Genetic Variation and Genotype by Environment Interaction for Heat Tolerance in Crisphead Lettuce

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Abstract. Lettuce (*Lactuca sativa* L.) is a cool season crop that is vulnerable to high temperature stress, which promotes bolting and decreases yield and quality. It is anticipated that climate change may lead to higher temperatures in current lettuce growing areas in the United States, thereby negatively affecting lettuce production and possibly resulting in adverse impacts on global food production. Therefore, it is important to identify lettuce germplasm with tolerance to temperatures higher than those that have occurred over the past century. We evaluated 25 crisphead lettuce cultivars for tolerance to high temperature stress in the San Joaquin, Imperial, and Salinas Valleys, CA. Genetic variation was identified for yield and horticultural traits, such as core length, head diameter, tipburn, bolting, and market maturity, of crisphead lettuce grown in warmer conditions. Significant genotype $\times$ environment interaction did not account for most of the variation; the main differences were found for environments and only a small proportion of the variation was due to genotypes. Cultivar Primetime is a good source of heat tolerance for crisphead lettuce, as it presented the best yield and exhibited other desirable characteristics across warmer conditions. These results provide insight into the cultivars that respond well to hot environments. Moreover, the data can be used by breeders to develop new heat-tolerant lettuce cultivars.

Lettuce (*L. sativa* L.) is a cool season crop that requires a daily average temperature of 18 °C for optimal development. Lettuce is planted year-round in temperate climates in many countries including the United States.

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China is the top producer in terms of area harvested (648,738 ha), followed by India (193,962 ha), the United States (117,032 ha), Italy (34,460 ha), Spain (33,674 ha), Turkey (22,576 ha), Japan (21,314 ha), and Mexico (20,527 ha) (FAOSTAT, 2018). The United States was the top producer in terms of harvested yield in 2018 with 31 t/ha followed by Spain, Japan, China, Mexico, Italy, Turkey, and India, which produced 28, 27, 24, 24, 22, 22, and 6 t/ha, respectively (FAOSTAT, 2018). Lettuce can be grown during the winter when temperatures are favorable in southern California, Arizona, and Florida. These three states account for $\approx 97\%$ of the total area planted in the United States and have a farmgate value of $\approx \$3.5$ billion in the United States (USDA-NASS, 2019).

The average worldwide temperature is predicted to increase due to climate change over the next few decades and this increase in temperature will affect agricultural production (Karl and Trenberth, 2003; Wurr et al., 1996). Climate change is already threatening the leafy vegetable industry in Florida and potentially may affect western production areas, especially southern California and Arizona. Therefore, breeding lettuce culti-

vars that tolerate warm temperatures is one of the priorities for leafy vegetable crops. The breeding process starts with screening available lettuce germplasm to identify lettuce cultivars that perform acceptably in warm conditions. Several leaf lettuce cultivars were identified to be heat tolerant in field conditions (Lafta et al., 2017). A few romaine cultivars performed well in greenhouses under warmer conditions (Holmes et al., 2019). Screening lettuce germplasm is particularly relevant for traits that are known to be affected by high temperature, such as physiological disorders like rib discoloration, tipburn, premature bolting, and ribbiness (Jenni, 2005; Jenni and Hayes, 2010; Jenni and Yan, 2009; Ryder, 1999). Rib discoloration is present in leaves as lettuce being exposed to warm conditions. This disorder is especially noticeable in older lettuce (Jenni and Yan, 2009). Tipburn is a physiological disorder associated with localized internal calcium deficiencies in lettuce (Barta and Tibbitts, 2000), which occurs during periods when rapid plant growth outpaces calcium transport (Barta and Tibbitts, 2000; Thibodeau and Minotti, 1969), and is more prominent in warm planting periods (Macias-González et al., 2019). Tipburn is especially detrimental to crisphead and romaine lettuce types, reducing the quality and shelf life of fresh and processed products. Many packing companies in the United States will reject entire fields of lettuce with a tipburn incidence greater than 5% because the symptoms are unacceptable to consumers and may lead to subsequent microbial rotting (Jenni and Hayes, 2010). Bolting is genetically controlled by several genes (Ryder and Milligan, 2005). The process is initiated by long photoperiods and high temperatures, and lettuce planted in warmer conditions tends to bolt faster (Silva et al., 1999). Early bolting shortens vegetative growth, resulting in reduced biomass. Once bolted, lettuce loses its marketability with poor head formation and bitter taste. Ribbiness is defined as protuberances of the outer ribs that prevents normal head development (Masarirambi et al., 2018), which is induced by high temperatures and long photoperiods (Jenni and Yan, 2009). These physiological disorders cause economic damage in all lettuce production areas (Barta and Tibbitts, 2000; Cox et al., 1976; Thibodeau and Minotti, 1969; Yanagi et al., 1983).

Yield of lettuce, especially in hot conditions, is controlled by genotype and environment but the influence by the interaction of both is still not well understood in crisphead (iceberg) lettuce. However, the yield of red and green leaf lettuce in hot conditions is controlled by this genotype $\times$ environment ($G \times E$) interaction and must be considered during the breeding process (Lafta et al., 2017). Physiological disorders have an important $G \times E$ interaction that breeders must take into considerations when designing breeding strategies to improve heat tolerance in lettuce (Jenni and Hayes, 2010; Jenni and Yan, 2009).

The genetic basis of heat tolerance of lettuce grown in field conditions has not been described in head lettuce, specifically crisphead cultivars, which account for ≈43% of the U.S. production (USDA-NASS, 2019). From the 1920s through the 1990s, crisphead lettuce dominated U.S. lettuce production, but its share of the market has steadily decreased over the past 30 years (Hayes, 2017). The yield of crisphead lettuce may decrease due to increasing temperatures in the current production areas. It is desirable to breed crisphead lettuce cultivars that develop uniform and marketable heads and do not bolt or present physiological disorders, such as tipburn, as consequence of excessive heat.

Therefore, the objectives of this study were to identify crisphead lettuce cultivars that tolerate high temperatures. To design a suitable breeding scheme for heat tolerance in lettuce, we investigated the cultivar × environment interactions that would maximize genetic gains in a breeding program. Our findings provide insight into the crisphead lettuce cultivars that respond well to hot environments. Moreover, these data can be used by breeders to develop new heat-tolerant lettuce cultivars.

Materials and Methods

Plant materials. Twenty-five crisphead lettuce cultivars (Table 1) were selected to test their responses to high temperature stress in five different environments based on their performances in preliminary growth chamber tests of more than 700 lettuce cultivars each with 16 3-week-old seedlings exposed to

43 °C 12 h day/35 °C 12 h night, 450 μmol·m⁻²·s⁻¹ photosynthetic photon flux density for 7 d, and then evaluated for leaf injury (rated on a 0–6 scale: 0, no injury; 1, <1/2 leaf edge injured; 2, >1/2 leaf edge injured; 3, <1/3 leaf area injured; 4, <2/3 leaf area injured; 5, >2/3 leaf area injured; 6, plant dead), reduction in leaf fresh weight, and percent seedling survival compared with controls at 25 °C day/17 °C night (Lafta and Mou, unpublished data). Several of the cultivars were developed outside the United States (e.g., ‘Reine des Glaces’). Controls were ‘Salinas’ and ‘Vanguard 75’, two cultivars well-adapted to the California central coast (Salinas Valley) and southwest United States (Imperial Valley, CA, and Yuma, AZ), respectively.

Experiments. Five field experiments were conducted in three different locations and different planting dates during 2012 and 2013. Two experiments were in the San Joaquin Valley at the University of California (UC) West Side Research and Extension Center (WSREC), Five Points, CA in Mar. 2013 and May 2012. Two experiments were planted in the Imperial Valley at the UC Desert Research and Extension Center (DREC), Holtville, CA, in Mar. 2013 and May 2012. One experiment was planted at the USDA-ARS, Salinas, CA, in June 2012. The experiments planted in March and May correspond with off-season plantings for southern production areas in California and Arizona. The June planting in Salinas, CA, corresponds with the summer in-season plantings in the Salinas Valley.

Each cultivar 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 planting. The experiments were maintained using standard practices for lettuce in the southern and central coast of California (Lafta et al., 2017). All experiments were arranged in a randomized complete block design with four replications.

Evaluation of crisphead quality. When controls (‘Salinas’ and ‘Vanguard 75’) reached market maturity, five plants of each cultivar were randomly harvested within a plot and evaluated for traits important to the crisphead lettuce industry. Yield was recorded on a plot basis and expressed as per head weight (HW) in grams. Core length (CL) was measured from the cut base to the apex of the head after splitting the lettuce head in half. These two measurements were then used for the ratio of HW to CL (g/mm), an indicator of maturity and is related to head quality. Head diameter (cm) was measured on the split heads at the widest side. Market maturity (firmness) was evaluated based on the following scale: 1 (soft or loose head); 2 (medium head filled); 3 (firm, good for the market); 4 (hard, past market maturity); and 5 (extra hard, over mature). Percentage bolted plants (%) was estimated per plot by counting the number of plants that showed signs of bolting (significant stem elongation). Tipburn was assessed by counting the number of leaves per plant with visible tipburn damage.

Statistical analysis. Analysis of variance was done using JMP program (SAS Institute Inc., Cary, NC) to determine the effects of environment (E), genotype (G), and G × E

Table 1. List of crisphead cultivars selected for heat tolerance trials from an initial screening of lettuce germplasm.

Cultivar	Adaptation	Breeder	Code ^c
Tested cultivars			
Anza	Central Coast, CA ^y	UC Davis/USDA Salinas, CA	Anz
Atlas Non-Bolting	Unknown	Unknown	Atn
Badger	Desert Southwest ^y	Seminis Veg Gen Corp	Bag
Baomach	Unknown	Unknown	Boa
Barcelona	Unknown	Nunhems	Bar
Barrier Reef	Unknown	Asgrow Seed Co	Baf
Calmar	Central Coast, CA ^y	UC Davis	Cal
Diamond	Central Coast, CA ^y	Coastal Seeds US	Dia
Empire	Muck land Northeast	USDA Beltsville, MD	Emp
Glacier	Central Coast, CA ^y	USDA Salinas, CA	Gla
Green Lake Butterhead	Unknown	Unknown	Grl
Green Prince	Unknown	Webbin, New Zealand	Grp
Hallmark WS	Unknown	Paragon Seed, Inc	Hal
Hanson Improved	Unknown	U.S. Dept. of Agriculture (USDA)	Han
Harvest Pride	Unknown	Henderson, United Kingdom	Har
Imperial 749	Unknown	Unknown	Imp
Invader	Coastal Area, CA ^y	Seminis Veg Gen Corp	Inv
Oasis	California, AZ ^y	Asgrow Seed Co.	Oas
Primetime	Unknown ^y	Asgrow Seed Co.	Pri
Reine des Glaces (Batavia)	Europe ^y	Unknown	Bat
Sniper	Coastal Area, CA ^y	Seminis Veg Gen Corp	Sni
Summertime	Unknown ^y	Oregon Agr. Exp. Sta.	Sum
Tiber	Coastal Area, CA ^x	USDA Salinas, CA	Tib
Control cultivars			
Salinas	Coastal Area, CA ^w	USDA Salinas, CA	Sal
Vanguard 75	Desert Southwest ^y	USDA Salinas, CA	Van

^cCode used for GGE biplot graph.

^yRyder et al. (2016).

^xRyder and Waycott (1998).

^wRyder (1979a).

^yRyder (1979b).

interaction on crisphead HW and other characteristics, where environments were considered using the location-year combination. Genotypes were considered fixed effects; replicates nested in the environment, and environments were considered random effects. Treatment means were separated by Fisher's protected least significant difference test at $P = 0.05$. A correlation analysis was also conducted to identify associations among the traits measured.

Analysis of genotype $\times$ G $\times$ E interaction (GGE) biplots (Yan and Kang, 2002; Yan et al., 2007) was conducted for all of the recorded traits. The biplot is based on the site regression linear-bilinear (multiplicative) model. For a full description of the equation, see Yan et al. (2007). Two analyses were performed on the recorded traits. The first analysis was the "which-won-where" method of the GGE biplot that was used to visualize the similarities among environments and to determine which cultivars are adequate for mega-environments. One mega-environment can be defined as the representative environment in which a cultivar 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). The GGE analysis was done using GGE biplot software (Yan, 2001).

Results and Discussion

The traits measured to determine heat tolerance in crisphead lettuce showed significant differences ($P < 0.0001$) among the tested cultivars (Table 2), indicating genetic variation for the traits under heat stress. These data confirmed a previous report of genetic variation for heat tolerance-related traits in eight crisphead lettuce cultivars (Jenni and Yan, 2009). Only a small proportion of this variation was explained by G with values ranging from 3% for bolted plants to 25% in number of tipburned leaves per plant (Table 2). Genetic variation for heat tolerance has been reported in rice (Ali et al., 2019), tomato (Driedonks et al., 2018), maize (Chen et al., 2012), and leaf lettuce (Lafta et al., 2017). Heat-tolerant romaine and butterhead lettuces have yet to be identified in field conditions, although there is indication of genetic variation for heat tolerance in romaine lettuce grown hydroponically in a greenhouse (Holmes et al., 2019).

A minor contribution to the variation for yield and related traits by G may be in part due to the narrow genetic variability in crisphead lettuce. The predominant ancestors of modern crisphead lettuce cultivars are Vanguard, Salinas, and Calmar (Mikel, 2007, 2013). 'Calmar' is a Great Lakes-type cultivar developed in the 1960s (Welch et al., 1965). 'Vanguard' has both *Lactuca saligna* and *Lactuca serriola* in its pedigree (Thompson and Ryder, 1961). 'Vanguard 75' was developed by the addition of resistance to lettuce mosaic virus to 'Vanguard' (Ryder, 1979b). 'Salinas' (Ryder, 1979a) was selected from a cross between a Vanguard-like breeding line and 'Calmar'. Among modern crisphead lettuce cultivars, the probabilities of 'Vanguard', 'Salinas', and 'Calmar' in their pedigrees are 24%, 12%, and 11%, respectively (Mikel, 2013). Hence, it can be assumed that most cultivars tested here may share common ancestors, explaining the small variation for G.

Compared with the cool environment in Salinas (S612), 10 of the 25 cultivars had higher HW when planted at warmer Five Points in May 2012 (W512): Atlas Non-Bolting, Baomach, Reine des Glaces, Empire, Green Prince, Hanson Improved, Harvest Pride, Imperial 749, Oasis, and Primetime (Table 3). The warmer conditions could have promoted the growth of these cultivars leading to higher HW. In addition, five cultivars had similar HW when planted in these two environments: Calmar, Diamond, Green Lake Butterhead, Invader, and Summertime. Among these cultivars, Atlas Non-Bolting and Primetime had higher HW in the Mar. 2013 planting at Holtville (D313) than the Salinas planting (S612), whereas Calmar and Hanson Improved showed no significant difference at the two environments. All cultivars suffered significant reductions in HW when planted at Holtville in May 2012 (D512) and at Five Points in Mar. 2013 (W313), as compared with the Salinas planting. 'Primetime' had the highest HW across environments with 734 g per head of lettuce. This was greater than the HW for the controls 'Salinas' (633 g) and 'Vanguard 75' (585 g) (Table 3). In three (D313, W512, and S612) of the five environments, 'Primetime' had HW of more than 900 g, higher than the HW of recently released crisphead lettuce breeding lines adapted to the California central coast which ranged from 567 to 848 g per head in field trials in Salinas (Hayes et al., 2018). Crossing cultivars with the highest

yield in warmer conditions with cultivars that have a high yield under normal conditions is recommended as a means to combine alleles for higher yield in both environments. 'Primetime' had the most acceptable head diameter, limited bolting, and fewer leaves with tipburn per plant compared with the other tested cultivars.

One caveat with the use of 'Primetime' as breeding material is its relatively long CL. Breeders could make crosses with 'Green Lake Butterhead' for short CL, as it had the shortest CL across all environments (Table 3). The core in conjunction with the total HW (HW/CL ratio) is an indicator of how much of the lettuce head is useful to the market. The highest ratio is the most desirable for the lettuce industry. 'Oasis', 'Baomach', and 'Invader' had mean ratios of 10.7, 10.5, and 10.3 g/mm (Table 4). These cultivars had greater values compared with 'Salinas' (7.1), 'Vanguard 75' (6.0), and 'Primetime' (7.3).

Although the traits mentioned previously are key to plant breeders for lettuce improvement, the crisphead breeding industry requires other characteristics to be considered. Head diameter in lettuce is an indicator of yield and certain size requirements must be met for the whole head market. 'Primetime', 'Hallmark WS', 'Diamond', and 'Salinas' had the largest diameter (Table 4). This supports the use of 'Primetime' as a source for breeding of heat tolerance.

Market maturity is a trait that indicates proper maturity at harvest. Harvesting occurred 65 d after planting on average, which is the standard number of days to harvest in the production locations between spring and summer in planting slots in southern California and the Salinas Valley. Despite the warmer conditions, 'Sniper' and 'Green Lake Butterhead' had mean market maturity ratings over 3 across the environments tested (Table 5). 'Glacier' and 'Oasis' had mean ratings of 2.9; 'Salinas', 'Summertime', and 'Tiber' had mean ratings of 2.8. The other cultivars did not reach the desirable maturity under the warmer conditions. No cultivar formed heads properly in the hot environment of May 2012 planting at Holtville (D512). Crisphead lettuce cultivars specifically bred for southern and inland California planting slots did not form heads when planted in the central coast of California, indicating lack of adaptability (Sandoya et al., 2017).

Acceptable heat-tolerant lettuce cultivars should not bolt under warmer conditions.

Table 2. Analyses of variance of yield, maturity, head width, core length, bolting, tipburn, and ratio of head weight to core length of 25 crisphead lettuce cultivars grown in five environments.

Source	df ^a	Head wt (HW)		Core length (CL)		HW:CL		Head diam		Market maturity		Bolting		Tipburn	
		P	SS ^b	P	SS	P	SS	P	SS	P	SS	P	SS	P	SS
G	24	<0.0001	11.0	<0.0001	12.7	<0.0001	8.9	<0.0001	17.6	<0.0001	17.7	<0.0001	3.3	<0.0001	25.1
E	4	<0.0001	80.4	<0.0001	71.9	<0.0001	82.7	<0.0001	66.5	<0.0001	69.4	<0.0001	88.6	<0.0001	42.4
G $\times$ E	96	<0.0001	8.6	<0.0001	15.4	<0.0001	8.4	<0.0003	15.8	<0.0001	12.8	<0.0001	8.0	<0.0001	32.5
Rep (E)	15	0.0169		<0.0001		0.0002		0.0001		0.0008		0.0314		0.0368	
Error	360														

^aDegrees of freedom.

^bSums of squares, percentage of the total sum of squares of genotype (G), environment (E), and genotype $\times$ environment interaction (G $\times$ E).

Table 3. Head weight and core length of 25 crisphead lettuce cultivars planted in five different environments: DREC Holtville Mar. 2013 (D313) and May 2012 (D512), WSREC Five Points Mar. 2013 (W313) and May 2012 (W512), and Salinas June 2012 (S612).

Genotype	Head wt (g)						Core length (cm)					
	D313	D512	W313	W512	S612	Mean (G)	D313	D512	W313	W512	S612	Mean (G)
Anza	557	199	439	662	759	523	18.0	48.3	4.5	37.2	3.1	22.2
Atlas Non-bolting	707	262	320	762	634	537	26.1	62.0	3.7	45.6	3.8	28.2
Badger	640	270	404	626	875	563	29.5	49.3	4.5	37.0	4.6	25.0
Baomach	668	218	351	998	760	599	5.4	31.8	2.8	17.8	3.9	12.3
Barcelona	514	91	349	724	962	528	8.3	26.5	4.6	29.8	6.5	15.1
Barrier Reef	722	220	435	833	1054	653	8.9	27.1	5.3	27.2	7.7	15.2
Calmar	750	153	553	807	783	609	7.3	34.4	4.0	19.5	4.1	13.9
Diamond	607	214	512	889	876	620	7.5	40.1	4.6	21.2	5.2	15.7
Empire	559	106	278	943	725	522	4.6	26.0	2.8	15.1	3.9	10.5
Glacier	711	141	477	913	1141	677	9.7	32.3	5.2	25.3	7.6	16.0
Green Lake Butterhead	335	83	253	643	678	399	3.5	13.8	3.0	9.5	3.9	6.7
Green Prince	634	270	465	908	824	620	6.3	35.6	3.8	19.8	4.0	13.9
Hallmark WS	673	267	489	982	1046	692	10.2	52.8	5.6	44.8	6.4	23.9
Hanson Improved	367	164	315	593	366	361	19.9	49.2	7.0	50.7	5.3	26.4
Harvest Pride	567	177	511	819	755	566	8.1	30.3	4.8	28.0	5.0	15.3
Imperial 749	624	192	383	958	899	611	11.9	45.5	4.5	42.3	5.2	21.9
Invader	647	276	352	856	825	591	5.2	47.2	3.7	9.4	4.2	13.9
Oasis	503	121	291	767	717	480	3.4	24.0	2.6	17.3	3.4	10.1
Primetime	978	280	498	990	925	734	13.1	30.0	5.6	28.8	5.9	16.7
Reine des Glaces	359	255	215	648	572	410	8.2	60.7	3.6	44.2	5.2	24.4
Salinas	700	188	445	857	975	633	10.2	27.8	4.9	32.0	6.1	16.2
Sniper	681	178	474	884	934	630	9.3	28.9	5.2	35.1	6.4	17.0
Summertime	559	158	349	694	683	489	4.9	26.3	3.5	16.8	4.4	11.2
Tiber	843	196	456	843	894	646	7.5	29.4	4.3	22.3	4.9	13.7
Vanguard 75	667	155	355	772	976	585	25.4	28.0	4.1	27.9	6.4	18.4
Mean (E)	623	193	399	815	825	571.1	10.9	36.3	4.3	28.2	5.1	17.0
LSD _{0.05} for E	50						5.3					
LSD _{0.05} for G	74						4.8					
LSD _{0.05} for E × G	165						10.8					

G = genotype; E = environment; LSD = least significant difference.

Table 4. Ratio of head weight/core length and head diameter of 25 lettuce cultivars planted in five different environments: DREC Holtville Mar. 2013 (D313) and May 2012 (D512), WSREC Five Points Mar. 2013 (W313) and May 2012 (W512), and Salinas June 2012 (S612).

Genotype	Head wt/core length (g/mm)						Head diam (cm)					
	D313	D512	W313	W512	S612	Mean (G)	D313	D512	W313	W512	S612	Mean (G)
Anza	3.5	0.5	9.9	1.8	25.0	8.1	12.8	8.8	13.2	13.6	13.6	12.4
Atlas Non-Bolting	2.8	0.4	8.6	1.7	16.5	6.0	12.9	11.2	12.3	12.8	10.9	12.0
Badger	2.2	0.6	9.5	1.8	19.4	6.7	14.3	10.8	12.9	13.8	14.3	13.2
Baomach	12.5	0.9	13.0	5.7	20.5	10.5	14.0	10.4	11.8	17.7	13.9	13.6
Barcelona	6.3	0.4	7.8	2.5	15.1	6.4	13.6	7.8	12.4	16.1	15.1	13.0
Barrier Reef	8.2	0.8	8.1	3.7	13.8	6.9	14.7	10.4	12.4	16.7	15.3	13.9
Calmar	10.3	0.5	14.0	4.3	19.7	9.8	14.9	11.9	13.0	15.4	14.2	13.9
Diamond	8.3	0.6	11.4	4.5	16.9	8.3	14.3	11.6	12.7	17.6	15.2	14.3
Empire	12.2	0.4	10.2	8.1	18.6	9.9	13.9	8.6	11.2	15.9	13.4	12.6
Glacier	7.4	0.5	9.1	3.7	15.0	7.1	14.3	10.1	13.4	16.6	15.3	13.9
Green Lake Butterhead	9.2	0.6	8.5	6.9	17.4	8.5	10.6	8.7	9.8	13.5	12.8	11.1
Green Prince	10.5	0.9	12.3	4.7	20.5	9.8	13.6	13.1	11.6	15.7	14.7	13.7
Hallmark WS	6.5	0.5	9.0	2.4	16.5	7.0	14.5	12.3	13.4	15.5	15.7	14.3
Hanson Improved	1.9	0.3	4.5	1.2	7.3	3.0	12.1	8.0	13.7	11.8	12.2	11.5
Harvest Pride	7.1	0.7	10.5	4.1	15.2	7.5	13.8	10.4	14.4	15.3	14.7	13.7
Imperial 749	5.4	0.6	8.6	2.5	17.5	6.9	14.0	11.6	12.8	15.5	14.6	13.7
Invader	12.6	0.7	9.5	9.3	19.6	10.3	13.9	10.0	13.0	14.6	14.3	13.2
Oasis	15.1	0.5	11.6	5.6	20.6	10.7	12.5	9.5	11.0	14.4	13.3	12.1
Primetime	7.5	0.9	8.9	3.5	15.5	7.3	15.2	10.4	13.5	17.6	14.8	14.3
Reine des Glaces	4.9	0.4	6.0	1.6	11.4	4.8	13.1	10.0	10.8	13.2	12.9	12.0
Salinas	7.1	0.7	9.1	2.7	16.2	7.1	14.4	12.4	12.6	15.3	15.8	14.1
Sniper	7.3	0.8	9.1	2.6	14.6	6.9	13.5	8.7	12.5	15.7	15.3	13.1
Summertime	11.4	0.7	9.9	4.3	15.5	8.4	13.4	9.3	11.8	14.3	14.2	12.6
Tiber	11.3	0.8	10.6	4.2	18.0	9.0	14.3	9.9	12.6	15.8	15.5	13.6
Vanguard 75	2.6	0.5	8.8	2.8	15.4	6.0	13.1	10.1	12.3	16.1	15.5	13.4
Mean (E)	7.8	0.6	9.5	3.8	16.9	7.7	13.7	10.2	12.4	15.2	14.3	13.2
LSD _{0.05} for E	0.9						1.2					
LSD _{0.05} for G	1.1						0.9					
LSD _{0.05} for E × G	2.4						2.0					

G = genotype; E = environment; LSD = least significant difference.

‘Invader’ (18%) and ‘Green Lake Butterhead’ (20%) had the fewest number of heads bolted and were significantly different from both controls (Table 5). Bolting is a complex

trait with an important genetic component. For example, PI 251246 flowers as early as 30 d from seeding (Ryder and Milligan, 2005), whereas *Lactuca virosa* species, a

wild relative of cultivated lettuce, bolts and flowers on a biennial basis (Ryder, 1999). The trait is also influenced by heat waves during the growing season, as

Table 5. Market maturity and percent bolted heads of 25 crisphead lettuce cultivars planted in five different environments: DREC Holtville Mar. 2013 (D313) and May 2012 (D512), WSREC Five Points Mar. 2013 (W313) and May 2012 (W512), and Salinas June 2012 (S612).

Genotype	Market maturity						Percent bolted heads					
	D313	D512	W313	W512	S612	Mean (G)	D313	D512	W313	W512	S612	Mean (G)
Anza	3.2	1.0	3.1	1.7	3.3	2.4	25	100	0	75	0	40
Atlas Non-Bolting	2.1	1.0	2.0	1.3	1.3	1.5	33	100	0	100	0	47
Badger	2.6	1.0	3.3	1.5	2.4	2.2	33	100	0	83	0	43
Baomach	3.5	1.0	3.1	2.9	2.9	2.7	0	100	0	33	0	27
Barcelona	3.3	1.0	2.9	2.9	3.7	2.7	0	100	0	50	8	32
Barrier Reef	3.4	1.0	3.2	2.2	3.8	2.7	0	100	0	58	0	32
Calmar	3.4	1.0	3.5	3.0	2.9	2.7	0	100	0	42	0	28
Diamond	3.1	1.0	3.4	2.5	3.4	2.7	0	100	0	33	0	27
Empire	2.7	1.0	2.5	2.3	2.7	2.2	0	100	0	25	0	25
Glacier	3.9	1.0	3.3	2.3	4.0	2.9	3	100	0	58	0	32
Green Lake Butterhead	3.7	1.0	3.5	3.6	3.7	3.1	0	100	0	0	0	20
Green Prince	3.5	1.0	3.4	2.3	3.1	2.7	0	100	0	58	0	32
Hallmark WS	3.5	1.0	3.3	1.6	3.6	2.6	3	100	0	100	8	42
Hanson Improved	2.0	1.0	2.3	1.0	1.5	1.6	28	100	0	100	0	46
Harvest Pride	3.3	1.0	3.5	2.0	3.3	2.6	0	100	0	67	0	33
Imperial 749	2.9	1.0	2.4	1.8	2.5	2.1	17	100	0	83	0	40
Invader	3.2	1.0	2.8	3.3	3.4	2.7	0	89	0	0	0	18
Oasis	3.7	1.0	3.3	3.2	3.1	2.9	0	100	0	25	0	25
Primetime	3.3	1.0	2.4	2.5	2.8	2.4	6	100	0	50	0	31
Reine des Glaces	2.6	1.0	2.3	1.4	1.3	1.7	3	100	0	100	0	41
Salinas	3.5	1.0	3.2	2.7	3.5	2.8	3	100	0	50	0	31
Sniper	3.8	1.0	3.5	3.0	3.5	3.0	0	100	0	83	0	37
Summertime	3.6	1.0	3.4	2.6	3.4	2.8	0	100	0	33	0	27
Tiber	3.7	1.0	3.5	2.3	3.4	2.8	0	100	0	33	0	27
Vanguard 75	2.2	1.0	2.6	2.0	3.6	2.3	31	100	0	58	0	38
Mean (E)	3.2	1.0	3.0	2.3	3.0	2.5	7	100	0	56	1	32.8
LSD _{0.05} for E	0.2						6					
LSD _{0.05} for G	0.3						10					
LSD _{0.05} for E × G	0.6						22					

G = genotype; E = environment; LSD = least significant difference.

demonstrated in this research; the percentage of bolted plants was ≈33% across the environments tested, with a maximum of 47% bolting.

It was expected that heat-tolerant cultivars would have the fewest number of leaves damaged by tipburn. The number of leaves with tipburn per plant ranged from 2.1 ('Tiber') to 11.3 ('Anza') (Table 6). 'Tiber' and 'Anza' differed significantly from 'Vanguard 75', which exhibited 8.6 leaves with tipburn per plant. The addition of 'Tiber' to the breeding pool is recommended, as it was selected for tipburn resistance (Ryder and Waycott, 1998). As expected, the heat-tolerant cultivars Tiber and Primetime were among the cultivars with the least number of tipburned leaves. Our results are consistent with the findings of Jenni and Hayes (2010), who identified 'Tiber' (34.3% of plants with tipburn on average), 'Diamond' (34.3), 'Salinas' (37.6), and 'Sniper' (39.1%) with stable tipburn resistance as compared with 'Vanguard 75' (49.3%) and 'Calmar' (51.5%) across four field trials in 2005 and 2006 in Salinas and Yuma, AZ, which is close to our experiment location Holtville, CA. Jenni and Yan (2009) also found 'Summertime' to be resistant to tipburn but susceptible to rib discoloration and bolting in Quebec, Canada.

Environmental effects accounted for the largest variation in all crisphead characteristics. The variations due to E were 80%, 72%, 83%, 67%, 69%, 89%, and 42% for HW, CL, HW/CL ratio, head diameter, market maturity, percentage of bolted plants, and total

Table 6. Tipburn incidence (number of leaves per plant) on 25 crisphead lettuce cultivars planted in five different environments: DREC Holtville Mar. 2013 (D313) and May 2012 (D512), WSREC Five Points Mar. 2013 (W313) and May 2012 (W512), and Salinas June 2012 (S612).

Genotype	Tipburn (leaves/plant)					
	D313	D512	W313	W512	S612	Mean (G)
Anza	15.5	9.8	9.6	21.4	0.0	11.3
Atlas Non-Bolting	14.8	11.7	4.7	14.6	0.0	9.2
Badger	20.9	13.3	8.1	11.8	0.4	10.9
Baomach	8.4	6.2	3.8	10.2	0.5	5.8
Barcelona	1.2	5.6	3.2	15.0	0.0	5.0
Barrier Reef	2.9	5.7	3.4	5.3	0.0	3.5
Calmar	4.4	11.3	3.8	6.5	0.0	5.2
Diamond	0.1	7.4	1.2	10.3	0.0	3.8
Empire	13.4	6.9	6.9	13.0	0.0	8.1
Glacier	4.3	6.1	3.6	17.6	0.0	6.3
Green Lake Butterhead	5.4	3.3	6.7	9.0	0.0	4.9
Green Prince	4.3	4.6	4.2	5.4	0.0	3.7
Hallmark WS	0.5	5.2	0.5	5.6	0.5	2.5
Hanson Improved	5.1	10.7	4.8	18.5	0.0	7.8
Harvest Pride	1.9	7.1	1.9	5.0	0.0	3.2
Imperial 749	12.8	6.8	6.3	8.3	0.8	7.0
Invader	0.4	11.7	2.5	1.5	0.0	3.2
Oasis	9.1	4.8	3.2	7.2	0.0	4.9
Primetime	0.9	6.7	1.8	3.7	0.0	2.6
Reine des Glaces	3.8	14.0	4.3	14.4	0.0	7.3
Salinas	1.4	8.4	1.2	5.3	0.0	3.3
Sniper	1.7	4.8	2.6	10.5	0.0	3.9
Summertime	1.8	7.1	0.6	8.0	0.0	3.5
Tiber	0.4	4.5	0.9	4.8	0.0	2.1
Vanguard 75	18.4	7.5	4.2	12.9	0.0	8.6
Mean (E)	6.2	7.6	3.7	9.8	0.1	5.5
LSD _{0.05} for E	1.5					
LSD _{0.05} for G	2.3					
LSD _{0.05} for E × G	5.1					

G = genotype; E = environment; LSD = least significant difference.

leaves with tipburn, respectively (Table 2). Two of the five environments were in the Imperial Valley (DREC, Mar. 2013; DREC, May 2012), two environments were in the

San Joaquin Valley (WSREC, Mar. 2013, WSREC, May 2012), and a fifth environment in the Salinas Valley (Salinas June 2012). Mean daily temperature at WSREC ranged

Table 7. Monthly averages of daily maximum, mean, and minimum temperatures (°C), wind velocity, daylength, and precipitation during field trials in Five Points (W313 and W512 at WSREC), Holtville (D313 and D512 at DREC), and Salinas (S612), California in 2012 and 2013.

Location	Month	Temperature (°C)						Wind velocity (km·h ⁻¹)		Day length	Precipitation (cm)	
		Maximum		Mean		Minimum		2012	2013	2012–13	2012	2013
		2012	2013	2012	2013	2012	2013					
WSREC	March	20	24	13	16	7	7	13	5	11h 57m	0.08	0.84
	April	24	28	16	19	8	9	13	7	13h 08m	0.08	0.02
	May	31	31	21	22	11	12	15	9	14h 07m	0.01	0.05
	June	33	36	23	26	13	15	16	9	14h 37m	0.00	0.00
	July	36	39	26	29	16	18	11	7	14h 23m	0.00	0.00
DREC	August	38	38	27	27	17	17	13	8	13h 31m	0.00	0.00
	March	26	29	18	20	9	10	11	7	11h 58m	0.00	0.03
	April	31	31	22	22	13	13	13	9	13h 00m	0.01	0.00
	May	38	35	27	29	18	17	11	9	13h 51m	0.00	0.00
	June	41	41	31	32	22	23	13	7	14h 18m	0.00	0.00
Salinas	July	41	41	33	34	26	28	11	7	14h 05m	0.08	0.00
	August	41	41	35	36	28	26	10	6	13h 20m	0.00	0.56
	June	22	–	17	–	11	–	15	–	14h 39m	0.01	–
	July	21	–	17	–	13	–	13	–	14h 24m	0.00	–
	August	22	–	17	–	13	–	13	–	13h 32m	0.00	–

Source: Weather Underground. Weather history. <https://www.wunderground.com/history>. Accessed 6 May 2020.

Fig. 1. Which-won-where view of the GGE biplot showing the performance of crisphead lettuce genotypes for head weight (A), core length (B), the ratio of head weight to core length (C), head diameter (D), market maturity (E), bolting (F), and tipburn (G). Environments are represented by D313 (Holtville, CA, March 2013 planting), D512 (Holtville, CA, May 2012 planting), W313 (Five Points, CA, March 2013 planting), W512 (Five Points, CA, May 2012 planting), and S612 (Salinas, CA, June 2012 planting).

from 13 to 27 °C from March to August in 2012 and 16 to 29 °C in 2013 with a maximum of 38 to 39 °C in both years (Table 7). Warmer temperatures were registered at DREC during these experiments, where mean daily temperatures ranged from 18 to 35 °C in 2012 and 20 to 36 °C in 2013 with a maximum of 41 °C.

Overall, temperatures in 2013 were higher than in 2012 (Table 7). Milder temperatures were observed in Salinas with steady mean temperatures of 17 °C from June to August (Table 7). As most of the variation in this experiment was explained by E, it is notable that the environment at DREC May 2012 experiment was the worst location-year combination in terms of head quality for production of crisphead lettuce. Between planting in May and the end of the experiment in July 2012, maximum temperatures ranged from 38 to 41 °C at DREC, making this location unacceptable for lettuce production at that time of the year. A significantly lower HW was obtained from DREC May 2012 planting (193 g). This environment also produced

lettuce with the longest CL (36.3 cm) on average, the smallest ratio (0.6 g/mm) of HW/CL, the smallest mean head diameter (10.2 cm), market maturity not reached (1.0), and 100% of the crisphead lettuce plants bolted. A greater number of leaves with tipburn per plant was registered in the WSREC May 2012 planting followed by DREC May 2012 (9.8 and 7.6, respectively) (Tables 3–6).

These findings indicate that planting crisphead lettuce in or around the summer, especially in DREC, is not recommended. However, planting in spring (March planting) when the weather is still not too hot for lettuce production may produce a suitable crisphead crop for lettuce growers. Currently, in southwestern locations, plantings generally occur from September to January and harvesting runs from November until April, with occasional harvests in May. Improvement of heat-tolerant cultivars may aid these production areas by extending their season until June each year.

The best environment to produce lettuce was Salinas in June 2012. This is the environment with the highest mean head weight

and better traits such as shorter CL, higher HW/CL ratio, larger diameter, perfect market maturity, and little bolting or tipburn. Although the experiment was planted in June, the average maximum temperature during this period in 2012 was 21 °C, which was lower than in the other two locations. The Salinas Valley of California is a unique environment for vegetable production with mild temperatures all year round, even in the summer (Table 7). In addition, most cultivars used in this study were improved for the California coast production areas and may be adapted to longer photoperiods.

Although it is true that central California coastal production regions (e.g., Santa Maria-Oxnard, Salinas, and Pajaro Valleys) provide lettuce to the entire U.S. markets from April through the fall (Simko et al., 2014), sometimes lettuce shortages occur due to weather-related issues (i.e., untimely rain, at the beginning and end of the coastal production season) and therefore there is a window of opportunity for the inland and deserts of California and the hot, subtropical area of Florida

Fig. 1. (Continued)

with heat-tolerant lettuce cultivars to provide for these shortages. Land value in the Salinas Valley is two to four times that of the land value in the San Joaquin and Imperial Valleys (Campbell, 2007); therefore, expanding the growing seasons of lettuce in hot but low land-cost areas, such as the San Joaquin Valley or Imperial Valley, may reduce land and production costs and improve the sustainability and profitability of U.S. lettuce crops.

G × E interaction for heat tolerance in lettuce. Variations due to $G \times E$ were relatively small at 9%, 15%, 8%, 16%, 13%, and 8% for HW, CL, HW/CL ratio, head diameter, market maturity, and percentage of bolted plants, respectively. $G \times E$ variation for tipburn was the highest in this study at 33% (Table 2).

The DREC environment (D313) was slightly positively correlated with D512 ($r = 0.42$; $P < 0.01$) and highly correlated with the rest of the environments (W313 $r = 0.67$, W512 $r = 0.64$, and S612 $r = 0.62$; $P < 0.01$)

for HW; the same tendency was observed for yield-related traits, CL, HW/CL, head diameter, and market maturity. These significantly positive correlations suggest the presence of non-crossover $G \times E$ interactions due to the consistency from site to site for yield under warmer environments. Although significant, these correlations are not so high to disregard the presence of any crossover interaction that may hinder progress in selections for yield in warmer areas. Similar to crisphead lettuce, correlations among environments were positive and significant for yield in warmer environments for green and red leaf lettuce (Lafta et al., 2017) suggesting non-crossover interactions. The improvement of yield and its related traits in a warmer environment could be done using a mega-environment that represents the targeted area of a cultivar release. However, crossover interactions were found in physiological disorders that commonly appear as a consequence of growing lettuce under warmer conditions; for example, tip-

burn, rib discoloration incidence, and ribbiness (Jenni and Hayes, 2010; Jenni and Yan, 2009). Although improvement of yield in warmer environments could be achieved using one mega-environment, precautions should be in place as physiological disorders may need multitrail, multiyear, multilocation evaluations because no consistent relationship was found among environments for tipburn and bolting in our study.

A different approach to understand $G \times E$ interaction is to graph (GGE biplot) singular value partitions using the which-won-where method that groups environment(s) into a mega-environment and identifying winning cultivar(s) for the specific target (mega) environment (Yan and Kang, 2002). The which-won-where method of the Biplot is presented for each trait (Fig. 1). The average tester coordinate method was used to identify the most representative environment for each trait (Fig. 2). When cultivars were grouped in a GGE biplot, one mega-environment for

Fig. 2. The average environment coordination (AEC) of the GGE biplot showing the performance of crisphead lettuce genotypes for head weight (A), core length (B), the head weight/core length ratio (C), head diameter (D), market maturity (E), bolting (F), and tipburn (G). Environments are represented by D313 (Holtville, CA, March 2013 planting), D512 (Holtville, CA, May 2012 planting), W313 (Five Points, CA, March 2013 planting), W512 (Five Points, CA, May 2012 planting), and S612 (Salinas, CA, June 2012 planting).

crisphead lettuce with higher HW is the Salinas June 2012 planting (S612), (Fig. 1A). In this environment, cultivar Glacier had the highest HW; other cultivars, such as Barrier Reef, Hallmark WS, Imperial 749, Salinas, Sniper, and Vanguard 75, had an acceptable HW. Nevertheless, this research was planned to find cultivars that could be planted in warmer environments. In the GGE biplot, DREC plantings in 2012 and 2013 (D512 and D313) and WSREC 2012 and 2013 plantings (W512 and W313) were grouped together in a second mega-environment for HW in warm environments. Cultivar Primetime had the highest HW in this mega-environment. The graphing of two mega-environments indicates that improvement for heat tolerance should be conducted under these warm environments and decision on selection should be based on mean performance and stability from experiments across several growing seasons, as previously described (Jenni and Hayes, 2010). Other yield-related traits, such as CL, HW/CL ratio, head diameter, and market maturity, also showed a similar grouping of these mega-environments (Fig. 1B–E); a variety of differ-

ent mega-environments in the GGE biplot (Fig. 1F and G) were graphed for traits that are disorders (tipburn and bolting) due to heat. This is further proof for the existence of crossover $G \times E$ interactions for traits that are consequences of heat in crisphead lettuce.

The average environment coordination of the GGE biplot ranking showed that Tiber is the most stable cultivar across the five environments. ‘Tiber’ was the closest to the abscissa x with little projection to the ordinate y (Fig. 2A). In contrast, ‘Glacier’, ‘Barrier Reef’, and ‘Vanguard 75’ were the least stable cultivars across the five environments. The closest environment to the average in this research was WSREC Mar. 2013, as indicated by the small dotted circle in Fig. 2A. Although ‘Primetime’ was not the most stable in terms of performance, it produced the highest HW in warmer conditions and proved its value for breeding. Similarly, stability across environments was cultivar-dependent for each of the studied traits. For yield-related traits, Hallmark was the most stable cultivar for core length, ‘Baomach’ and ‘Diamond’ were the most stable for the HW/CL ratio,

and ‘Harvest Pride’ was most stable for head diameter (Fig. 2B–D). ‘Primetime’ was the most stable across all environments tested for percentage of bolted plants and ‘Anza’ for the number of leaves with tipburn (Fig. 2F and G).

Our data provided clear evidence of $G \times E$ interactions and environments are separated in warmer locations (Holtville and Five Points, CA) and a less warm location (the Salinas Valley in California in the summer planting in central California). Although a non-crossover $G \times E$ interaction was detected for HW, the negatively related traits, bolting and tipburn, exhibited differently across these environments, suggesting that breeding for heat tolerance should take into consideration these important characteristics during multilocation trials.

The Salinas Valley has a milder climate than Holtville and Five Points, meaning that selection for heat tolerance traits should be conducted in warmer conditions for lettuce. It is likely that the material improved for the central coastal California (e.g., the Salinas Valley) may not be immediately suitable for

Fig. 2. (Continued)

production in the warmer environments (i.e., San Joaquin and Imperial Valleys). Five Points Mar. 2013 may be an appropriate location with conditions that can improve lettuce for heat tolerance because it graphed near the ideal environment (x exe) when considering yield and other related traits, although for traits such as bolting, tipburn, and CL, the Five Points Mar. 2013 is graphed farther from the x exe. Planting lettuce in March with harvest occurring until May could be adopted in warmer production areas.

Although the tested crisphead material presented genetic variability for HW and other physiological disorders as indicators of heat tolerance, there is room for improvement of crisphead lettuce to be adapted to warmer conditions. However, in this experiment, only a subset of available genotypes was tested in field conditions and there may be lettuce cultivars with higher heat tolerance that remain unidentified. Hence, additional tests of lettuce cultivars in different environments, especially lines that have been improved for bolting and tipburn, should be carried out. Breeding of heat-tolerant crisphead lettuce should be conducted in multitrail and multilocations because of the crossover interaction identified in physiological disorders that occur in part because of warmer environments.

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