Obe
PI 678902	Skyway	Sky	NA	Okayama	Oka
PI 665207	Sweet Valentine	Swt	NA	Oleta	Ole
PI 665208	Tall Guzmaine	Tal	NA	Parmanta	Par
PI 543959	Valmaine	Val	PI 381933	Ga 1-9-1-2b	P38
PI 536774	Winter Density	Win	PI 617948	Red Riding Hood	Rrh
			NA	Sensation	Sen
			PI 612659	Summer Bibb	Sum

[i] Code used for genotypes to generate a biplot for the genotype × environment interaction analysis.
[ii] Not available.
[iii] This PI number is reported for this accession, one in lowercase and one in uppercase. The one in uppercase is 'BUTTERCRUNCH' (PI W6 3743) according to https://npgsweb.ars-grin.gov/gringlobal/search.

Table 2. Specifications (locations and month) for experiments conducted for heat tolerance for romaine and butterhead lettuce accessions, including temperature (maximum, mean, and minimum), growing degree days, wind velocity, daylength, and precipitation for where these experiments were conducted.[i]

		Temperature (°C)						
Location	Mo.	Maximum	Mean	Minimum	GDD[ii]	Wind velocity (km/h)	Daylength	Precipitation (cm)
Five Points, CA, USA								
P313[iii]	March	20.0	13.3	6.7	3.3	12.9	11 h 57 min	0.08
	April	24.4	16.1	8.3	6.1	12.9	13 h 8 min	0.08
P512	May	30.6	20.6	11.1	10.6	14.5	14 h 7 min	0.01
	June	33.3	23.3	13.3	13.3	16.1	14 h 37 min	0
	July	36.1	26.1	16.1	16.1	11.3	14 h 23 min	0
	August	37.8	27.2	17.2	17.2	12.9	13 h 31 min	0
Holtville, CA, USA								
E313	March	26.1	17.8	9.4	7.8	11.3	11 h 58 min	0
	April	30.6	21.7	13.3	11.7	12.9	13 h 00 min	0.01
E512	May	36.7	27.2	18.3	17.2	11.3	13 h 51 min	0
	June	40.6	31.1	22.2	21.1	12.9	14 h 18 min	0
	July	41.1	32.8	25.6	22.8	11.3	14 h 5 min	0.08
	August	41.1	35.0	28.3	25.0	9.7	13 h 20 min	0
Salinas, CA, USA								
S612	June	21.7	16.7	11.1	6.1	14.5	14 h 39 min	0.01
	July	21.1	17.2	12.8	6.7	12.9	14 h 24 min	0
	August	21.7	17.2	12.8	6.7	12.9	13 h 32 min	0
	September	21.7	17.2	12.2	6.7	9.7	12 h 23 min	0

[i] Environmental data were retrieved from Weather Underground (https://www.wunderground.com/history).
[ii] Growing degree days based on 10 °C.
[iii] Code used for environments to generate a biplot for the genotype × environment interaction analysis. The location of the code in the table indicates the month the experiments were planted.

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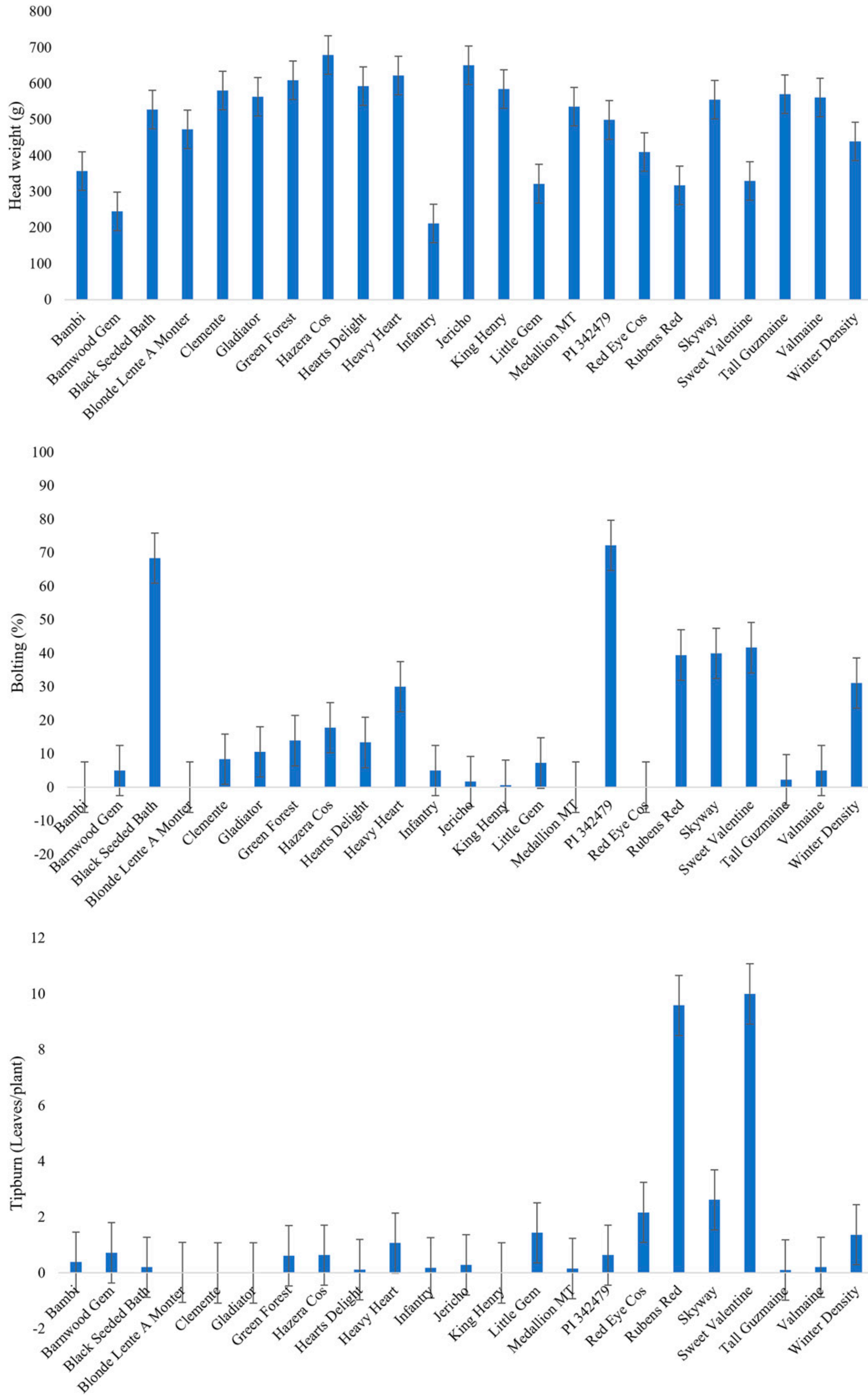

Fig. 1. Least square means for head weight (**top**) and associated heat-related traits of bolting (**middle**) and tipburn (**bottom**), in romaine lettuce grown in five experiments conducted in Holtville, CA, USA, Mar 2013 (E313) and May 2012 (E512); Five Points, CA, USA, Mar 2013 (P313) and May 2012 (P512); and Salinas, CA, USA, Jun 2012 (S612). The bars indicate the average (n = 200) of four replicates and 10 plants per replicate on each of the five experiments. Error bars are 95% confidence intervals. Least significant difference values are for head weight (= 70.9), bolting (= 10.5), and tipburn (= 1.5).

planting. The experiments were maintained using standard practices for lettuce in the southern and central coast of California (Lafta et al. 2017).

Data collected. Ten plants of each genotype were harvested randomly within a plot and evaluated for several traits. All data collection occurred at harvest on each of the experiments at different days postplanting (DPP). Harvest occurred on 15 May 2013 for P313 (71 DPP), 10 Jul 2012 for P512 (63 DPP), 9 May 2013 for E313 (63 DPP),

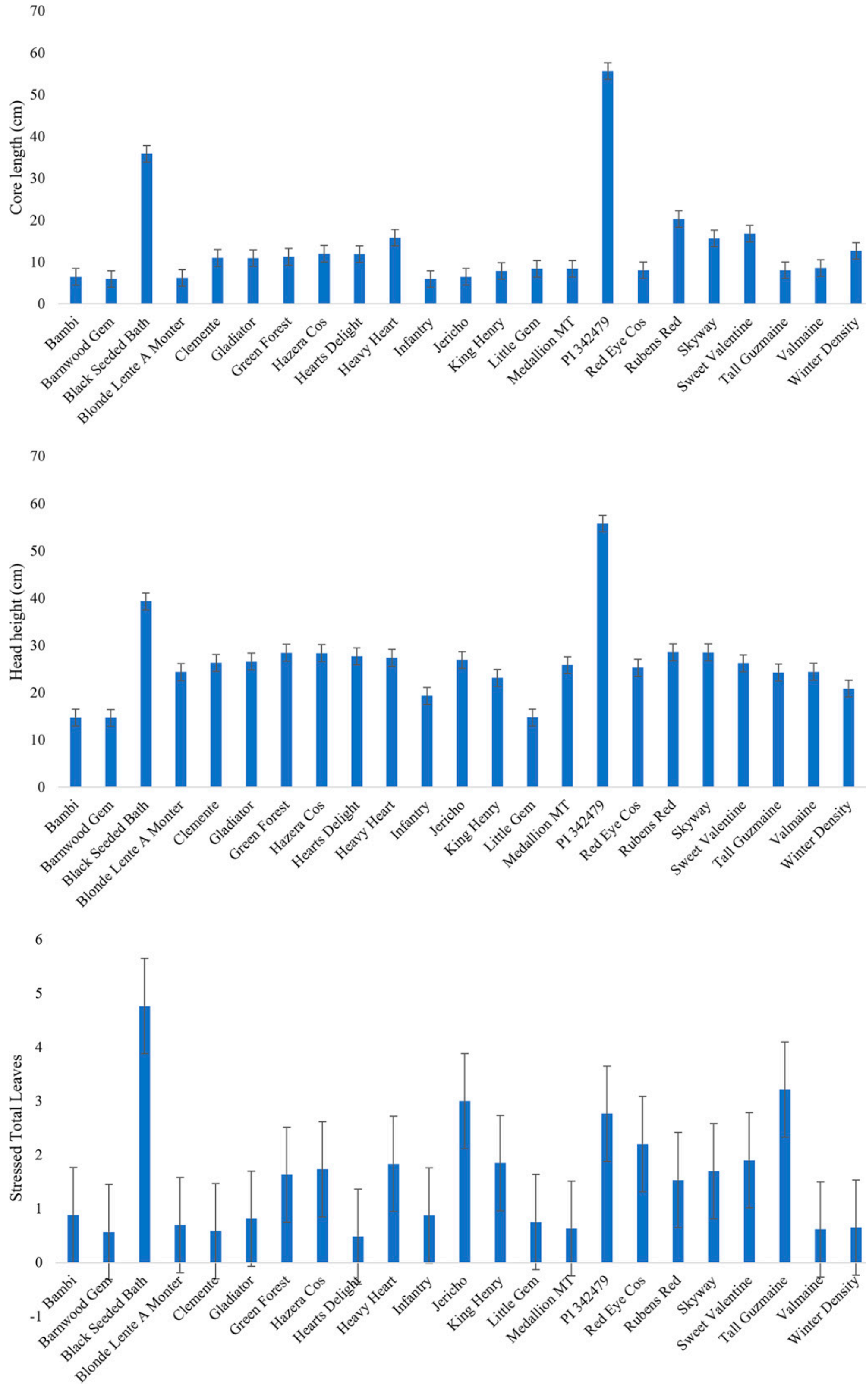

Fig. 2. Least square means for core length (**top**), head height (**middle**), and stressed total leaves (**bottom**) of romaine lettuce planted in in five experiments conducted in Holtville, CA, USA, Mar 2013 (E313) and May 2012 (E512); Five Points, CA, USA, Mar 2013 (P313) and May 2012 (P512); and Salinas, CA, USA, Jun 2012 (S612). The bars indicate the average (n = 200) of the five experiments. Error bars are 95% confidence intervals. Least significant difference values are for core length (= 2.7), head height (= 2.4), and stressed total leaves (= 1.1).

2 Jul 2012 for E512 (54 DPP), and 20 Aug 2012 for S612 (55 DPP).

Specific data were collected in both types of lettuce. With regard to yield (fresh HW), this trait was recorded on 10 plants on per-plot basis and is expressed per HW in grams. Core length (CL) was measured from the cut base to the apex of the stem after splitting the lettuce head in half. Bolting was recorded by counting the number of plants that showed signs of bolting (stem elongation) and is expressed as percentage of bolted plants on a per-plot basis.

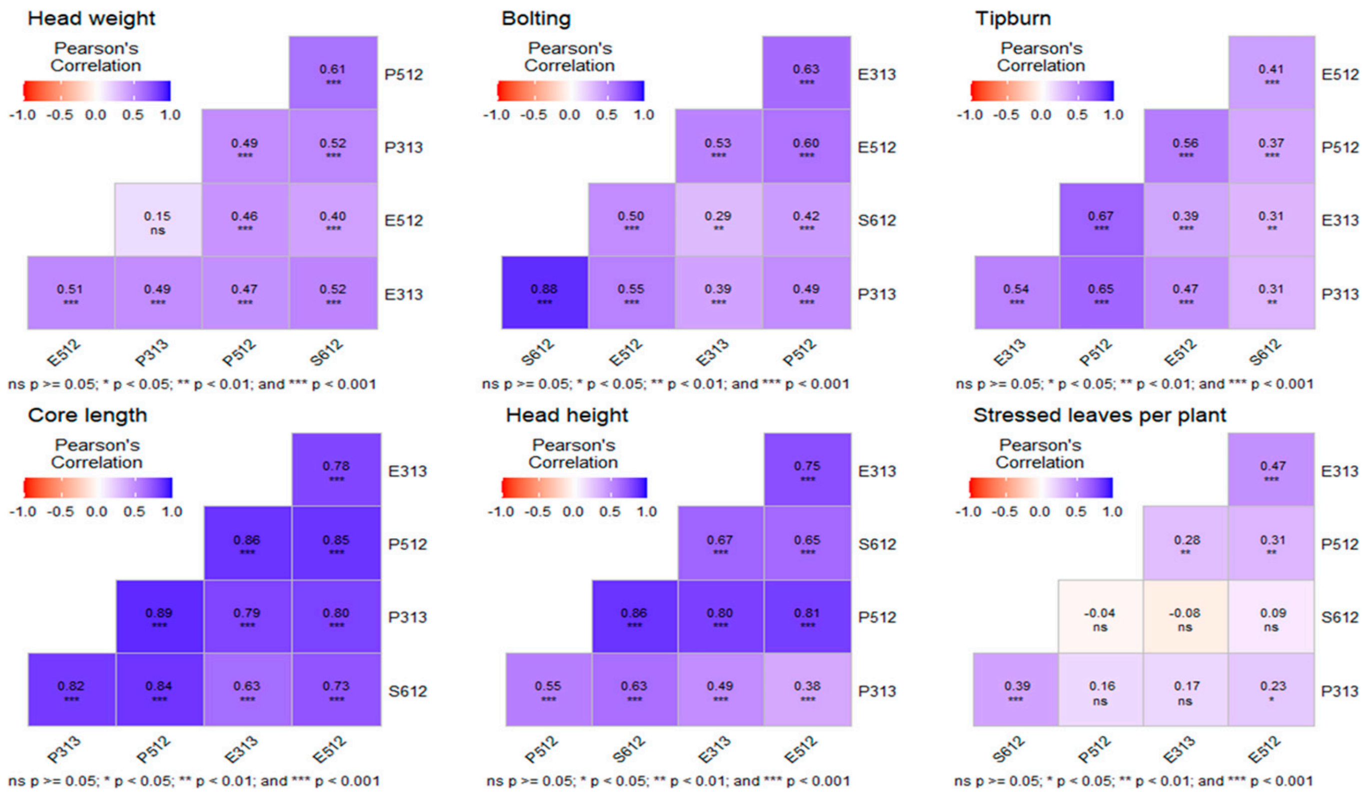

Fig. 3. Correlation coefficients for traits including head weight (**top left**), head height (**bottom middle**), core length (**bottom left**), bolting (**top middle**), tipburn (**top right**), and stressed leaves per plant (**bottom right**) registered in romaine lettuce across five experiments conducted in Holtville, CA, USA, Mar 2013 (E313) and May 2012 (E512); Five Points, CA, USA, Mar 2013 (P313) and May 2012 (P512); and Salinas, CA, USA, Jun 2012 (S612).

The physiological disorder of tipburn was assessed by counting the number of leaves per plant with visible tipburn damage.

Specific data were collected for romaine lettuce. Head height was determined using 10 plants per plot and is recorded in centimeters. Stressed total leaves, or the number of leaves with lesions caused by the heat, on a plant was recorded on a per-plant basis.

Specific data were collected for butterhead lettuce. The ratio of HW to CL (measured in grams per millimeter) is an indicator of maturity and is related to head quality; the higher the ratio, the more mature and more filled the lettuce head. Market maturity (firmness) was evaluated based on the following scale: 1 point, soft or loose head; 2 points, medium head filled; 3 points, firm, good for the market; 4 points, hard, past market maturity; and 5 points, extra hard, overmature.

Experimental design and statistical analysis. Each experiment was planted as a randomized complete block design (RCBD) with four blocks or replicates. For each replicate, a sample of 10 plants per plot were chosen randomly to record the different traits under investigation. Analysis of variance (ANOVA) was conducted using statistical software (JMP, ver. 9.4; SAS Institute Inc., Cary, NC, USA) and was computed as an RCBD to determine the effects of E, G, and G × E interaction on HW and other characteristics of romaine and butterhead lettuce. During the ANOVA, environment was considered using a location-year combination. Genotypes were considered fixed effects, and replicates nested to the environment were considered random effects. Additional ANOVAs were calculated for each experiment, and means (genotype) were separated by Fisher's least significant difference at $P = 0.05$ and by 95% confidence intervals. A Pearson's correlation analysis was also conducted to identify associations among the environments (experiments) for the traits measured.

Analysis of G + G × E interaction (GGE) biplots (Yan and Kang 2003; Yan et al. 2007) was conducted for all of the recorded traits. The biplot is based on the site regression linear–bilinear (multiplicative) model (Yan et al. 2007). In the biplot, a polygon is created by connecting the genotypes that are in the extremes across the four sides of the biplot. Then, a perpendicular line is graphed from the origin to each one the sides of the polygon (forming a 90° angle) to construct mega environments. Two analyses were performed on the recorded traits in the biplot.

The first analysis was the "which-won-where" method of the GGE biplot that was used to visualize the similarities among the environments within a mega-environment and to determine which genotypes are adequate for a specific mega-environment. One mega-environment can be defined as the representative environment in which a genotype has the best performance.

The second analysis was mean vs. stability of the GGE biplot because it facilitates genotype comparisons based on mean performance and stability across environments within a mega-environment (Yan et al. 2007). This method is based on an "ideal" hypothetical environment. The GGE analysis was done using a statistical software [GGE biplot (Yan 2001)].

Results

Romaine lettuce germplasm is tolerant to warmer plantings. Romaine genotypes were statistically ($P < 0.000$) different for HW and heat-related traits such as tipburn, bolting, and the number of stressed leaves per plant in lettuce produced in different environmental conditions (Supplemental Table 1). The HW of 'Hazera Cos' was the greatest across the five environments tested in our study (Fig. 1). 'Hazera Cos' had little tipburn, but a significant number of bolted plants and few stressed leaves (Figs. 1 and 2). Genotypes with an acceptable HW, with significantly less HW than 'Hazera Cos', such as Blonde Lente A Monter, Medallion MT, and Red Eye Cos, had no bolted plants and little tipburn, and very few stressed leaves per plant (Figs. 1 and 2). Likewise, other genotypes such as King Henry, Jericho, Infantry, Tall Guzmaine, and Valmaine had very few bolted plants, and minor tipburn and stressed leaves, but their HW was not as high as the genotypes mentioned previously. In our study, germplasms with a high percentage of bolted plants, tipburn, and stressed leaves were detected; these disorders were associated with lower HW for several accessions, such as Black Seeded Bath and PI 342479 (Figs. 1 and 2).

Statistical ($P < 0.000$) differences were observed for head height and CL (Supplemental Table 1). Romaine lettuce is marketed for whole-head consumption and for packaging of "romaine hearts." Therefore, head height depends on the market requirement, and there was significant variation among the romaine genotypes tested for this trait in our study. Head height varied from 15 cm in Bambi, 'Barnwood Gem', and 'Little Gem' to 56 cm in PI 342479 (Fig. 2). However, the most heat-tolerant genotypes, the ones with no signs of bolting and minimum tipburn (Fig. 2) (Blonde Lente a Monter, Red Eye Cos, and King Henry), had a medium head height of 25 cm, except for Bambi, which had a shorter head height. Similarly, these genotypes had an average CL (range, 6–8 cm) for the industry, which places them as ideal candidates for breeding romaine lettuce with a shorter CL (Fig. 2).

Romaine lettuce performs differently according to the planted environment. Significant differences ($P < 0.000$) were observed among the environments tested for all traits, including HW, plant height, CL, bolting, tipburn, and stressed leaves in the analysis of variance (ANOVA) (Supplemental Table 1). The environment was the most important component of the total variation based on the percentage of their phenotypic variance during these experiments. A particularly greater variance was detected for HW, bolting, and number of stressed leaves than for plant height, CL, and tipburn (Supplemental Table 1).

Planting lettuce in May 2012 in Holtville, CA, USA (E512), and in Five Points, CA, USA (P512), and in Mar 2013 in Five Points, CA, USA (P313), resulted in less HW, more bolted plants, and a greater tipburn percentage (Supplemental Tables 2 and 3). Mean temperatures in these environments were more than 26 °C during the growing season, except for the experiment in P313 (Table 2). The lettuce crop faced the warmest temperatures between 30 and 36 °C in Holtville, CA, USA, in the May planting as this was the environment (E512) that resulted in the greatest number of bolted plants and more plants with stressed lettuce leaves (Supplemental Table 3). It was also noted that the minimum temperatures were also the lowest in E512, especially from Jun to Aug 2012 (Table 2). Bambi produced heavier heads in the warmer environments, except the May planting at Holtville, CA, USA, than in the Salinas Valley (Supplemental Table 2), with no or little increase in bolting or tipburn (Supplemental Table 3).

Stable romaine lettuce germplasm was seen for warmer environments. The interaction between G and E was significant ($P < 0.000$) for all the studied characteristics in romaine lettuce (Supplemental Table 1). The similarity of these environments was studied further by understanding how related these environments (experiments) were for the studied traits. Head weight, head height, and CL correlated mostly highly ($P < 0.000$, n = 92) among the different experiments (Fig. 3). Except for one correlation that was not significant ($r = 0.15$, $P = 0.147$, n = 92) between P313 and E512 for HW (Fig. 3). These correlations were also highly significant for bolting and tipburn ($P < 0.000$, n = 92) among experiments (Fig. 3). The fewest relationships found among the tested experiments corresponded with the number of total stressed leaves, with very few significant correlations reported (Fig. 3).

Two mega environments were graphed in the GGE biplot as representative of the five experiments planted in warmer conditions for HW of romaine lettuce. Mega environment 1 was composed of experiments with P313, E512, P512, and S612, in which romaine genotypes Hazera Cos (highest performer for HW) was accompanied by Heavy Heart, Jericho, Hearts Delight, Gladiator, Skyway, and Clemente (Fig. 4A). Mega environment 2 comprised E313 only, with several genotypes falling into this area of the polygon, including Winter Density, PI 342479, Bambi, Medallion MT, King Henry, and Tall Guzmaine (Fig. 4A). The rest of the genotypes did not fall into any mega environment for HW (Fig. 4A). However, Hazera Cos was the most

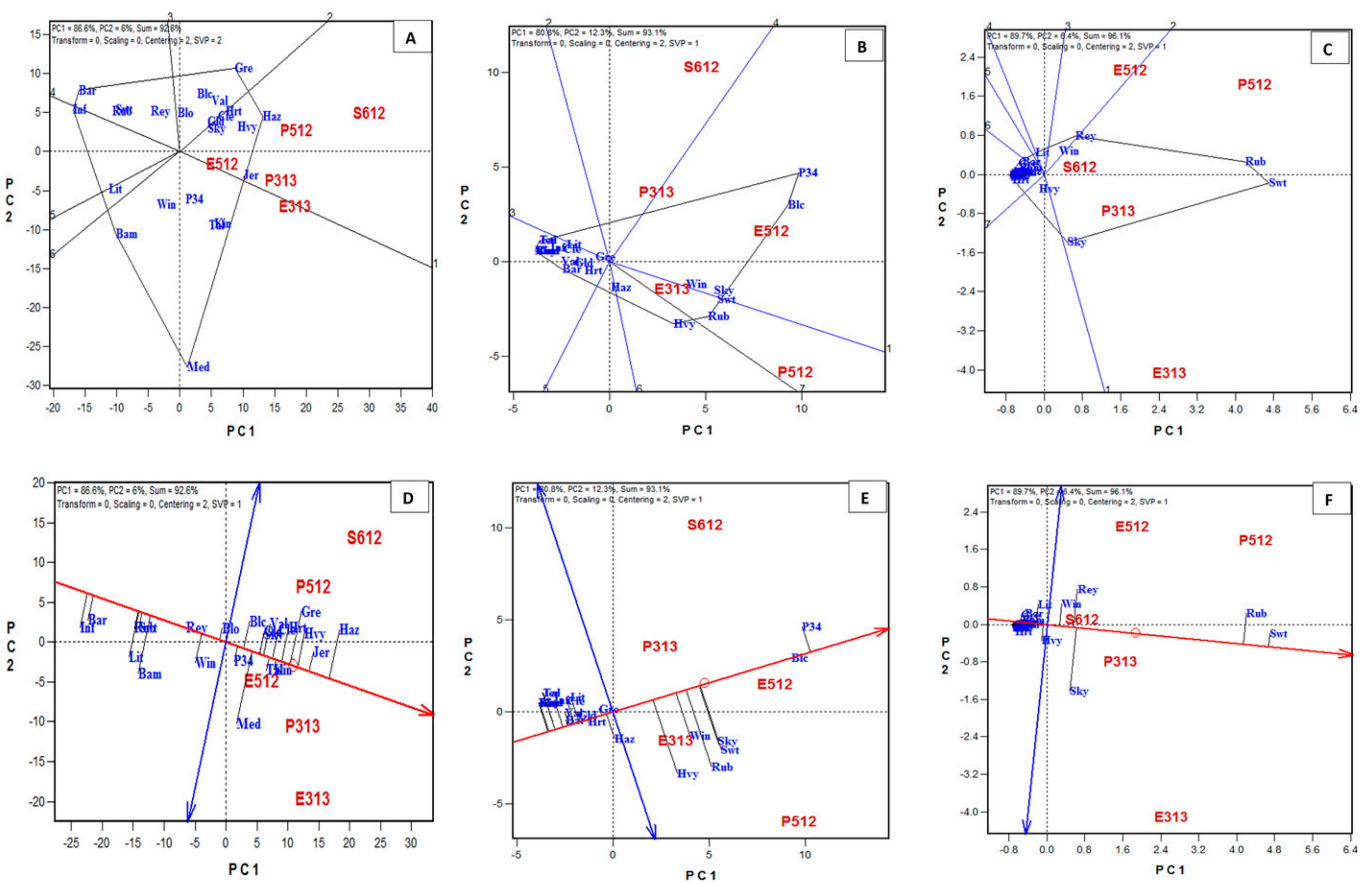

Fig. 4. Which-won-where view of the genetics + genetics × environment biplot showing the performance of romaine lettuce genotypes for head weight (**A**), bolting (**B**), and tipburn (**C**). Average environment coordinates for head weight (**D**), bolting (**E**), and tipburn (**F**) are also shown. Environments are represented by E313 (Holtville, CA, USA; Mar 2013 planting), E512 (Holtville, CA, USA; May 2012 planting), P313 (Five Points, CA, USA; Mar 2013 planting), P512 (Five Points, CA, USA; May 2012 planting), and S612 (Salinas, CA, USA; Jun 2012 planting).

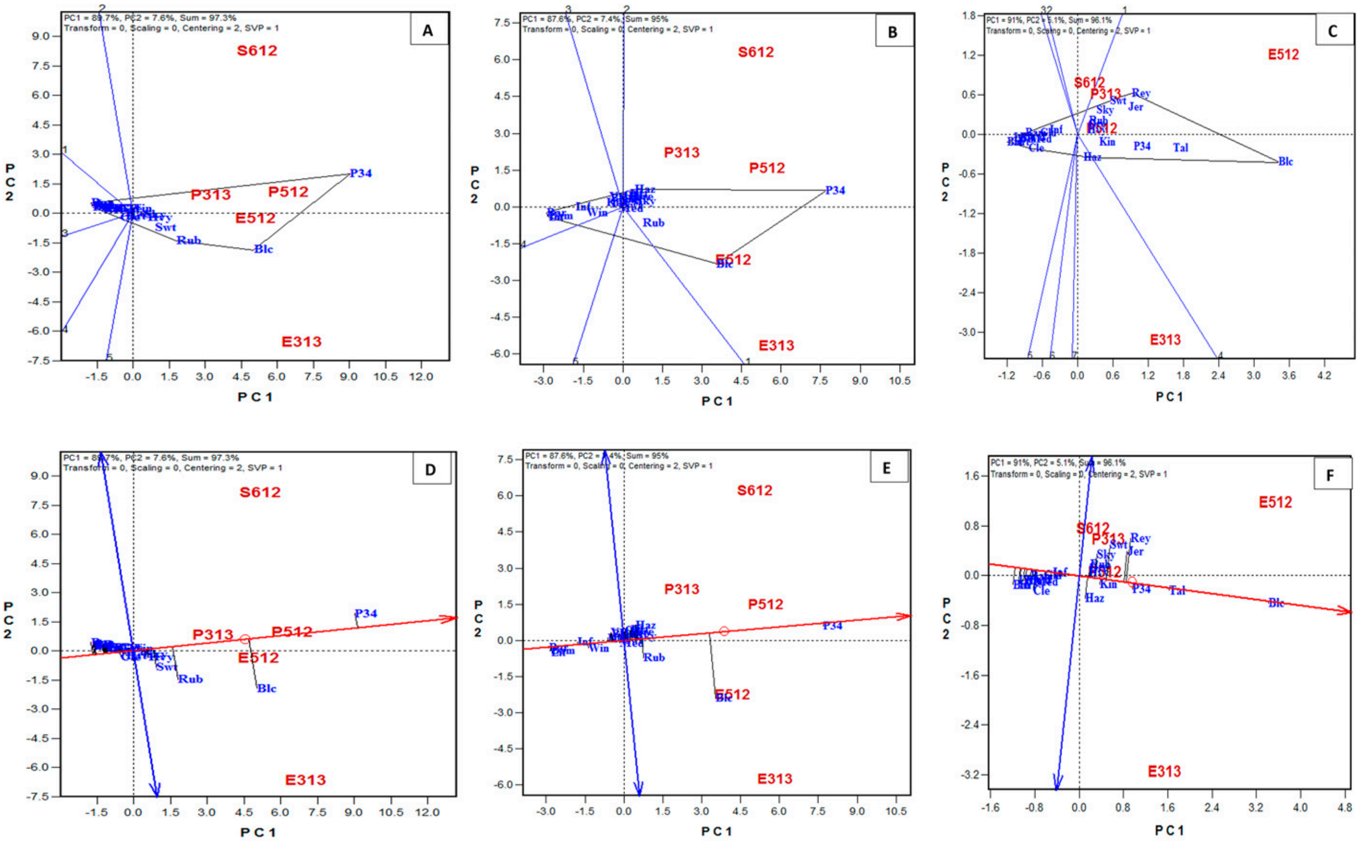

Fig. 5. Which-won-where view of the genetics + genetics × environment biplot showing the performance of romaine lettuce genotypes for core length (**A**), head height (**B**), and stressed leaves per plant (**C**). Average environment coordinate for core length (**D**), head height (**E**), and stressed leaves per plant (**F**) are also shown. Environments are represented by E313 (Holtville, CA, USA; Mar 2013 planting), E512 (Holtville, CA, USA; May 2012 planting), P313 (Five Points, CA, USA; Mar 2013 planting), P512 (Five Points, CA, USA; May 2012 planting), and S612 (Salinas, CA, USA; Jun 2012 planting).

stable genotype for HW, and environment E512 was ideal for selecting for HW. This environment was the closest to the average environment coordinates (AECs) (Fig. 4D).

Three mega environments were identified for the percentage of bolted plants. The first mega environment was comprised of P313 and S612, with no genotypes positioned in this area of the polygon (Fig. 4B); both were environments with the fewest bolted plants. A second mega environment was constructed with E512 only, and PI 342479, Black Seeded Bath, Winter Density, and Skyway were the genotypes, which performed similarly with a greater percentage of bolted plants (Fig. 4B). The third mega environment was comprised of E313 and P512, along with genotypes Hazera Cos, Heavy Heart, Rubens Red, and Sweet Valentine as the genotypes, and these had a somewhat greater percentage of bolted plants. The rest of genotypes performed similarly with a lower percentage of bolted plants and were not positioned in any mega environment (Fig. 4B). Lettuce accession PI 342479 was the one with the most consistent value of bolted plants across the environments tested, and E512 was the environment more representative for this trait (Fig. 4E).

A different situation was graphed for the percentage of plants with tipburn. Only one mega environment was detected. The genotypes with greatest number of plants with tipburn were Rubens Red and Sweet Valentine in all environments (P512, S612, P313, and E313) (Fig. 4C). The rest of the genotypes were not positioned in any mega environment because had lower percentage of plants with tipburn (Fig. 4F). Rubens Red and Sweet Valentine were the genotypes with the greatest consistency of plants with tipburn across environments; environment P313 had the most tipburn in these experiments (Fig. 4F).

Only one mega environment was graphed for CL and head height, with PI 342479 as the accession with the tallest head height and longest CL (Fig. 5A and B). Likewise, PI 342479 was the genotype with consistent values for both traits across the studied environments, and P512 was the environment that increased the value of both traits consistently (Fig. 5D–F).

Three mega environments were graphed for the number of stressed leaves. In the first mega environment (comprised of P313, E512, and P512), the genotypes Red Eye Cos and Black Seeded Bath were the ones with the greatest value (Fig. 5C). A second mega environment was comprised of E313 only, with genotype Hazera Cos as the accession with the most stressed leaves (Fig. 5C). S612 was positioned alone, with no genotype falling within this third mega environment (Fig. 5C). Black Seeded Bath was the genotype that consistently had the greatest number of stressed leaved in all environments (Fig. 5F).

Butterhead lettuce tolerant to warmer plantings. Similarly, HW for butterhead lettuce was detected to be significantly ($P < 0.00$) different when planted in these five environments (Supplemental Table 4). Characteristics such as CL, HW:CL, marketability, bolting, and tipburn were also significantly ($P < 0.0001$) different among the tested accessions (Supplemental Table 4). Genotype Butter King had the greatest HW, with no bolting and 8% of tipburn on average (Fig. 6). Margarita had a significantly lower HW, but no bolting and little tipburn (Fig. 6). Butter King, Margarita, and Sensation had no bolting plants in these five experiments, whereas Novir had more than 50% of bolted plants on average (Fig. 6). Margarita and 'Oberto' had very little tipburn whereas PI 381933 had more than 10% of plants with tipburn (Fig. 6).

There was also variation in lettuce CL and HW:CL (Fig. 7). Margarita had the shortest cores, at 3.3 cm on average, whereas 'Edogawa Salad' had the longest cores, with an average of 26.1 cm (Fig. 7). Butter King had the greatest HW:CL, at 7.4 on average, whereas 'Edogawa Salad' had the smallest ratio, at 1.1 (Fig. 7). Butter King was the only genotype with an average of 2.9 for marketability, which for butterhead lettuce is

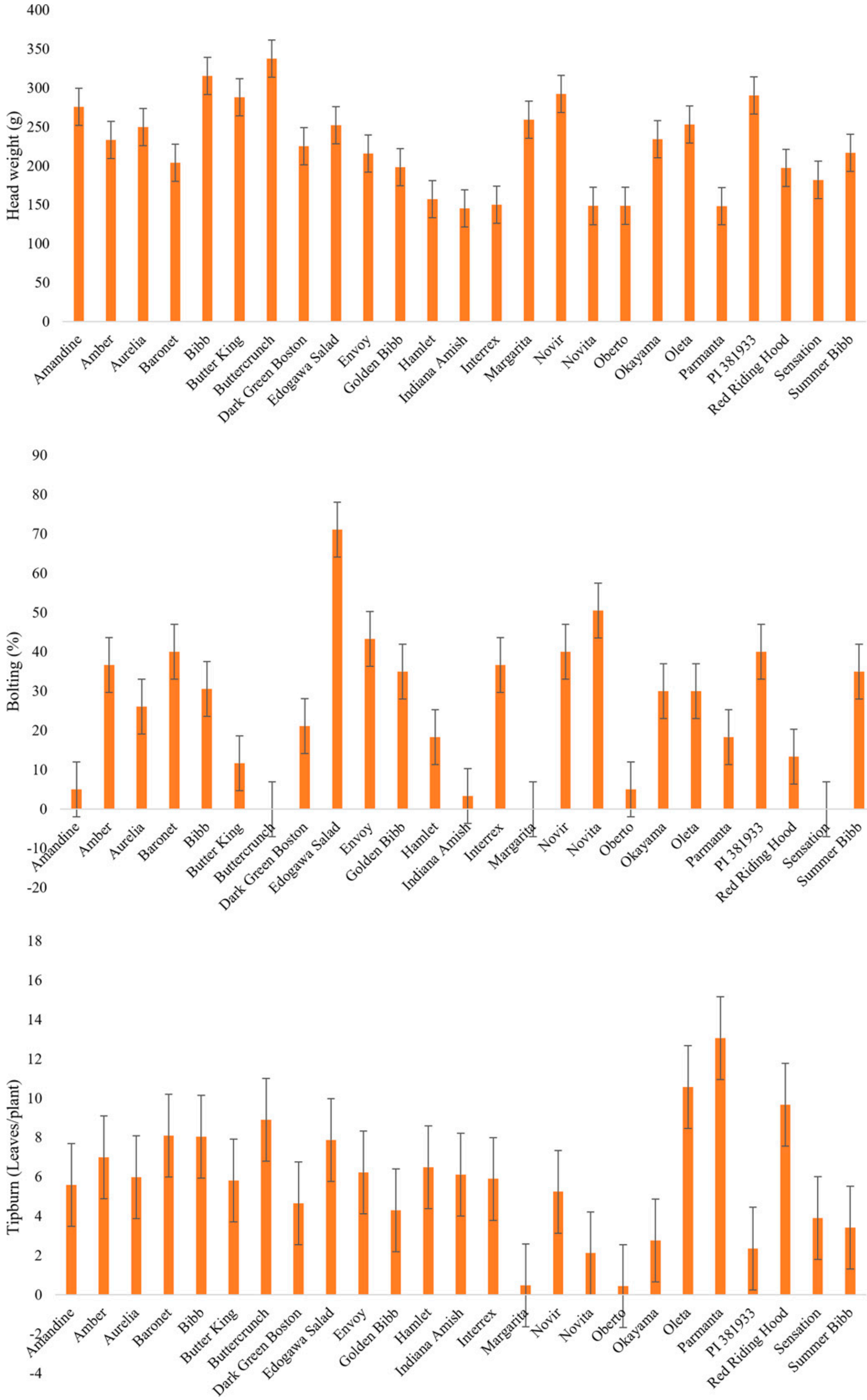

Fig. 6. Least square means for head weight (**top**) and associated heat-related traits of bolting (**middle**) and tipburn (**bottom**) in butterhead lettuce grown in five experiments conducted in in Holtville, CA, USA, Mar 2013 (E313) and May 2012 (E512); Five Points, CA, USA, Mar 2013 (P313) and May 2012 (P512); and Salinas, CA, USA, Jun 2012 (S612). The bars indicate the average (n = 200) of the five experiments. Error bars are 95% confidence intervals. Least significant difference values are for head weight (= 32.3), bolting (= 9.9), and tipburn (= 2.9).

considered marketable when heads are rated as 3.0 (Fig. 7). The rest of the butterhead genotypes had values less than 3.0, which are considered to have poor marketability (Fig. 7).

Butterhead lettuce performs differently according to the planted environment. Significant differences ($P < 0.000$) were observed among the environments tested for all traits including HW, marketability, CL, HW:CL, bolting, and tipburn (Supplemental Table 4). For most of the studied traits in these experiments, the environment was the most important

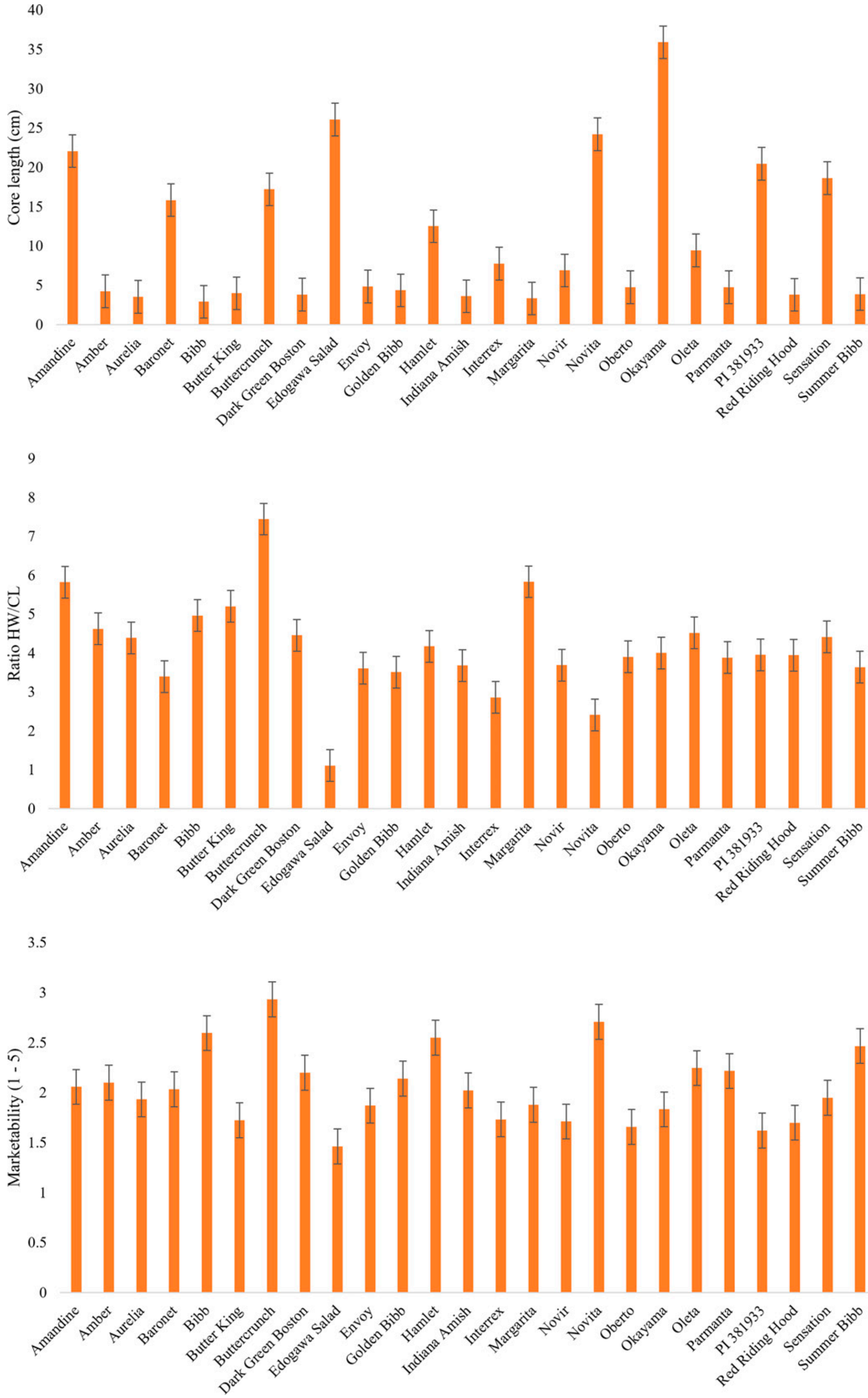

Fig. 7. Least square means for core length (CL) (**top**), head weight (HW):CL (**middle**), and marketability (**bottom**) of butterhead lettuce planted in five experiments conducted in Holtville, CA, USA, Mar 2013 (E313) and May 2012 (E512); Five Points, CA, USA, Mar 2013 (P313) and May 2012 (P512); and Salinas, CA, USA, Jun 2012 (S612). The bars indicate the average (n = 200) of the five experiments. Error bars are 95% confidence intervals. Least significant difference values are for CL (= 2.9), HW:CL (= 0.56), and marketability (= 0.24).

component of the total variation, as greater phenotypic variation was detected for those traits except for head width (Supplemental Table 4).

Planting lettuce in May 2012 in Holtville, CA, USA (E512), and in Salinas, CA, USA, in Jun 2012 (S612) produced the fewest marketable heads (Supplemental Tables 5 and 6). Similarly, CL (longest cores) and HW:CL (smallest ratio) were affected in these experiments, especially for the May 2012 planting in Holtville, CA, USA (E512) (Supplemental Table 6). More bolted plants and a greater tipburn percentage was identified when lettuce was planted in May 2012 in Holtville, CA, USA (E512), and in May 2012 in Five

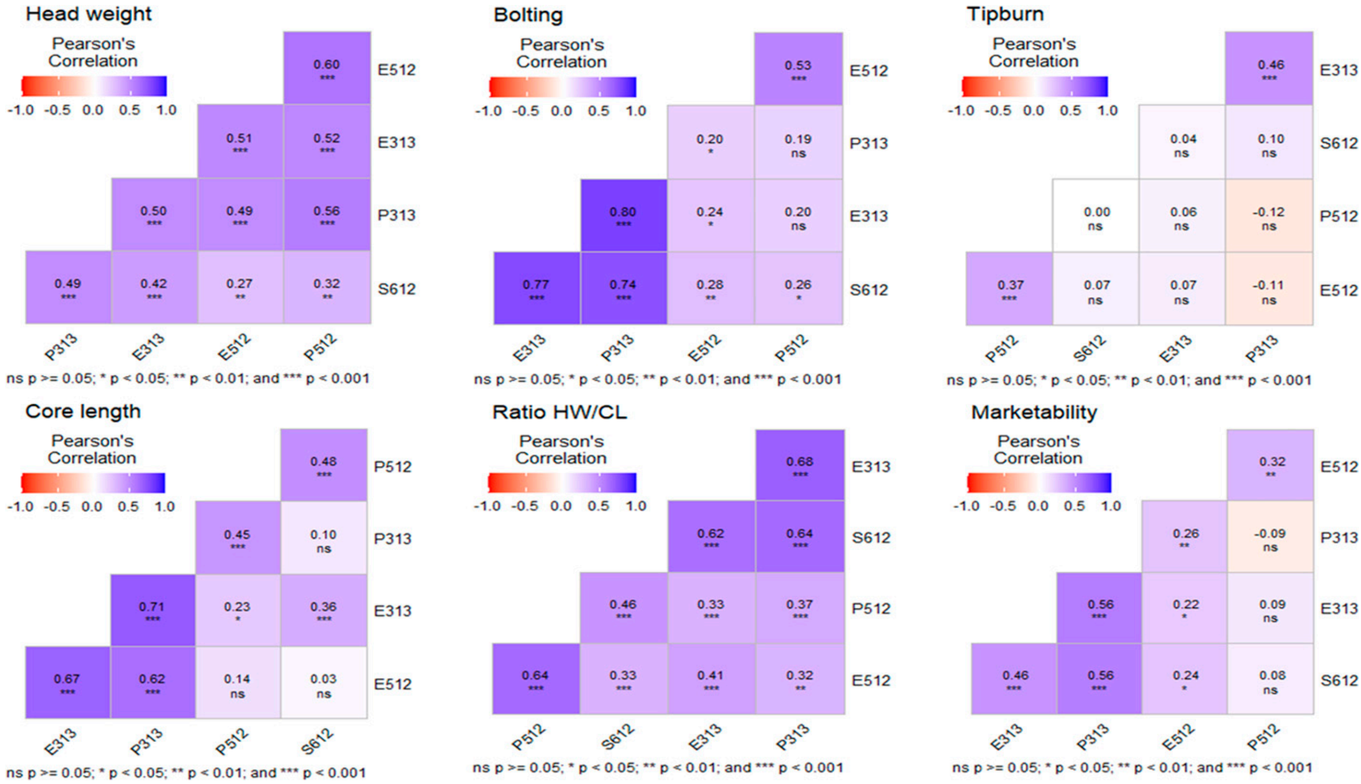

Fig. 8. Correlation coefficients for traits including head weight (HW) (**top left**), bolting (**top middle**), tipburn (**top right**), core length (CL) (**bottom left**), HW:CL (**bottom middle**), and marketability (**bottom right**) registered in butterhead lettuce across five experiments conducted in E313 (Holtville, CA, USA; Mar 2013 planting), E512 (Holtville, CA, USA; May 2012 planting), P313 (Five Points, CA, USA; Mar 2013 planting), P512 (Five Points, CA, USA; May 2012 planting), and S612 (Salinas, CA, USA; Jun 2012 planting).

Points, CA, USA (P512) (Supplemental Table 5). As stated previously, May planting was affected by the warmest minimum and maximum temperatures (Table 2).

For butterhead lettuce, HW was greater in the warmer environments, except the May planting in Holtville, CA, USA, than in the Salinas Valley (Supplemental Table 5), but tipburn surged in all warmer environments and bolting increased in the two May plantings (Supplemental Table 5). However, Butter King, Margarita, 'Oberto', and Sensation had no or little increase in bolting or tipburn or both when planted in the warmer environments (Supplemental Table 5).

Stable Butterhead lettuce was seen in warmer environments. The interaction between G and E was significant ($P < 0.000$) for all studied characteristics in butterhead lettuce (Supplemental Table 2). Relationships for the different traits registered among the different environments (experiments) in butterhead lettuce were significant ($P < 0.000$, n = 92), with some exceptions (Fig. 8). Although these correlations were significant ($P < 0.000$, n = 92), their relationship level was not as strong. For instance, significant correlations for HW reached values of $r = 0.27$ ($P = 0.007$, n = 92) between E512 and S612, and $r = 0.60$ ($P < 0.000$, n = 92) between E512 and P512 (Fig. 8). For CL, these relationships were found to range from nonsignificant ($r = 0.03$, $P = 0.737$, n = 92) between E512 and S612 to highly significant ($4 = 0.71$, $P < 0.000$, n = 92) between E313 and P313. Although all experiments were highly correlated ($P < 0.000$, n = 92) for HW:CL, with values of $r = 0.32$ between E512 and P313, and r = 0.68 between E313 and P313 (Fig. 8). Most of the relationships for tipburn were not significant among all the experiments conducted in our study (Fig. 8), although traits such marketability and bolting presented a few significant relationships among the environments tested (Fig. 8).

Two mega environments were graphed for HW. The first one was comprised of E512 and P512 (Holtville and Five Points, CA, USA; May 2012 planting) and the second one included E313, P313, and S612 (Holtville and Five Points, CA, USA, Mar 2013 planting; and Salinas, CA, USA, Jun 2012 planting) (Fig. 9A). Genotypes Novir and Buttercrunch had the greatest HW in these two mega environments, respectively. Two mega environments were detected for bolting. Mega environment 1 was comprised of E512; mega environment 2 was comprised of E313, P313, P512, and S612 (Fig. 9B). Genotypes Edogawa Salad and Bibb were the accessions with the most bolting in these two mega environments, respectively (Fig. 9B). Environments E313 and P313 were the experiments with the least bolting and were the most stable (Fig. 9E). Similarly, two mega environments were graphed for tipburn. Mega environment 1 was comprised of S612, E512, and P512; mega environment 2, with E313 and P313, for tipburn (Fig. 9C). Genotype Oleta was positioned in extremes in mega environment 1, indicating that this was the genotype with the greatest tipburn percentage. 'Oleta' also showed a high, consistent tipburn percentage across the five experiments (Fig. 9F). Genotype Edogawa Salad, positioned in mega environment 2, had the greatest tipburn percentage in that environment (Fig. 9C).

Edogawa Salad was the genotype with the longest CL and was positioned as the representative accession in a single mega environment comprised of E313, P313, E512, P512, and S612 (Fig. 10A). No environment was positioned as a near-ideal environment among the five locations tested according to the AECs (Fig. 10D). With regard to HW:CL, only one mega environment was detected (Fig. 10B). Buttercrunch was the genotype with the most stable values for HW:CL in all the environments tested, and was the closest to the AECs (Figs. 10E). Similarly, mega environment distribution was detected for marketability (Fig. 10C), with Buttercrunch and Novita having the greatest in mega environment 1 (comprised of E512 and P512) and mega environment 2 (comprised of E313, P313, and S612), respectively (Fig. 10C). Genotype Hamlet was the most stable for marketability (Fig. 10F) and was the closest to the AECs (Fig. 10F).

Discussion

The results of our study show that is genetic variation in romaine and butterhead lettuce for plant breeders to improve heat tolerance in these types of lettuce. Leaf (Lafta et al. 2017),

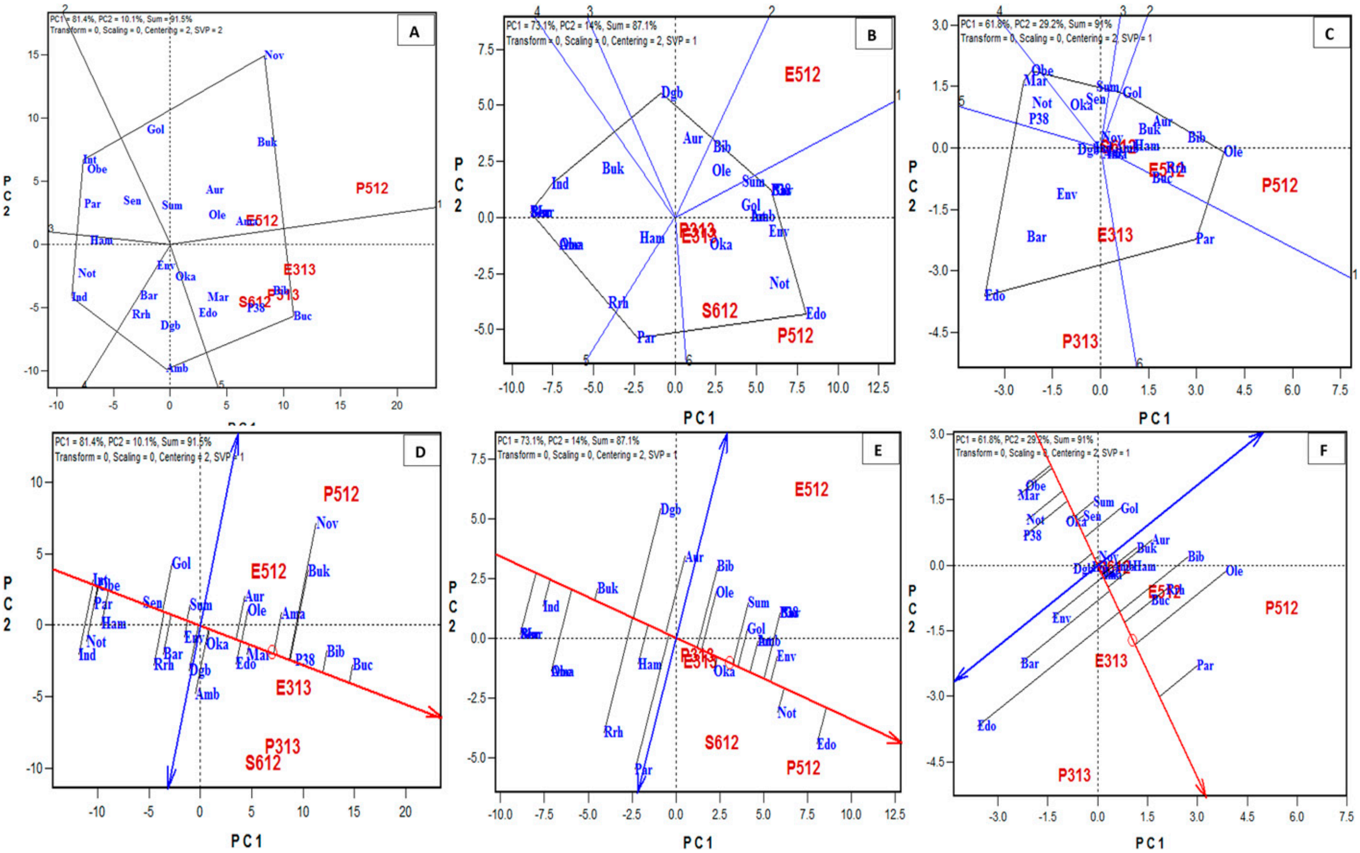

Fig. 9. Which-won-where view of the genetics + genetics × environment biplot showing the performance of butterhead lettuce genotypes for head weight (**A**), bolting (**B**), and tipburn (**C**). Average environment coordinates for head weight (**D**), bolting (**E**), and tipburn (**F**) are also shown. Environments are represented by E313 (Holtville, CA, USA; Mar 2013 planting), E512 (Holtville, CA, USA; May 2012 planting), P313 (Five Points, CA, USA; Mar 2013 planting), P512 (Five Points, CA, USA; May 2012 planting), and S612 (Salinas, CA, USA; Jun 2012 planting).

crisphead (Lafta et al. 2021), romaine, and butterhead lettuce types (identified in our study) present genetic variation in HW, bolting, and tipburn in warmer environments, indicating the possibility of improving heat tolerance in all lettuce types. Romaine lettuce currently represents 36% of the total area planted in the United States, whereas butterhead and iceberg lettuce occupy 39% (US Department of Agriculture, National Agricultural Statistics Service 2022). Therefore, it is vital to improve heat tolerance in all lettuce, including romaine and butterhead, to ensure long-term sustainability of these types in the market.

Historically, romaine lettuce was planted in small proportions until the 1990s, but the increase in area has been exponential since the 2000s (Hayes 2018a), which is boosted by consumer awareness of its greater nutritional value compared with iceberg lettuce (Mou 2008; Mou and Ryder 2004) and the popularity of Caesar salad. Butterhead lettuce is also known as Boston lettuce and its production has also increased steadily in the United States (Hayes 2018a). Both types of lettuce are also preferred for the greenhouse industry in controlled environments, including hydroponics and aquaponics (Resh 2022). The heat tolerance presented in our study was identified in fields experiments in warmer environments where lettuce is currently planted in wintertime. However, additional research should focus on investigating interactions between field and greenhouse because the environment is the main component of the variation for heat tolerance in lettuce, as demonstrated previously (Lafta et al. 2017, 2021).

Genotypes Bambi, Blonde Lente a Monter, Medallion MT, and Red Eye Cos within romaine lettuce showed a tolerance to bolting in warmer environments, as described previously for other lettuce types (Lafta et al. 2017, 2021). These accessions could be also less sensitive to daylength, because planting occurred in the off season. Bolting is highly influenced by photoperiod in addition to temperature (Rosental et al. 2021). These accessions also had an overall fewer number of leaves stressed by heat, confirming their tolerance to warmer conditions. Overall, romaine lettuce showed the fewest heat disorders (tipburn and number of stressed leaves) as a consequence of being planted in May in Holtville and Five Points, CA, USA, compared with early planting in these two regions, as reported previously for other lettuce types (Lafta et al. 2017, 2021). Overall, romaine lettuce had fewer bolted plants and can be crossed with other types, including butterhead, crisphead, and leaf lettuce, which seem to be more sensitive to bolting when planted when temperatures are not favorable for lettuce production (Lafta et al. 2017, 2021). These romaine genotypes were not the best performers in terms of greater and stable HW; however, they had short cores and an average head height. Therefore, crossing accessions with less bolting and accessions with good performance in terms of yield (HW and its related traits) could produce offspring that have a tolerance to heat stress disorders, such as bolting and tipburn, with horticulturally acceptable characteristics.

The challenge for plant breeders is to select lettuce that are heat tolerant in the right environment during the breeding process. Although it seems that HW and its related traits (marketability, CL, and head width) could be selected for in a single environment because of a minimal G × E interaction, the opposite occurs with heat-related disorders such as bolting and tipburn. The G × E interaction seems to be a crossover type for heat-related disorders, as correlations among environments were not significant. It is likely that breeders would have to evaluate offspring in multiple environment trials, as suggested previously when selecting against these two disorders (bolting and tipburn) in warmer environments (Jenni 2005; Jenni and Hayes 2010; Jenni and Yan 2009; Lafta et al. 2017, 2021). However, the G × E interaction in our study was evaluated when lettuce experienced greater amounts of daylight, which may have contributed to greater variation in these experiments and the crossover interaction.

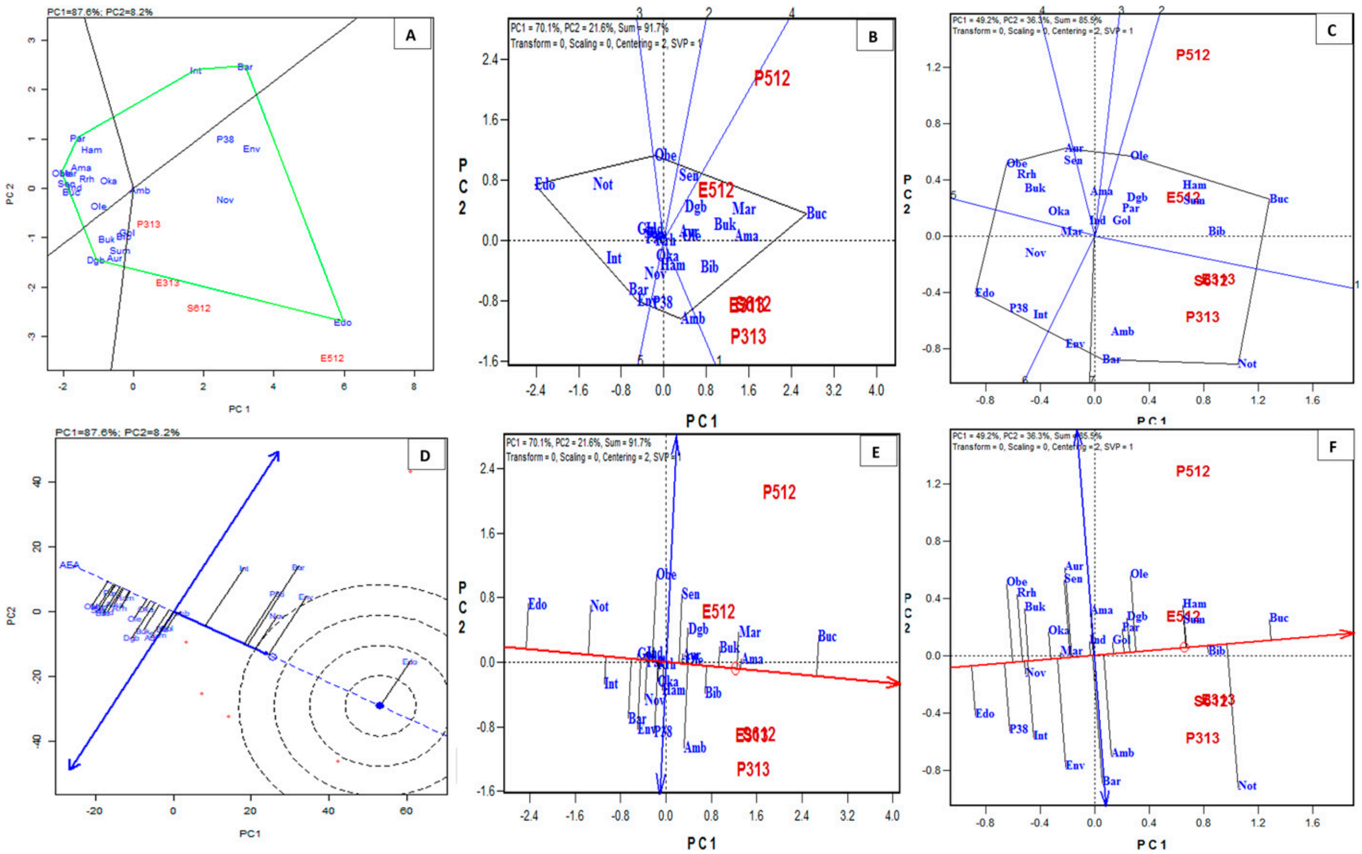

Fig. 10. Which-won-where view of the genetics + genetics × environment biplot showing the performance of butterhead lettuce genotypes for core length (**A**), head height to core length ratio (**B**), and marketability (**C**). Average environment coordinates for core length (**D**), head height to core length ratio (**E**), and marketability (**F**) are also presented. Environments are represented by E313 (Holtville, CA, USA; Mar 2013 planting), E512 (Holtville, CA, USA; May 2012 planting), P313 (Five Points, CA, USA; Mar 2013 planting), P512 (Five Points, CA, USA; May 2012 planting), and S612 (Salinas, CA, USA; Jun 2012 planting).

Lettuce performance is highly sensitive to photoperiod (Ryder 1999).

Unlike romaine lettuce, butterhead lettuce had a greater percentage of bolted plants (E512 and EP12) and greater tipburn incidence (P512) when planted in warmer environments. However, two genotypes showed less bolting and tipburn when planted in warmer environments. One of these genotypes is Butter King, which is described by the USDA-GRIN-NPGS to be a slow-to-bolt accession that develops less tipburn (US Department of Agriculture, Agricultural Marketing Service 2017). Despite genetics, tipburn is a characteristic controlled by many environmental factors, including warmer temperatures, fertilization, and transpiration rates (Hayes 2006, 2018b; Jenni and Hayes 2010). Margarita was developed to resist the pathogen causing lettuce downy mildew (*Bremia lactuca*) and was discovered to have good tolerance to tipburn and bolting (US Department of Agriculture, Agricultural Marketing Service 2017). Both accessions are candidates to improve butterhead lettuce for warmer temperatures. However, their use as a source of breeding for heat tolerance may limit marketability, because Butter King had barely good market maturity and Margarita did not reach an acceptable market maturity. Producing breeding populations between these two accessions may yield butterhead lettuce with acceptable HW and less disorders, including bolting and tipburn. The heat-tolerant offspring may then be crossed with more horticulturally acceptable butterhead breeding lines and backcrossed as many generations as needed.

Unlike in other studied lettuce types for field heat tolerance (Lafta et al. 2017, 2021), environment S612 (Salinas, CA, USA; Jun 2012 planting) did not register the greatest HW in butterhead lettuce. On the contrary, environment P512 (Five Points, CA, USA; May 2012 planting) was the one with the greatest HW. The extra daylight and heat likely induced excessive plant growth, which translated into greater weight in P512 than in S612. Plants change their organization in cellular structure and membrane function as consequence of being exposed to warmer conditions (Weis and Berry 1988). Planting in late spring in E512 in Holtville, CA, USA, in May 2012, and in P512 resulted in a high incidence of bolting and tipburn. Specific genotypes such as Amandine, Butter King, Margarita, PI 381933, Red Riding Hood, and Sensation had no tipburn in E512, whereas Butter King, Margarita, and Sensation had no tipburn in P512, indicating that the trait is highly complex and not only temperature affected its expression (Hayes 2018b).

Heat-tolerant cultivars could reduce production costs by extending the growing season in the San Joaquin Valley and southern desert valleys of California and Arizona, USA, where land costs are much less than in the coastal Salinas Valley, the major main-season (spring to fall) lettuce-producing region of the United States (Lafta et al. 2017). Some romaine varieties had greater or similar HW when planted in some of the warmer environments compared with the cooler Salinas Valley (Table 2). Through selection and/or breeding for heat-tolerant cultivars, it seems feasible to extend the growing season of lettuce in the San Joaquin and southern valleys.

Conclusion

In conclusion, genetic variation for HW in lettuce planted in warmer environments was identified for both romaine and butterhead lettuce. Romaine genotypes Bambi, Blonde Lente a Monter, Medallion MT, and Red Eye Cos, and butterhead genotypes Butter King and Margarita had 0% bolting across five experiments conducted in warmer environments. Of these, Medallion MT and Butter King had the greatest HW within each horticultural type—romaine and butterhead, respectively. Moreover, these genotypes also presented short cores and acceptable head height within their respective types. These genotypes can be used as parents to breed lettuce for heat tolerance. However, the environment explained a high proportion of the phenotypic variation and should be taken into consideration when selecting for heat tolerance. This variation made the G × E interaction significant and requires a detailed analysis to understand the crossover or noncrossover nature of this interaction, and whether selection for heat tolerance can be achieved easily. In addition, a detailed analysis is needed to detect all the genotypic and environmental factors influencing G × E for heat tolerance in lettuce. Stable genotypes for less bolting and tipburn, and other horticultural characteristics within each type, were also identified. Such germplasm should yield heat-tolerant cultivars that can be planted across a wider region in warmer regions in the western United States. Similarly, stable genotypes for breeding lettuce across multiple warmer locations in the western United States were identified in green and red leaf lettuce (Lafta et al. 2017) and in crisphead lettuce (Jenni and Hayes 2010; Jenni and Yan 2009; Lafta et al. 2021) based on HW and heat-related disorders such as tipburn and bolting. Our data suggest that selecting and/or breeding for heat-tolerant cultivars may be feasible and may extend the growing season of lettuce in the San Joaquin and southern valleys, in addition to reducing production costs. However, how long the season can be extended is yet to be determined. At this point, it is not known whether such an extension can be achieved for $\leq$1 week (or month) beyond the current lettuce production in southern California. Currently, lettuce production ends by the end of March in the Imperial Valley and by the end of April in the San Joaquin Valley (Simko et al. 2014). Heat-tolerant cultivars are paramount to the lettuce industry in the western United States and everywhere else where this vegetable will be affected by global warming.

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