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Tocopherols, Phylloquinone, Ascorbic Acid, and Sugar Contents in Hydroponically Grown Lettuce

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Abstract. Growing vegetables in controlled environments (CEs), such as hydroponics, aquaponics, and vertical structures, is a rapidly expanding industry in Florida and the United States, especially in nearby urban areas. Although lettuce (Lactuca sativa) is still mostly produced in fields, growing in CEs proximal to urban areas has become increasingly popular because it may facilitate reduced transportation time and associated postharvest degradation. Lettuce is among the top-most consumed vegetables in the United States and could provide some of the nutrition missing in the US diet. This research was planned to understand the levels of some vitamins that are key for human health, including vitamin E (tocopherols), vitamin K_1 (phylloquinone), and vitamin C (ascorbic acid), in lettuce grown in greenhouse hydroponics. Lettuce germplasm was grown using the hydroponic nutrient film technique system in three greenhouse experiments: at the beginning, middle, and end of the Florida, USA, growing season (from Aug 2020 to Mar 2021). Genetic variation for these vitamins were found among the germplasm tested in the four morphological types of lettuce, romaine, Boston, Latin, and leaf. In addition, a sugar analysis was conducted in this germplasm, of which fructose was the most abundant sugar. A significant genotype × environment (G × E) interaction was observed, indicating that the levels of these compounds, especially vitamins, was environment dependent. However, the presence of certain non-crossover G × E interactions indicates that selecting lettuce in a representative environment could result in new cultivars with higher vitamin content. This research marks the initial steps to improve lettuce for these vitamins, which can contribute to better health of US consumers, not for the highest amount of these compounds in lettuce but for the offset due to its high consumption.

Lettuce (Lactuca sativa) is among the top-most consumed vegetables in the United States, planted on 139,000 ha, and worth $3.5 billion annually [US Department of Agriculture (USDA), National Agricultural Statistics Service (NASS) 2019]. Although there are at least five different morphological types within the cultivated species L. sativa (Ryder 1999), only a few are commercially planted in the United States. The most commonly planted lettuce types are head (iceberg and Boston), Cos (romaine), and leaf lettuce, accounting for 43%, 35%, and 22% of the total planted area, respectively (USDA NASS 2019). These statistics are for US field-produced lettuce, which concentrates mostly in California (73%), Arizona (21%), and Florida (3%), although lettuce is produced in smaller operations (3%) throughout the United States, especially in the summer (USDA NASS 2019). In addition to field production, there are 402,270 m^2 of lettuce cultivated in areas under protection (Walters et al. 2020).

In Florida, USA, alone, an increase in the area of fresh fruits and vegetables produced in controlled environments (CEs) was reported from less than 41 ha before 2000 to nearly 160 ha in 2013 (Hochmuth and Toro 2014). The size of Florida's CE industry in 2013 included 240 grower operations producing at least 10 ha of lettuce and leafy greens (Hochmuth and Toro 2014). One of the drivers for this increase is that consumers are increasingly concerned with the quality of fresh produce, including locally produced fresh vegetables (Gordon et al. 2019). Traditionally in the United States, lettuce is supplied year-round from western states to the rest of the country and must travel long distances in refrigerated containers (Hayes 2018). Therefore, nutrients such as vitamin C (ascorbic acid), for example, could degrade before consumption due to long-distance transportation (Martínez-Ispizua et al. 2022a). One of the potential benefits of lettuce grown in local CEs is shorter transportation to distributors and consumers. This could allow for lettuce to be harvested and consumed fresher, potentially providing consumers with lettuce that has less degradation of ascorbic acid or other nutrients. In addition to fresher food, CEs can complement the current food supply grown in fields. As the human population continues to grow toward an estimated 10 billion people by 2050 (United Nations 2015), producing food in CEs could play a vital role in helping alleviate the increased food demand in future years.

Lettuce is commonly linked to a healthy diet because of its low caloric content, but it is not considered as a top provider of vitamins and minerals (Murray et al. 2021). However, lettuce possesses nutrients essential to humans including provitamin A (b-carotene), vitamin C (ascorbic acid), vitamin K_1 (phylloquinone), vitamin E (a- and g-tocopherol), and other minerals including Fe, Ca, and K (Mou 2008; Murray et al. 2021). Even though several vitamins and minerals in lettuce are not as high as in other leafy greens such as spinach (Spinacia oleracea) or kale (Brassica oleracea var. acephala), the annual per capita consumption of lettuce is more than 4.5 kg, compared with 0.68 kg for spinach and less than 0.5 kg for kale (USDA Economic Research Service 2020).

A commonly studied vitamin is ascorbic acid that helps prevent scurvy disease (Buxeraud and Faure 2021). Ascorbic acid is a very unstable and easily degradable vitamin; although, its significant concentration in leafy greens has been documented (Mou 2008; Simko 2019; Simonne et al. 2002). Tocopherols and phylloquinone are two fat-soluble nutrients that possess free-radical scavenging properties. Tocopherol is an antioxidant protecting cellular components like cell membranes from oxidative degeneration and can improve postharvest shelf life of foods (Liu et al. 2015). Dietary consumption of tocopherols can help protect against high cholesterol, cancer, heart disease and inflammation-related illnesses (Stacey et al. 2016). Phylloquinone is an electron carrier of photosystem I (van Oostende et al. 2008). In humans, phylloquinone is necessary for blood coagulation, as well as producing proteins that are required for bone formation, inhibiting calcification of arterial walls, regulating cell growth, and others with unknown function (Vermeer 2012). Additionally, tocopherols and ascorbic acid are antioxidants in plants that can potentially enhance tolerance to stresses, and little is known how increased phylloquinone might improve plant health.

The concentration of vitamins and minerals in lettuce seems to be morphological type dependent (Mou 2008). For instance, iceberg lettuce has minimal amounts of vitamins and minerals, but stalk (or known as stem) lettuce has the highest amounts of these compounds among types (Mou 2008). Differences for ascorbic acid, folate, b-carotene, and lutein were associated with specific lettuce types including loose-leaf, crisphead, butterhead, and romaine lettuce grown to full maturity in fields (Simonne et al. 2002). Furthermore, differences among accessions within lettuce types have been identified for ascorbic acid, b-carotene, and anthocyanins in baby leaf lettuce (Simko 2019), as well as polyphenols, antioxidants, and lutein in mature head lettuce (Llorach et al. 2008; Mou 2005). Additionally, vitamin concentrations in lettuce depend on the phenological stage and are influenced by the environment (Martínez-Ispizua et al. 2022b; Mou 2005; Yang et al. 2021). In field production, ascorbic acid content changes through seasons, and lower values of ascorbic acid are found in lettuce produced in warmer environments (Mou 2005). In greenhouse, differences in b-carotene content between winter and summer seasons were genotype-dependent (de Souza et al. 2022). Field-grown lettuce is likely to have higher vitamin content compared with lettuce produced in CEs largely due to the exposure of direct sunlight and ultraviolet radiation (Alves et al. 2022), but growing conditions in CEs can be manipulated so that the content of vitamins and minerals could be increased (Mou 2008). For instance, ascorbic acid and b-carotene content on one romaine lettuce cultivar was similar in both field and hydroponically grown lettuce (Lei and Engeseth 2021).

Breeders could focus on improving the content of tocopherols, phylloquinone, and ascorbic acid in lettuce to help improve human diets and plant stress tolerances, adding extra benefit to growers (Asensi-Fabado and Munné-Bosch 2010). Unfortunately, little information exists regarding genetics and its interaction with environmental factors influencing the selection of lettuce with improved vitamin content. Therefore, this research was planned to determine genetic differences in ascorbic acid, a- and g-tocopherols, and phylloquinone in a set of lettuce germplasm cultivated in a greenhouse hydroponic system. Sugars were also measured because they remain one of the key features attracting consumer preferences and ideally would not be diminished by breeding for increased nutrient content (Chadwick et al. 2016). An additional goal of this research was to establish the genotype × environment (G × E) interaction for these compounds among a diverse set of four lettuce types to provide an indication for how breeding schemes might be designed for improving vitamin contents in lettuce.

Materials and Methods

Plant material

Cultivars and breeding lines of different morphological types were used to conduct this study and comprised nine romaine, eight Boston, six Latin, and seven leaf germplasm accessions (Table 1, Fig. 1A–D). Thirteen of these accessions were commercial cultivars and seventeen were advanced breeding lines from the University of Florida, Institute of Food and Agricultural Science

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Table 1. Breeding status, type, source, and color of 30 lettuce accessions representing four morphological types measured for nutrients in three nutrient film technique hydroponic experiments. All 30 accessions were green in color and absent of anthocyanin or other visible pigmentation.

Code[i]	Status	Genotype	Type	Source[ii]	Color[iii]
G1	Breeding line	BG18–0588	Romaine	UF/IFAS	MG
G2	Breeding line	60182	Romaine	UF/IFAS	MG
G3	Breeding line	60184	Romaine	UF/IFAS	MG
G4	Breeding line	60183	Romaine	UF/IFAS	MG
G5	Breeding line	C1145	Romaine	UF/IFAS	MG
G6	Breeding line	43007	Latin	UF/IFAS	MG
G7	Cultivar	Salvius	Romaine	JSS	MG
G8	Cultivar	Sparx	Romaine	JSS	MG
G9	Cultivar	Monte Carlo	Romaine	JSS	DG
G10	Cultivar	Green Forrest	Romaine	JSS	MG
G11	Breeding line	B1196	Boston	UF/IFAS	LG
G12	Breeding line	70202	Boston	UF/IFAS	LG
G13	Breeding line	70882	Boston	UF/IFAS	LG
G14	Breeding line	50111	Boston	UF/IFAS	MG
G15	Cultivar	Rex	Boston	JSS	LG
G16	Cultivar	Salanova Green Butter	Boston	JSS	MG
G17	Cultivar	Mirlo	Boston	JSS	LG
G18	Cultivar	Palmetto	Boston	JSS	MG
G19	Breeding line	BG19-0255	Leaf	UF/IFAS	MG
G20	Breeding line	BG19-0200	Leaf	UF/IFAS	MG
G21	Breeding line	BG19-0208	Leaf	UF/IFAS	DG
G22	Breeding line	H1059	Leaf	UF/IFAS	MG
G23	Cultivar	Muir	Leaf	JSS	LG
G24	Cultivar	North Star	Leaf	Unknown	DG
G25	Cultivar	Salanova Green Oakleaf	Leaf	JSS	MG
G26	Breeding line	C1146	Latin	UF/IFAS	LG/DG
G27	Breeding line	C1148	Latin	UF/IFAS	DG
G28	Breeding line	45060	Latin	UF/IFAS	DG
G29	Cultivar	Newham	Latin	JSS	MG
G30	Cultivar	Little Gem	Latin	JSS	DG

[i] Codes used for graphing genotypes for the genotype **1** genotype × environment biplot analysis figures.
[ii] UF/IFAS5 University of Florida/Institute for Food and Agricultural Science, FL, USA; JSS 5 Johnny's Selected Seeds, Winslow, ME, USA.
[iii] MG 5 medium green, DG 5 dark green, LG 5 light green.

(UF-IFAS) lettuce breeding program (Table 1). Before this research, the UF-IFAS germplasm was increased at the Everglades Research and Education Center in Belle Glade, FL, USA. Seeds of the commercial cultivars were provided by Johnny's Selected Seeds (Winslow, ME, USA).

Experiment description

Three experiments were conducted using a nutrient film technique (NFT) hydroponic system (CropKing Inc., Lodi, OH, USA) at the North Florida Research and Education Center—Suwannee Valley (NFREC-SV) near Live Oak, FL, USA. Plants were grown in a 162-m^2 passively ventilated greenhouse with a roof consisting of two layers of clear polyethylene (Table 2). The greenhouse has an automated ridge vent and roll-up sidewalls to facilitate ventilation and was covered with high-performing shading material with 50% shade level (Aluminet; Signature Supply, Lakeland, FL, USA). The NFT system consisted of 30 channels that were 3.05 m long and 11.4 cm wide, with each channel holding 15 plants (450 plants total) at 25.4-cm spacing between plants. The three experiments were separately conducted during three different planting times. Expt. 1 was started by seeding in rockwool on 6 Aug 2020, Expt. 2 was seeded on 6 Oct 2020, and Expt. 3 was seeded on 22 Jan 2021 (Table 2). Fifteen seedlings for each genotype were transplanted between 20 to 24 d after seeding in the NFT channels and distributed as a randomized complete block design (RCBD) with three replicates. Each replicate consisted of five plants per genotype (30 accessions × 3 replicates × 5 plants 5 450 plants total).

Plants were grown with roots contained in a plastic NFT channel (CropKing Inc.) for another 19, 24, and 25 d until harvest for Expts. 1, 2, and 3, respectively. The pH of the nutrient solution was maintained at 5.8 and the EC at 1.2 $mS\cdot cm^{-1}$ using an automated nutrient and pH monitoring system (Fertroller, CropKing Inc.) and an automated nutrient and pH management dosing controller (Bluelab Dostronic; Tauranga, Gisborne, New Zealand). This system used two concentrated nutrient tanks with a formulation of individual mineral salts and one tank with acid (Hochmuth and Hochmuth 1990). The main reservoir was 378.5 L filled with a nutrient solution that provided 120 $mg\cdot L^{-1}$ nitrogen, 50 $mg\cdot L^{-1}$ phosphorus, 200 $mg\cdot L^{-1}$ potassium, 48 $mg\cdot L^{-1}$ magnesium, 220 $mg\cdot L^{-1}$ calcium, 60 $mg\cdot L^{-1}$ sulfur, 3.5 $mg\cdot L^{-1}$ iron, 0.3 $mg\cdot L^{-1}$ copper, 1.3 $mg\cdot L^{-1}$ manganese, 0.3 $mg\cdot L^{-1}$ zinc, 0.7 $mg\cdot L^{-1}$ boron, and 0.05 $mg\cdot L^{-1}$ molybdenum (Hochmuth and Hochmuth 1990). A 746-W water chiller (Penguin Chillers;

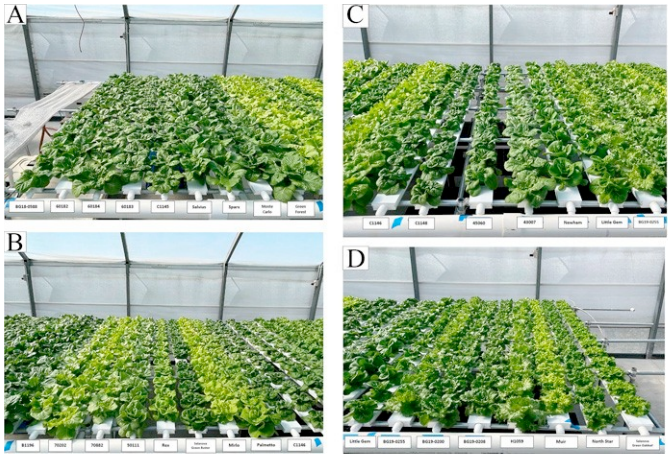

Fig. 1. Illustration of the experimental conditions for the three nutrient film technique hydroponic experiments and morphology of the 30 lettuce genotypes approaching physiological maturity tested in this study. (A–D) Each row of 15 plants was divided among three replicates running the perpendicular length of the system.

Knoxville, TN, USA) was used to keep the reservoir at 20 °C during night hours. The nutrient solution continuously circulated through the system at a flow rate of 591.5 mL·min^{-1}.

Weather data were retrieved from a Florida Automated Weather Network (FAWN) station located at the NFREC-SV 350 m from the greenhouse (University of Florida 2021). Average, minimum and maximum daily temperatures were registered for the duration of Expts. 1, 2, and 3 (Table 2). Similarly, average solar radiation was also retrieved from FAWN (Table 2). Although not continuously monitored, the conditions inside the greenhouse were generally warmer at night and warmer in direct sunlight, relative to the surrounding environment. Solar radiation was also reduced due to the polyethylene and shading materials.

Ascorbic acid, tocopherols, phylloquinone, and moisture analysis

At harvest (41, 43, and 53 d after seeding for Expts. 1, 2, and 3, respectively), one lettuce head per cultivar from each of three replications was collected, maintained on ice, and transported to the laboratories of the Horticultural Sciences and the Family, Youth and Community Science Departments in Gainesville, FL, USA. Each head of lettuce was divided in two equal halves, with one half processed for α- and γ-tocopherol and phylloquinone and the other half processed for ascorbic acid and moisture analysis at the two different laboratories.

Ascorbic acid and moisture. Due to the smaller size of some lettuce cultivars, the edible portions of one-half lettuce from two or three replicates were homogenized into one composite sample. Two independent subsamples were obtained for both moisture and ascorbic acid protocols. For measuring ascorbic acid, the two composite subsamples were individually blended in metaphosphoric-acetic acid extractant and analyzed by the titrimetric method according to the Association of Official Analytical Chemists (AOAC) method 967.22 (AOAC International 1995). Additional sample protection protocols were similar to the previous works by Simonne et al. (2002) to minimize conversion of ascorbic acid to dehydroascorbic acid. Moisture was analyzed on two separate composite subsamples according to the AOAC method 920.251 (AOAC International 1995).

Tocopherols and phylloquinone. Leaf tissues were collected from the top, middle and base of freshly harvested whole lettuce heads using a circular punch biopsy tool (6 mm diameter) run perpendicular throughout the plant. For each lettuce head, leaf samples [0.17–0.63 g fresh weight (FW)] were pooled and homogenized in 0.8 mL of 95% (v/v) ethanol using a 5-mL Pyrex tissue grinder. The grinder was rinsed with 0.2 mL of 95% (v/v) ethanol, and the wash was combined to the original extract. The samples were then centrifuged (5 min, 21,000 g_n) and immediately injected (5 μL) on a high-performance liquid chromatography (HPLC) column [5 μM, 250 × 4.6 mm (Supelco Discovery C-18; Sigma-Aldrich Corp., St. Louis, MO, USA)] thermostated at 30 °C and developed in isocratic mode at a flow rate of 0.8 mL·min^{-1} with 80% methanol: 20% of 95% ethanol (v/v) containing 2 mM $ZnCl_2$, 2 mM acetic acid, and 1 mM sodium acetate using HPLC (1260 Infinity II; Agilent Technologies, Santa Clara, CA, USA). Tocopherols were detected by fluorometry (290 nm excitation/330 nm emission). Phylloquinone was also detected fluorometrically (238 nm excitation/426 nm emission) after online reduction into a post-column chemical reactor (70 × 1.5 mm) packed with 100-mesh zinc dust (Sigma-Aldrich Corp.). Retention times were 9.9 min for γ-tocopherol, 11 min for α-tocopherol, and 17.3 min for phylloquinone. These compounds were quantified according to corresponding external calibration standards as previously described (Block et al. 2013; van Oostende et al. 2008).

Relative value of vitamins

An overall total relative vitamin content (TRVC) was calculated to identify accessions with the highest accumulation of tocopherols, phylloquinone, and ascorbic acid. Relative values were calculated for all vitamins using the following formula:

$$TRVC = \sum \frac{\bar{X}\,VitCon}{\mu\,VitCon},$$

where TRVC is the summation of the average value of each vitamin content per genotype ($\bar{X}\,VitCon$) and the overall mean of each vitamin content ($\mu VitCon$).

Sugar content analysis

For sugar analysis, three plants per genotype were evaluated for each of the three replicates on each experiment for soluble solids. Similarly, three plants per genotype were sampled to

Table 2. Experiment dates, average temperatures, and average solar radiation for three experiments conducted in a greenhouse nutrient film technique system located at the North Florida Research and Education Center—Suwanee Valley, Live Oak, FL, USA. Weather data were retrieved from the Florida Automated Weather Network station located at the same station (University of Florida 2021).

	Dates			Daily avg temp (°C)			
Expt.	Planting	Transplanting	Harvesting	Avg	Minimum	Maximum	Avg solar radiation (W·m^{-2})
1	6 Aug 2020	25 Aug 2020	16 Sep 2020	25	22	31	167.8
2	6 Oct 2020	30 Oct 2020	18 Nov 2020	20	15	26	131.2
3	22 Jan 2021	16 Feb 2021	16 Mar 2021	15	9	22	134.1

measure individual sugars including fructose, glucose, and sucrose; due to COVID-19 related restrictions, data were not collected for individual sugars in Expt. 1.

Soluble solids. Soluble solids (percent) were measured to determine the approximate concentration of dissolved soluble solids in the lettuce using a handheld analog refractometer (Thermo Fisher Scientific, Waltham, MA, USA). A hydraulic press was used to mash and squeeze the lettuce cores/stem samples from the bases of middle-aged leaves to provide liquid material for the soluble solid measurements.

Individual sugars: sample collection and extraction. Samples for sugar analysis were likewise collected from the same area as described for soluble solids, and from three plants for each genotype and replicate. A total of 2 to 3 g of sample was ground with 35 mL water (total volume), and incubated at 100 °C for 30 min. After centrifugation at 16,000 g_n for 20 min, 100 mL of supernatant was transferred into the new tube and mixed with 100 mL of acetonitrile.

Compound optimization and quantitation of metabolite. The compounds were optimized using triple quadrupole mass spectrometers (TSQ Altis, Thermo Fisher Scientific) coupled with an ultra high-performance liquid chromatography (UHPLC) system (Vanquish Horizon, Thermo Fisher Scientific) and the internal standards fructose (D-fructose, catalog no. F0127; purity > 99%), glucose (D-glucose, catalog no. G8270; purity > 99%), and sucrose (catalog no. S9378; purity > 99%) were purchased from Sigma-Aldrich Corp. The triple-quadrupole mass spectrometer was housed with a heated-electrospray ionization source using the following settings with sheath gas flow with 50 arbitrary units, auxiliary gas flow with 10 arbitrary units, sweep gas flow with 1 arbitrary unit, ion transfer tube temperature at 325 °C, and vaporizer temperature at 350 °C. The spray voltage was 4.0 kV under the negative polarity. A 1-s cycle time was used. The quadropoles Q1 and Q3 resolution of full width at half maximum were both 0.7; 1.5 mTorr was used for the coalition-activated dissociation gas pressure. To find the optimal fragments and collision energies to be used for multiple reaction monitoring transitions, the data acquisition and processing software (Xcalibur 4.0, Thermo Fisher Scientific) was applied for the Quant optimization under the following conditions: fructose 179 m (mass)/z (charge number of ions) → 89.1 m/z at from 4.5 min to 5.5 min, glucose 179 m/z → 89.1 m/z at from 5.51 min to 7 min, and sucrose 341.1 m/z → 179 m/z at from 8 min to 9.5 min.

The compounds were separated using ACQUITY UPLC BEH Amide Column, 130Å, 1.7 mm, 2.1 mm × 100 mm (Waters, Milford, MA, USA). To accomplish LC separation, the UHPLC system was set for the column compartment temperature at 35 °C, and the flow rate was set to 125 mL·min^{-1}. The mobile phases consisted of [80% acetonitrile (v/v), 19.8% water (v/v), and 0.2% triethylamine (v/v)] and the mobile phase B [30% acetonitrile (v/v), 69.8% water (v/v), and 0.2% triethylamine (v/v)]. The following linear gradient was applied from 8% to 60% solvent B in 14 min, hold 60% solvent B in 1 min, and ramping down 8% solvent B in 0.1 min, and stayed 8% solvent B for 4.9 min as a re-equilibration. The samples were kept at 6 °C in the autosampler. The injection volume was 1 mL.

Data processing. A data acquisition and processing software (Xcalibur 4.0) was used to determine the compounds and processed the quantification using the Quant Brower. The data were normalized by the initial number of samples.

Statistical analysis

Analysis of variance. The data on vitamins, sugars, and moisture were analyzed as an RCBD with three replicates to obtain an analysis of variance (ANOVA). In the ANOVA, replicates nested to the experiments, accessions, experiments, and the interaction accession × experiments were considered fixed effects. Meanwhile replicates were considered the random effect. Furthermore, the sum of squares was divided by types to detect differences within each type. Least square means were obtained and compared with Tukey's test at $P \leq 0.05$. All analysis were conducted using PROC GLIMMIX of SAS (ver. 9.4; SAS Institute Inc., Cary, NC, USA).

Pearson simple correlations. Pearson simple correlations were calculated on two occasions. The first correlations were calculated to understand the relationship among tocopherols, phylloquinone, and ascorbic acid. The second correlations were calculated among % soluble solids and sugars (fructose, glucose, and sucrose) to understand if soluble solids concentrations are related to sugars. Additional correlations were calculated among experiments for all the recorded traits. These correlations were used to understand whether the G × E interactions are of cross-over or non-crossover type.

Genotype and G × E interaction. A genotype + genotype × environment (GGE) biplot analysis was conducted to identify the most stable lettuce accessions across the three environments (experiments), as proposed by Yan et al. (2000). In the GGE biplot, higher values of the trait would fall into the +x and +y axis where germplasm with the best score and more stable across experiments are positioned. The which-won-where biplot is used to identify the winning accession in all environments (Yan and Kang 2003). Accessions positioned farthest from the biplot origin are connected to form a polygon. Lines are drawn from the biplot origin, perpendicular to the polygon sides, to separate the tested environments into mega-environments or environments that perform similarly. Within a mega-environment, the accession graphed at the extreme is the "winning" cultivar that normally has the highest trait value across the environments (experiments) tested (Jenni and Hayes 2010).

Furthermore, the average-environment coordination (AEC) view of the GGE biplot was graphed to rank lettuce accessions where the arrows point. The AEC graphs the ideal accession and the ideal environment for the best performance of a trait; accession(s) and environment(s) closer to both are the ones to be considered. Accessions on the right side of the AEC have above average values whereas the ones on the left have below average values of the analyzed trait.

Both GGE biplots and AEC view were graphed for the content of tocopherols, phylloquinone, ascorbic acid and relative value of vitamins across all experiments. The GGE biplot analysis was conducted with PBTools software (ver. 14; International Rice Research Institute, Los Baños, Laguna, Philippines) was used to perform and to generate which-won-where biplots for all vitamins. The accessions, experiments, and their respective codes used in the GGE biplots are listed in Tables 1 and 2.

Results

Differences on vitamins and sugars detected in lettuce accessions. The tested germplasm had statistically significant ($P < 0.001$) different levels of α-tocopherol, γ-tocopherol, phylloquinone, ascorbic acid, and moisture when grown in greenhouse in a NFT hydroponic system. Such differences were observed within a lettuce type except for g-tocopherol in Boston (P 5 0.376), a-tocopherol in Latin (P 5 0.199), phylloquinone in Boston (P 5 0.247) and leaf (P 5 0.759), and for moisture in leaf (P 5 0.291) (Supplemental Table 1).

Cultivar North Star was the accession displaying the highest amount of g-tocopherol and consequently the highest overall average of this vitamin was found in leaf lettuce (Table 3). Meanwhile, the highest value of a-tocopherol was found in romaine lettuce and in particular in breeding line BG18-0588 (Table 3). Romaine cultivar Monte Carlo had the highest concentration of phylloquinone (Table 3). Likewise, the highest value of ascorbic acid was found in romaine cultivar Green Forrest and the highest moisture content in cultivar Muir (Table 3).

An additional analysis revealed that these differences were statistically significant when all relative values of the four vitamins in this study were combined into TRVC (Supplemental Table 2). Cultivar North Star had the highest TRVC followed by breeding lines BG18-0588 and 43007 (Fig. 2). Romaine lettuce had the highest overall TRVC in this study, but values among types were slightly different to one another (Fig. 1).

The soluble solids concentration of the 30 lettuce accessions evaluated in hydroponics varied significantly (P < 0.05), and this variation was observed within each type (Supplemental Table 3). Conversely, no significant (P > 0.05) differences were found for fructose, glucose, sucrose, and total sugar (Supplemental Table 3). The romaine breeding line BG18-0588 had the highest soluble solids concentration, and two cultivars, Salanova Green Butter and North Star, had the lowest soluble solids concentration (Table 4). Meanwhile, the leaf breeding line BG19-0200 had the highest values of fructose and glucose, whereas 'Muir' had the highest value of sucrose (Table 4).

Positive correlations were found between the concentration of g-tocopherol, a-tocopherol, phylloquinone, and ascorbic acid and among each other (Table 5). Likewise, significant correlations of these compounds were found positively associated with soluble solids, although the strongest relationship was found between ascorbic acid and soluble solids (Table 5). In contrast, g- and a-tocopherols, ascorbic acid, and soluble solids were negatively correlated to moisture; the only positive correlation determined was between phylloquinone and moisture (Table 5). Statistically nonsignificant (P > 0.05) correlations were found between soluble solids and the sugars (fructose, glucose, and sucrose) measured in these experiments (Table 5). Significant (P < 0.05) correlations were found between fructose and glucose, as well as fructose and glucose with total sugars (Table 5).

Planting time influences the content of vitamins and other compounds in lettuce. g-Tocopherol, a-tocopherol, phylloquinone, and soluble solids displayed statistically significant (P < 0.05) differences for the G × E interaction, indicating that the levels of these compounds were experiment dependent (Supplemental Table 1). These interactions were also statistically significant (P < 0.05) within a type with few exceptions. Statistically nonsignificant (P > 0.05) differences were detected for the Boston × experiment and leaf × experiment interactions for g-tocopherol; for the Boston × experiment and Latin × experiment interactions for a-tocopherol; the Boston × experiment, Latin × experiment, and leaf × experiment for phylloquinone; and the Latin × experiment and leaf × experiment interactions for moisture (Supplemental Table 1). All interactions between experiments × accessions and experiment × type (e.g., romaine × experiment) were statistically significant for soluble solids (Supplemental Table 2). No calculations of interactions were determined between accessions and experiments for fructose (F), glucose (G), sucrose (S), and F1G1S because data for these traits was recorded on pooled samples (Supplemental Table 2).

The highest concentrations of g-tocopherol, a-tocopherol, and ascorbic acid were detected in Expt. 2 in all the tested accessions with few exceptions (Supplemental Figs. 1–3). According to the environmental data, Expt. 2 experienced the lowest average solar radiation and intermediate temperatures approximately halfway between Expts. 1 and 3 for the daily average temperature, daily minimum temperature and daily maximum temperature (Table 2). The phylloquinone levels were more variable in the three experiments conducted in this research, with the coldest experiment, Expt. 3, resulting in the lowest phylloquinone levels overall (Table 2, Supplemental Fig. 4). Unlike vitamins, moisture in almost all 30 lettuce accessions were higher in Expts. 1 and 3, except for cultivar Muir, which had a higher percentage of moisture content detected in Expt. 2 (Supplemental Fig. 5). Expt. 1 was the experiment with the highest average temperatures and average solar radiation, whereas Expt. 3 had the lowest temperatures and similar solar radiation as Expt. 2. Soluble solids and S were generally higher in Expt. 2 (Supplemental Fig. 6); however, higher values of F, G, and F1G1S were detected in Expt. 3 with fewer exceptions (Supplemental Fig. 7A–D).

GGE interaction and stability of accessions for vitamins and other compounds in hydroponic grown lettuce. Vitamin content including g-tocopherol, a-tocopherol, and phylloquinone levels were dependent on the environmental conditions (Supplemental Table 1). Both principal components PC1 and PC2 explained a large proportion of the GGE variation 90.6%, 86.7%, and 89.4%, for g-tocopherol, a-tocopherol, and phylloquinone levels, respectively (Fig. 2). Expts. 1 and 2 (r 5 0.34), Expts. 1 and 3 (r 5 0.28), and Expts. 2 and 3 (r 5 0.31) were positively correlated (P < 0.05, n 5 87) indicating a possible non-crossover G × E interaction for g-tocopherol, as differences among the three experiments tended to impact g-tocopherol content similarly amongst the accessions. Expts. 1 and 2 (r 5 0.02) and Expts. 1 and 3 (r 5 0.19) were not correlated (P > 0.05, n 5 87), and Expts. 2 and 3 (r 5 0.25) were slightly correlated (P 5 0.023, n 5 87) for a-tocopherol. Similarly, Expts. 1 and 2 (r 5 0.02) and Expts. 1 and 3 (r 5 –0.17) did not correlate (P > 0.05, n 5 87), and Expts. 2 and 3 (r 5 0.23) were slightly correlated (P 5 0.035, n 5 87) for phylloquinone. The lack of correlations and/or inconsistent correlations between experiments suggests the presence of a crossover interaction for both a-tocopherol and phylloquinone, as the differences among the three experiments tended to have unequal impacts amongst the accessions.

The "which-won-where" graph identified G24 ('North Star') as the accession with consistent levels of g-tocopherol in all three environments (experiments). The three experiments fell into only one mega-environment for values of g-tocopherol (Fig. 3A). Oppositely, the which-won-where method divided the three environments (experiments) in two mega-environments for a-tocopherol (Fig. 3B); Expts. 2 and 3 in a first mega-environment with G15 ('Rex') as the wining accession and Expt. 1 in a second mega-environment with G1 (BG18–0558) as the winning accession (Fig. 3B). Meanwhile only one mega-environment was detected for phylloquinone, and the winning accession was G30 ('Little Gem') (Fig. 3C).

The AEC view of the GGE biplot indicates that G24 ('North Star'), G30 ('Little Gem'), G22 (H1059), and G1 (BG18-0558)

Table 3. Least square means (LSM) and 95% confidence intervals (CI) for content of tocopherols, phylloquinone, and ascorbic acid, and moisture percentage, measured in 30 lettuce accessions grown in three nutrient film technique hydroponic experiments. Lettuce accessions are grouped by type and overall values are provided for romaine, Boston, leaf, and Latin lettuce tested in this study. Tocopherols and phylloquinone were measured via high-performance liquid chromatography system according to (Block et al. 2013). Ascorbic acid and percent moisture were analyzed using protocols from Association of Official Analytical Chemist International (1995) methods 967.22 and 920.151, respectively (Simonne et al. 2002).

		g-Tocopherol (µg/100 g)		a-Tocopherol (µg/100 g)		Phylloquinone (µg/100 g)		Ascorbic acid (mg/100 g)		Moisture (%)	
Status	Accession	LSM	95% CI	LSM	95% CI	LSM	95% CI	LSM	95% CI	LSM	95% CI
BL	BG18–0588	444.1	(370.1–518.0)	698.0	(572.1–823.9)	68.1	(49.4–86.7)	9.37	(8.8–9.9)	94.49	(94.0–95.0)
BL	60182	500.3	(404.2–596.5)	398.4	(234.7–562.0)	90.6	(66.4–114.9)	6.70	(5.7–7.7)	93.97	(93.0–94.9)
BL	60184	291.8	(217.9–365.8)	443.7	(317.8–569.6)	71.3	(52.6–89.9)	7.07	(6.5–7.6)	94.01	(93.5–94.5)
BL	60183	415.0	(341.0–488.9)	411.9	(286.0–537.8)	51.9	(33.2–70.5)	4.98	(4.4–5.5)	94.86	(94.4–95.3)
BL	C1145	377.1	(303.1–451.0)	434.8	(308.9–560.7)	48.8	(30.1–67.4)	5.81	(5.3–6.4)	94.07	(93.6–94.5)
BL	43007	522.5	(448.5–596.4)	661.1	(535.2–787.0)	66.8	(48.1–85.5)	6.26	(5.9–6.7)	93.93	(93.5–94.4)
CC	Salvius	351.2	(277.3–425.2)	364.8	(238.8–490.7)	64.6	(45.9–83.3)	5.82	(5.3–6.4)	94.37	(93.9–94.9)
CC	Sparx	359.6	(285.7–433.8)	443.3	(317.4–569.2)	45.8	(27.2–64.5)	5.81	(5.3–6.4)	94.21	(93.7–94.7)
CC	Monte Carlo	436.5	(362.6–510.5)	280.2	(154.3–406.1)	95.5	(76.8–114.2)	6.20	(5.7–6.7)	94.15	(93.7–94.6)
CC	Green Forrest	465.1	(391.1–539.1)	392.5	(266.6–518.4)	61.2	(42.5–79.9)	10.33	(9.8–10.9)	94.34	(93.9–94.8)
	Romaine	416.3	(340.1–492.5)	452.9	(323.2–582.6)	66.5	(47.2–85.7)	6.83	(6.3–7.4)	94.23	(93.7–94.8)
BL	B1196	383.7	(309.7–457.7)	328.9	(203.0–454.8)	76.3	(57.6–94.9)	5.59	(5.0–6.2)	94.13	(93.7–94.6)
BL	70202	319.9	(245.9–393.8)	248.0	(122.1–373.9)	55.5	(36.8–74.1)	6.37	(5.7–7.0)	94.18	(93.6–94.7)
BL	70882	361.3	(287.3–435.3)	285.0	(159.1–410.9)	60.6	(41.9–79.2)	4.01	(3.4–4.6)	94.64	(94.2–95.1)
BL	50111	352.3	(278.3–426.3)	407.1	(281.1–533.0)	82.0	(63.3–100.7)	5.22	(4.6–5.8)	94.47	(94.0–95.0)
CC	Rex	361.7	(287.7–435.6)	592.4	(466.5–718.3)	72.3	(53.7–91.0)	8.89	(8.3–9.5)	94.72	(94.2–95.2)
CC	SGB	288.6	(214.6–362.6)	282.3	(156.4–408.2)	55.2	(36.5–73.8)	4.82	(4.2–5.5)	95.19	(94.7–95.7)
CC	Mirlo	365.8	(291.8–439.8)	288.9	(163.0–414.8)	81.1	(62.4–99.8)	5.92	(5.3–6.5)	94.55	(94.0–95.1)
CC	Palmetto	281.1	(207.2–355.1)	212.0	(86.0–337.9)	68.4	(49.8–87.1)	7.45	(6.8–8.1)	94.60	(94.1–95.1)
	Boston	339.3	(265.3–413.3)	330.6	(204.6–456.5)	68.9	(50.3–87.6)	6.03	(5.4–6.7)	94.62	(94.1–95.1)
BL	BG19–0255	427.3	(347.2–507.4)	304.5	(168.1–440.9)	65.7	(45.5–86.0)	3.58	(2.8–4.3)	95.04	(94.5–95.6)
BL	BG19–0200	347.8	(273.8–421.8)	225.2	(99.3–351.1)	69.1	(50.4–87.7)	6.78	(6.1–7.5)	94.85	(94.4–95.3)
BL	BG19–0208	407.0	(326.9–487.1)	262.4	(126.1–398.8)	62.7	(42.5–82.9)	6.32	(5.6–7.0)	94.26	(93.8–94.7)
BL	H1059	477.5	(397.4–557.6)	361.8	(225.5–498.2)	68.0	(47.8–88.2)	5.71	(5.2–6.4)	94.23	(93.8–94.7)
CC	Muir	396.9	(322.9–470.9)	224.2	(98.3–350.1)	72.9	(54.3–91.6)	6.19	(5.5–6.9)	95.40	(94.9–95.9)
CC	North Star	732.2	(658.3–806.2)	452.3	(326.4–578.2)	90.1	(71.4–108.8)	7.77	(7.1–8.5)	93.84	(93.4–94.3)
CC	SGO	293.5	(219.6–367.5)	285.7	(159.8–411.6)	77.7	(59.0–96.4)	7.09	(6.4–7.8)	94.69	(94.2–95.2)
	Leaf	440.3	(363.7–516.9)	302.3	(171.9–432.7)	72.3	(53.0–91.6)	6.21	(5.5–6.9)	94.61	(94.1–95.1)
BL	C1146	295.8	(221.8–369.8)	332.4	(206.5–458.3)	45.6	(26.9–64.2)	6.24	(5.8–6.6)	94.33	(93.9–94.8)
BL	C1148	333.3	(259.3–407.2)	473.0	(347.0–598.9)	61.4	(42.7–80.0)	5.91	(5.5–6.3)	94.54	(94.0–95.1)
BL	45060	363.3	(289.3–437.2)	383.5	(257.6–509.4)	65.2	(46.5–83.8)	7.28	(6.9–7.7)	93.95	(93.5–94.4)
CC	Newham	384.5	(310.5–458.5)	413.6	(287.6–539.5)	59.8	(41.1–78.4)	6.25	(5.9–6.7)	94.38	(93.9–94.9)
CC	Little Gem							2.20	(0.5–3.9)	.	.
	Latin	344.2	(270.2–418.2)	400.6	(274.7–526.5)	58.0	(39.3–76.6)	5.58	(4.9–6.2)	94.30	(93.8–94.8)

BL 5 breeding line; CC 5 commercial cultivar.

were stable accessions for the highest content of g-tocopherol (Fig. 3D). Similarly, a representative environment where lettuce with the highest content of g-tocopherol could be detected was the one with similar characteristics to the environmental conditions of Expt. 1 (Fig. 3D). Although G1 (BG18-0558), G15 ('Rex'), and G6 ('Salvius') are the accessions with the highest value of a-tocopherol, not all of them were stable across the three experiments in the NFT system. There were other accessions with more stable performance across these experiments including G18 ('Palmetto'), G30 ('Little Gem'), G23 ('Muir'), G21 (BG19-0208), and G12 (70202) according to the AEC view of the GGE biplot (Fig. 3E). Likewise, the average environment to produce lettuce with higher content of a-tocopherol is located near Expt. 1 (Fig. 3E). Most of the lettuce accessions presented a less stable performance for phylloquinone; however, G2 (60182) and G27 (C1148) seemed to have consistent performance across environments. The ideal environment to produce lettuce for higher content of phylloquinone was not near any of the tested environments, Expts. 1, 2, or 3 (Fig. 3F).

The contents of ascorbic acid, moisture, and soluble solids were experimentally (environment) dependent as differences among experiments were detected (Supplemental Tables 1 and 2). However, weak and nonsignificant correlations ($P > 0.05$, n 5 58) were detected between Expts. 1 and 2 (r 5 0.07), Expts. 1 and 3 (r 5 0.14), and Expts. 2 and 3 (r 5 0.22) for ascorbic acid, which suggests a crossover interaction. Only one positive significant correlation between Expts. 1 and 2 ($P < 0.05$, n 5 53) was detected for moisture. The remaining two correlations between Expts. 1 and 3 (r 5 –0.06) and Expts. 2 and 3 (r 5 0.07) were not significant ($P > 0.05$, n 5 58). Correlations between Expts. 1 and 2 (r 5 0.12) and Expts. 1 and 3 (r 5 –0.02) were not significant

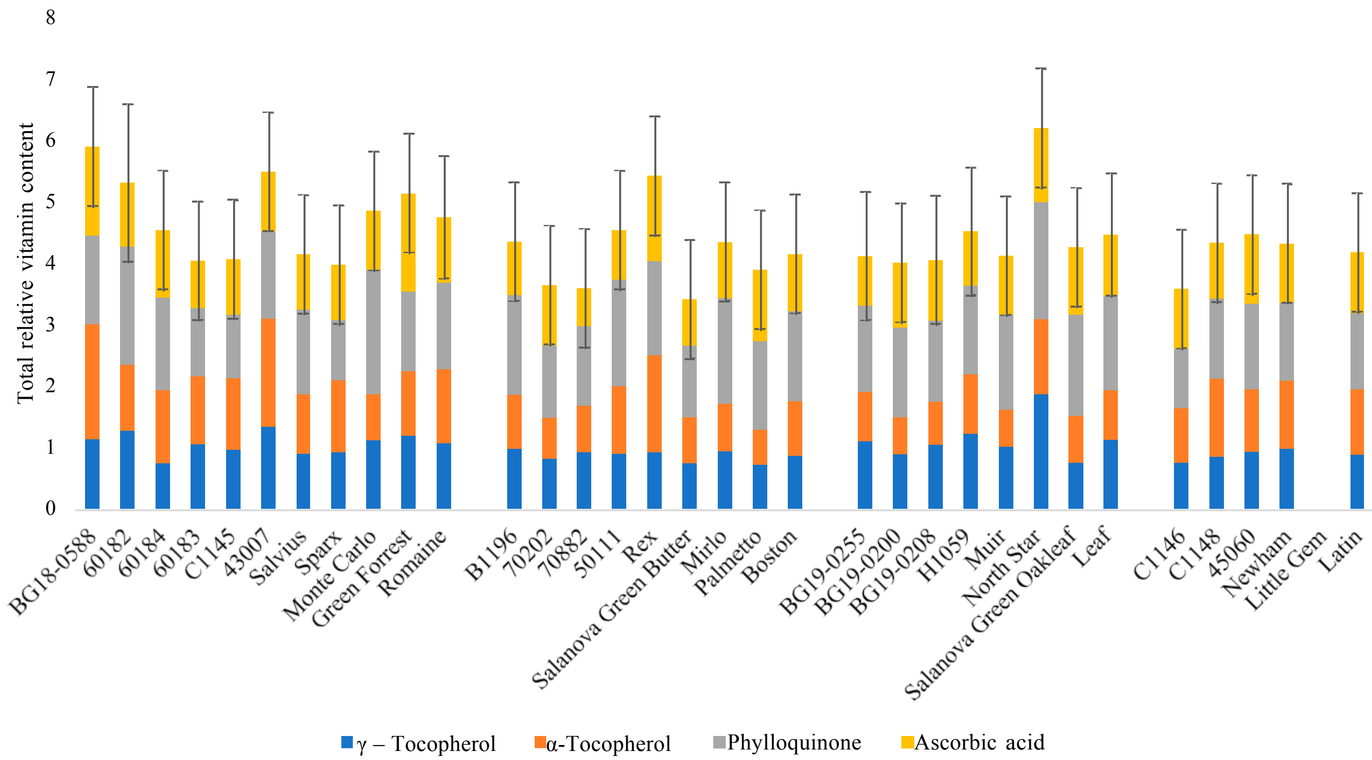

Fig. 2. Relative content of individual vitamins (colored bars) and total relative vitamin content presented as least square means for 30 lettuce accessions tested in three nutrient film technique hydroponic experiments. Error bars represent 95% confidence intervals for the least square means of total relative vitamin content. Relative values are calculated for comparison purposes only because contents of different nutrients may vary by order of magnitude.

for soluble solids ($P > 0.05$, n 5 58), whereas a significant ($P <$ 0.05, n 5 90) correlation was found between Expts. 2 and 3 (r 5 0.29). The lack of correlations, or the inconsistent pattern for correlations between experiments, suggests the presence of a crossover interaction for ascorbic acid, moisture, and soluble solids.

The which-won-where graph situated G10 (‘Green Forrest’), G15 (‘Rex’), and G18 (‘Palmetto’) as the winning accessions with the highest content of ascorbic acid in the first mega-environment (Expt. 2); two other accessions, G24 (‘North Star’) and G25 (‘Salanova Green Oakleaf’) fell within the same mega-environment. A second mega-environment was constructed with Expts. 1 and 3, with G1 (BG18–0558) as the winning accession (Fig. 4A). The which-won-where method divided the three experiments in two mega-environments for moisture. The first mega-environment was composed of Expts. 1 and 2, with G23 (‘Muir’) as the winning accession and the second mega-environment of Expt. 3 with G20 (BG19-0200) as the wining accession (Fig. 4B). Similarly, Expts. 1 and 2 were grouped as one mega-environment and Expt. 3 as a second mega-environment for soluble solids; both G28 (45060) and G12 (70202) were the wining accessions in mega-environments 1 and 2, respectively (Fig. 4C).

The AEC view of the biplot positioned G10 (‘Green Forrest’) as the ideal accession for ascorbic acid content, but other accessions including G1 (BG18–0588) were more stable across Expts. 1, 2, and 3 (Fig. 4D). Most of the environments (experiments) were located far away from the ideal environment in the AEC but the closest was Expt. 1 (Fig. 4D). G23 was the accession with the highest value for moisture (Fig. 4E) but not the most stable across environments (Expts. 1, 2, and 3) according to the AEC of the biplot. More accessions were stable for soluble solids similar to G26 (C1146) that was positioned closer to the ideal environment or Expt. 1 (Fig. 4F).

A joint analysis of all vitamins showed a lack of correlation among experiments. Expts. 1 and 2 (r 5 0.13) and Expts. 1 and 3 (r 5 0.05) were not correlated ($P > 0.05$, n 5 85), and Expts. 2 and 3 (r 5 0.23) were slightly correlated (P 5 0.037, n 5 85), suggesting a crossover interaction. Interestingly, the which-won-where method separated Expts. 1, 2, and 3 in three independent mega-environments in which G1 (BG18-0558), G24 (‘North Star’), and G15 (‘Rex’) were the winners, respectively (Fig. 5A). While the AEC of the biplot graphs Expt. 3 as the environment closest to the ideal conditions to detect lettuce germplasm with the highest content of vitamins and G24 (‘North Star’) as the accession with most stable relative values of vitamins (Fig. 5B).

Discussion

VARIATION OF VITAMIN CONTENT IN HYDROPONIC LETTUCE. Vitamin E, detected as g-tocopherol and a-tocopherol, and vitamin K_1, detected as phylloquinone have been less studied in lettuce relative to other nutrients (Kim et al. 2016; Shi et al. 2022; Simonne et al. 2002). Differences among accessions identified among three experiments in this research for both forms of tocopherols and phylloquinone, indicate the existence of genetic variation for these traits. g-tocopherol was overall slightly higher than a-tocopherol, in contrast to prior research, where a-tocopherol was higher in accessions tested of crisphead, Boston, romaine, and leaf lettuce (Simonne et al. 2002). Leaf lettuce had the highest overall content of g-tocopherol, but romaine lettuce was higher in a-tocopherol, the more bioavailable form of tocopherol for

Table 4. Least square means (LSM) and 95% confidence (CI) intervals for soluble solids concentration, fructose (F), glucose (G), sucrose (S), and total sugars (F1G1S) measured in 30 lettuce accessions grown in three nutrient film technique hydroponic experiments. Lettuce accessions are grouped by type and overall means are provided for the romaine, Boston, leaf, and Latin lettuce tested in this study. Soluble solids concentrations were measured using a refractometer and individual sugars were analyzed using ultra high-performance liquid chromatography system.

		Soluble solids (%)		F ($mg \cdot g^{-1}$ tissue)		G ($mg \cdot g^{-1}$ tissue)		S ($mg \cdot g^{-1}$ tissue)		F+G+S ($mg \cdot g^{-1}$ tissue)	
Status	Accession	LSM	95% CI	LSM	95% CI	LSM	95% CI	LSM	95% CI	LSM	95% CI
BL	BG18–0588	2.8	(2.7–2.9)	10.1	(7.3–12.8)	5.3	(3.6–7.0)	1.3	(0.4–2.1)	16.6	(12.4–20.9)
BL	60182	2.7	(2.6–2.8)	10.7	(7.9–13.4)	6.0	(4.3–7.6)	2.1	(1.3–3.0)	18.7	(14.5–23.0)
BL	60184	2.4	(2.2–2.5)	8.0	(5.3–10.8)	4.1	(2.4–5.8)	1.0	(0.2–1.9)	13.2	(8.9–17.4)
BL	60183	2.3	(2.2–2.4)	9.4	(6.7–12.2)	5.1	(3.5–6.8)	1.2	(0.3–2.0)	15.7	(11.4–20.0)
BL	C1145	2.5	(2.4–2.7)	9.2	(6.5–12.0)	5.1	(3.4–6.7)	1.0	(0.1–1.8)	15.3	(11.0–19.6)
BL	43007	2.4	(2.3–2.5)	11.0	(8.2–13.7)	6.1	(4.5–7.8)	1.0	(0.1–1.8)	18.1	(13.8–22.3)
CC	Salvius	2.3	(2.2–2.5)	9.6	(6.9–12.4)	5.0	(3.3–6.6)	0.5	(− 0.4–1.3)	15.1	(10.8–19.4)
CC	Sparx	2.3	(2.2–2.4)	8.5	(5.7–11.2)	4.3	(2.7–6.0)	0.5	(− 0.3–1.4)	13.3	(9.1–17.6)
CC	Monte Carlo	2.7	(2.6–2.8)	10.5	(7.8–13.2)	5.8	(4.1–7.4)	1.2	(0.4–2.1)	17.5	(13.2–21.8)
CC	Green Forrest	2.2	(2.1–2.3)	8.5	(5.8–11.3)	4.7	(3.0–6.3)	0.7	(− 0.1–1.6)	13.9	(9.6–18.2)
	Romaine	2.5	(2.3–2.6)	9.6	(6.8–12.3)	5.1	(3.5–6.8)	1.0	(0.2–1.9)	15.7	(11.5–20.0)
BL	B1196	2.4	(2.3–2.6)	11.1	(5.5–16.7)	5.9	(2.7–9.0)	1.3	(0.1–2.6)	18.3	(9.4–27.2)
BL	70202	2.6	(2.5–2.8)	3.3	(− 2.3–8.8)	1.5	(− 1.6–4.7)	0.6	(− 0.7–1.9)	5.4	(− 3.5–14.3)
BL	70882	2.5	(2.3–2.6)	9.1	(3.6–14.7)	4.4	(1.2–7.6)	1.2	(− 0.1–2.5)	14.7	(5.8–23.6)
BL	50111	2.3	(2.1–2.4)	7.0	(1.5–12.6)	3.3	(0.2–6.5)	1.7	(0.4–2.9)	12.0	(3.1–20.0)
CC	Rex	2.5	(2.4–2.6)	8.7	(3.1–14.2)	4.6	(1.5–7.8)	2.1	(0.9–3.4)	15.4	(6.6–24.3)
CC	Salanova Green Butter	2.1	(2.0–2.2)	7.5	(1.9–13.0)	3.7	(0.5–6.8)	1.2	(− 0.1–2.4)	12.3	(3.4–21.2)
CC	Mirlo	2.2	(2.1–2.3)	7.3	(1.7–12.9)	3.9	(0.7–7.0)	2.1	(0.8–3.3)	13.3	(4.4–22.2)
CC	Palmetto	2.3	(2.2–2.4)	6.8	(1.2–12.3)	3.2	(0.1–6.4)	0.5	(− 0.7–1.8)	10.5	(1.6–19.4)
	Boston	2.4	(2.2–2.5)	7.6	(2.0–13.2)	3.8	(0.6–7.0)	1.3	(0.1–2.6)	12.7	(3.9–21.6)
BL	BG19–0255	2.5	(2.4–2.6)	9.01	(4.9–13.2)	4.5	(2.1–6.8)	0.9	(− 0.9–2.6)	14.3	(7.4–21.3)
BL	BG19–0200	2.5	(2.4–2.6)	11.8	(7.6–15.9)	6.9	(4.5–9.2)	1.2	(− 0.6–2.9)	19.8	(12.8–26.7)
BL	BG19–0208	2.3	(2.2–2.4)	4.8	(0.7–9.0)	2.2	(− 0.2–4.5)	1.2	(−0.6–3.0)	8.2	(1.2–15.1)
BL	H1059	2.5	(2.4–2.6)	7.6	(3.4–11.7)	3.9	(1.6–6.3)	0.7	(− 1.1–2.4)	12.1	(5.2–19.1)
CC	Muir	2.6	(2.5–2.7)	8.7	(4.5–12.8)	4.4	(2.0–6.7)	2.4	(0.6–4.2)	15.4	(8.5–22.3)
CC	North Star	2.1	(2.0–2.2)	4.6	(0.4–8.8)	2.1	(− 0.3–4.5)	1.9	(0.1–3.6)	8.6	(1.6–15.5)
CC	Salanova Green Oakleaf	2.3	(2.2–2.4)	6.3	(2.1–10.4)	3.1	(0.7–5.4)	0.6	(− 1.2–2.4)	10.0	(3.0–16.9)
	Leaf	2.4	(2.3–2.5)	7.52	(3.4–11.7)	3.9	(1.5–6.2)	1.3	(− 0.5–3.0)	12.6	(5.7–19.6)
BL	C1146	2.4	(2.3–2.6)	10.9	(8.3–13.5)	6.0	(4.2–7.9)	0.4	(− 0.8–1.7)	17.4	(12.5–22.3)
BL	C1148	2.3	(2.1–2.4)	8.4	(5.9–11.0)	4.4	(2.5–6.3)	0.9	(− 0.3–2.2)	13.8	(8.9–18.7)
BL	45060	2.7	(2.6–2.9)	11.2	(8.6–13.8)	6.3	(4.4–8.2)	1.2	(− 0.1–2.5)	18.8	(13.9–23.6)
CC	Newham	2.5	(2.3–2.6)	8.4	(5.8–11.0)	4.2	(2.3–6.1)	1.6	(0.3–2.8)	14.2	(9.3–19.0)
CC	Little Gem	2.2	(2.0–2.3)	10.3	(7.7–12.9)	5.7	(3.8–7.6)	1.2	(− 0.1–2.4)	17.2	(12.3–22.0)
	Latin	2.4	(2.3–2.6)	9.9	(7.3–12.4)	5.3	(3.4–7.2)	1.1	(− 0.2–2.3)	16.3	(11.4–21.1)

human diets. Both tocopherols were higher in green leaf, followed by romaine, red leaf, butterhead, and iceberg lettuce (Simonne et al. 2002). The quantities and ratios of a- to c-tocopherol reported for lettuce grown in greenhouse or laboratory conditions has varied significantly among several studies, and in general the a- and c-tocopherol contents have both been shown to vary between 100 and 1000 mg/100 g FW, similar to this research (Byrdwell et al. 2021; Kim et al. 2016; Mou 2012).

Phylloquinone was found in variable amounts for the accessions tested, with no clear association for morphological types. Although little research exists for phylloquinone contents in lettuce, the values presented here fall within the 24 to 140 mg/100 g FW previously summarized for five major lettuce types (Mou

Table 5. Pearson correlations for vitamins, moisture content, and soluble solids concentration among 30 lettuce accessions tested in three nutrient film technique hydroponic experiments. Probability values are included in parentheses.

Trait	g-Tocopherol	a-Tocopherol	Phylloquinone	Ascorbic acid	Moisture	Soluble solids
g-tocopherol	1.00	0.74 (<0.001)	0.44 (<0.001)	0.72 (<0.001)	− 0.37 (<0.001)	0.68 (<0.001)
a-tocopherol		1.00	0.29 (<0.001)	0.66 (<0.001)	− 0.37 (<0.001)	0.63 (<0.001)
Phylloquinone			1.00	0.42 (<0.001)	0.33 (<0.001)	0.49 (<0.001)
Ascorbic acid				1.00	− 0.44 (<0.001)	0.99 (<0.001)
Moisture					1.00	− 0.29 (<0.001)
Soluble solids						1.00

Table 6. Pearson correlations for soluble solids concentration, fructose (F), glucose (G), sucrose (S), and total sugars (F1G1S) among 30 lettuce accessions tested in three nutrient film technique hydroponic experiments. Probabilty values are included in parentheses.

Trait	Soluble solids	F	G	S	F1G1S
Soluble solids	1.00	0.20 (0.122)	0.24 (0.066)	−0.25 (0.053)	0.18 (0.172)
F		1.00	0.99 (<0.001)	−0.12 (0.364)	0.99 (<0.001)
G			1.00	−0.15 (0.255)	0.98 (<0.001)
S				1.00	0.03 (0.837)
F1G1S					1.00

2012). Similarly, vitamin C detected as ascorbic acid was found to be variable in different lettuce accessions in this research as in prior reports (Simko 2019; Simonne et al. 2002; van Treuren et al. 2018). Ascorbic acid values were reported between 2 and 11 mg/100 g FW in greenhouse in the current research compared with other research where these values were within 2 to 30 mg/100 g FW in fields (Kim et al. 2016; Mou 2012).

Variation of moisture and sugar contents in hydroponic lettuce. In addition to vitamins, moisture, soluble solids concentration, and sugars (fructose, glucose, and sucrose) were variable across accessions and experiments. Moisture content was remarkably similar among all four lettuce types analyzed, and the greatest experimental differences were observed in the lower moisture contents measured in Expt. 2. Higher moisture content seemed to occur during Expts. 1 and 3, with the higher and lower average temperatures, respectively; however, it remains unclear how changes in temperatures or other environmental factors may influence moisture content in lettuce. Results from the correlation analysis suggest that moisture content was positively associated with phylloquinone, whereas the other vitamins and soluble solids concentration were negatively correlated. Ascorbic acid is a water-soluble vitamin that was shown to have higher content in baby leaf lettuce, a phenological stage associated with much lower moisture content, agreeing with the current analysis (Martínez-Ispizua et al. 2022b; Simko 2019). g-tocopherol and a-tocopherol are fat-soluble and tend to be associated with thylakoid membranes in photosynthetic tissue. Presence of lower moisture content, and higher dry matter content, could be associated with more structural components that require tocopherols, as was the trend observed in previous research (Simonne et al.

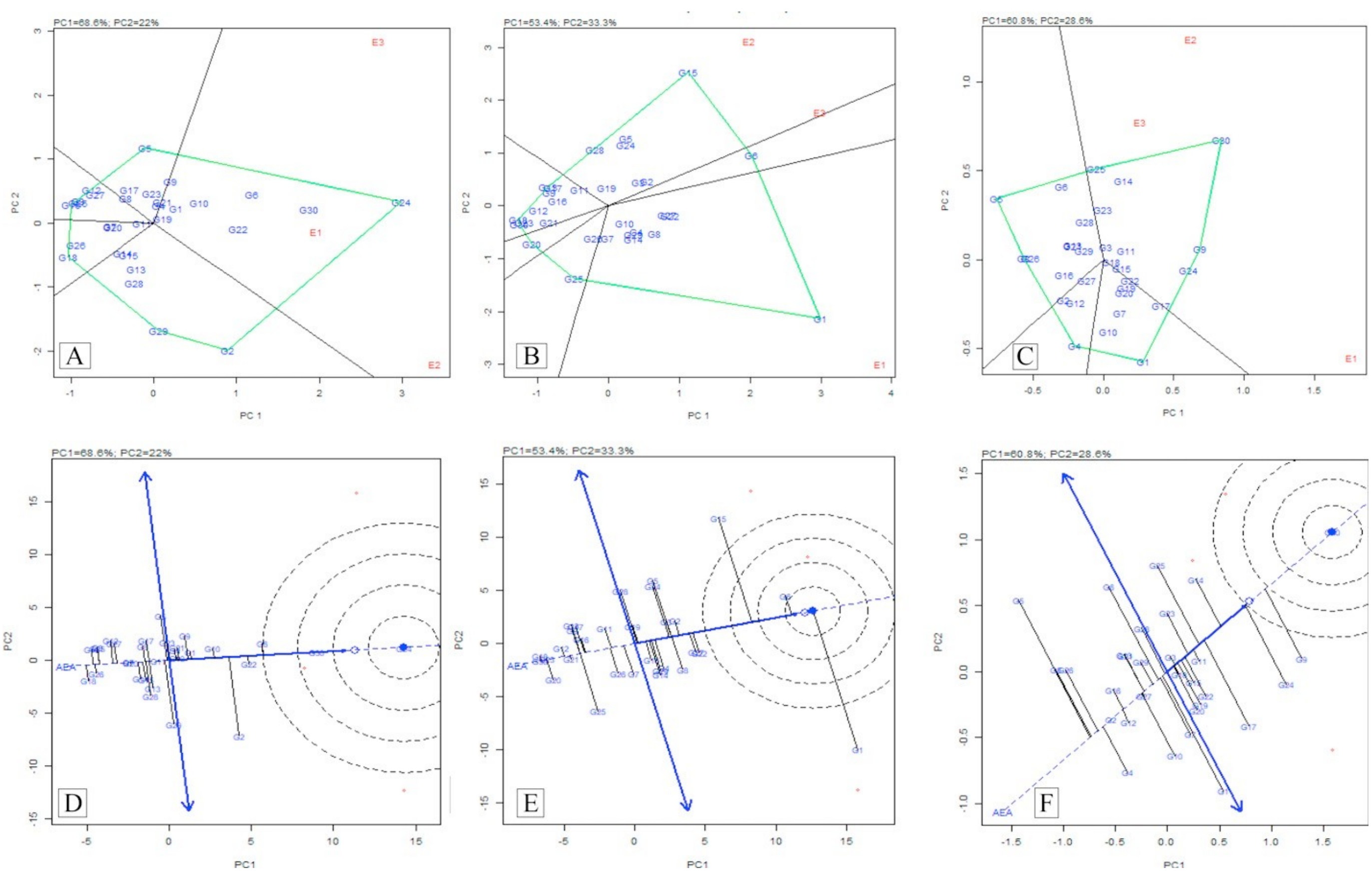

Fig. 3. Which-won-where view of the genotype 1 genotype × environment (GGE) interaction biplot (Yan et al. 2000) for g-tocopherol (A), a-tocopherol (B), and phylloquinone (C) and stability analyses for these compounds, respectively (D–F), presented in the average-environment coordination (AEC) view. G1 to G30 in blue corresponds with lettuce accessions listed in Table 1, and Expt. 1 (E1) to Expt. 3 (E3) in red corresponds with the three experiments conducted in Table 2. Black lines drawn on the which-won-where GGE biplots (A–C) group environments into mega-environments where similar performance of the respective trait was observed, and the genotypes with the most extreme position within each mega-environment have the highest trait value. The genotype and environment positioned closest to the bullseye in the AEC view (D–F) are considered the most ideal for each respective trait, and genotypes positioned closest to the x-axis are considered most stable.

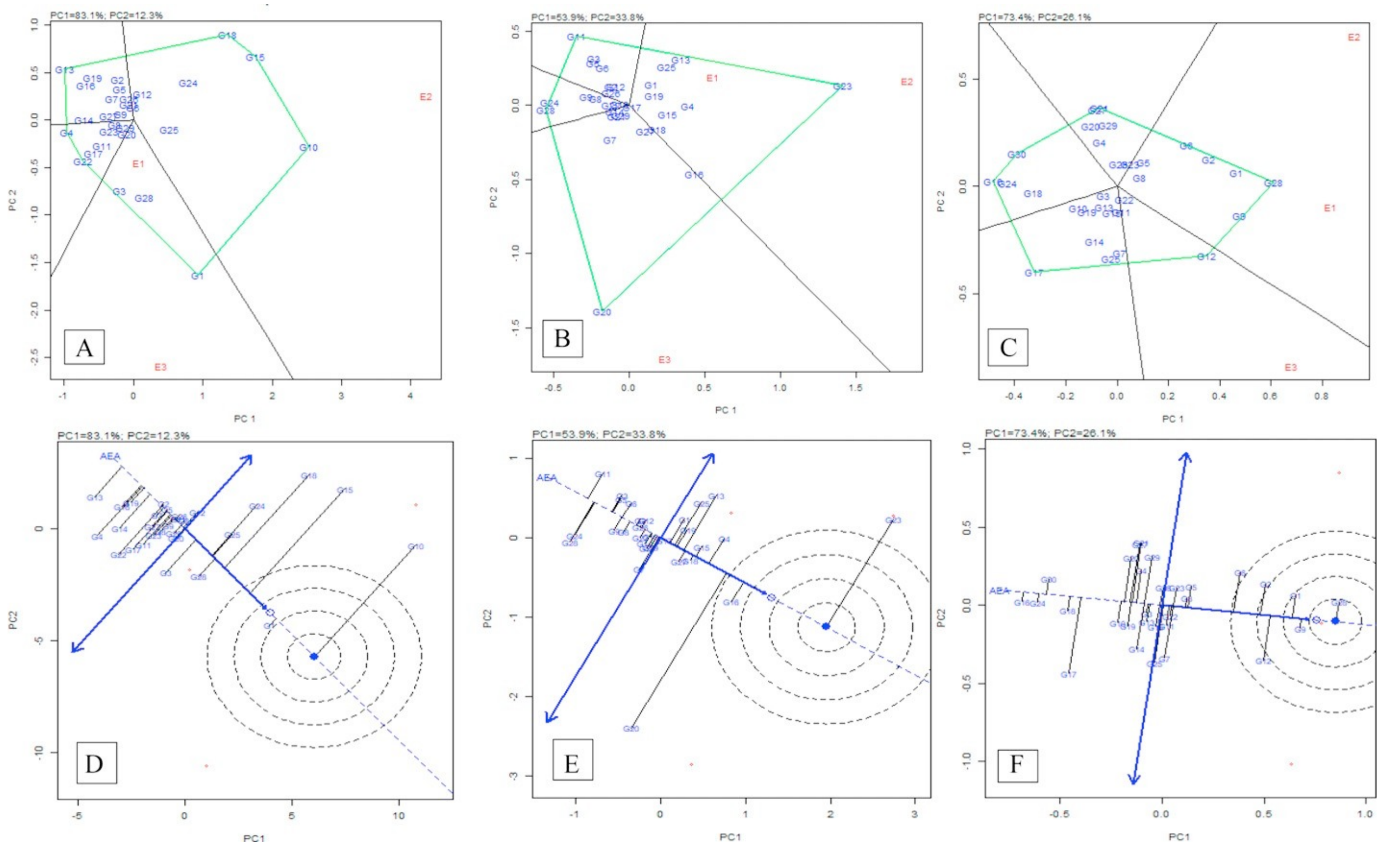

Fig. 4. Which-won-where view of the genotype 1 genotype × environment (GGE) interaction biplot (Yan et al. 2000) for ascorbic acid (A), moisture (B), soluble solids (C) and stability analysis for these compounds, respectively (D–F), presented in the average-environment coordination (AEC) view. G1 to G30 corresponds with lettuce accessions listed in Table 1 and Expt. 1 (E1) to Expt. 3 (E3) corresponds with experiments in the nutrient film technique hydroponic system listed in Table 2. Black lines drawn on the which-won-where GGE biplots (A–C) group environments into mega-environments where similar performance of the respective traits was observed, and the genotypes with the most extreme position within each mega-environment have the highest trait value. The genotype and environment positioned closest to the bullseye in the AEC view (D–F) are considered the most ideal for each respective trait, and genotypes positioned closest to the x-axis are considered most stable.

2002). Phylloquinone, on the other hand, is a structural component of the photosystem I thylakoid membrane, and it is unclear how its composition would relate to increased moisture content.

Soluble solids concentration did not correlate to sugar (fructose, glucose, and sucrose) content in lettuce, and therefore, soluble solids measurements as performed in this study potentially may not be a useful estimate of sugar content in lettuce (Table 6). Mature lettuce accessions tested in this research were mostly composed of fructose, as found in heat-tolerant lettuce (Alves et al. 2022) and the early stages of plant development in baby leaf lettuce (Simko 2019). Among the three experiments, Expt. 2 with the most intermediate temperatures generally resulted in the highest concentration of soluble solids and sucrose, whereas the generally cooler Expt. 3 resulted in higher contents of F, G, and F1G1S. Previous research has shown that temperature affects accumulation of total and individual sugars; however, other environmental changes between seasons remain unexplored for their effect on sugar content (Alves et al. 2022). The temperatures in Expt. 3 were within the range of conditions for better cultivation (Hayes 2018), and higher sugar concentration will accumulate when the crop is cultivated under these ideal temperatures. Unfortunately, these sugars were not measured in Expt. 1 when lettuce was cultivated fairly outside the temperature range.

Furthermore, these sugars were type dependent, with the highest content of fructose and glucose in Latin lettuce, whereas highest content of sucrose was found for Boston and leaf lettuce. Higher content of fructose and glucose were found in romaine lettuce than leaf or Boston, as in previous research (López et al. 2014; Simko 2019). Preference by consumers for one lettuce type over the others may be related to the sugar content. Sensory panels or a more accurate measurement of these sugars should be employed in breeding programs, in parallel to the increase of tocopherols, phylloquinone, and ascorbic acid, to ensure that higher vitamin lettuce will be adopted by consumers (Chadwick et al. 2016).

IDENTIFYING GERMPLASM FOR BREEDING LETTUCE WITH IMPROVED VITAMINS. The high positive correlations found among tocopherols, phylloquinone, and ascorbic acid in this study suggests that breeding lettuce for higher content of a- and g-tocopherol, phylloquinone, and ascorbic acid simultaneously could be possible. Furthermore, it seems that breeders could efficiently identify specific accessions that may accumulate high content of multiple vitamins to be used for further breeding and research. Specific accessions in this research demonstrated high TRVC, associated with relatively high content of multiple vitamins, as well as stability of vitamin content in multiple environments. These include leaf cultivar North Star (high for g-tocopherol, phylloquinone, and ascorbic acid), romaine breeding line BG18–0558 (g-tocopherol, a-tocopherol, and ascorbic acid), Boston cultivar Rex (a-tocopherol and ascorbic acid), and romaine cultivar Green Forrest (g-tocopherol and ascorbic acid). A

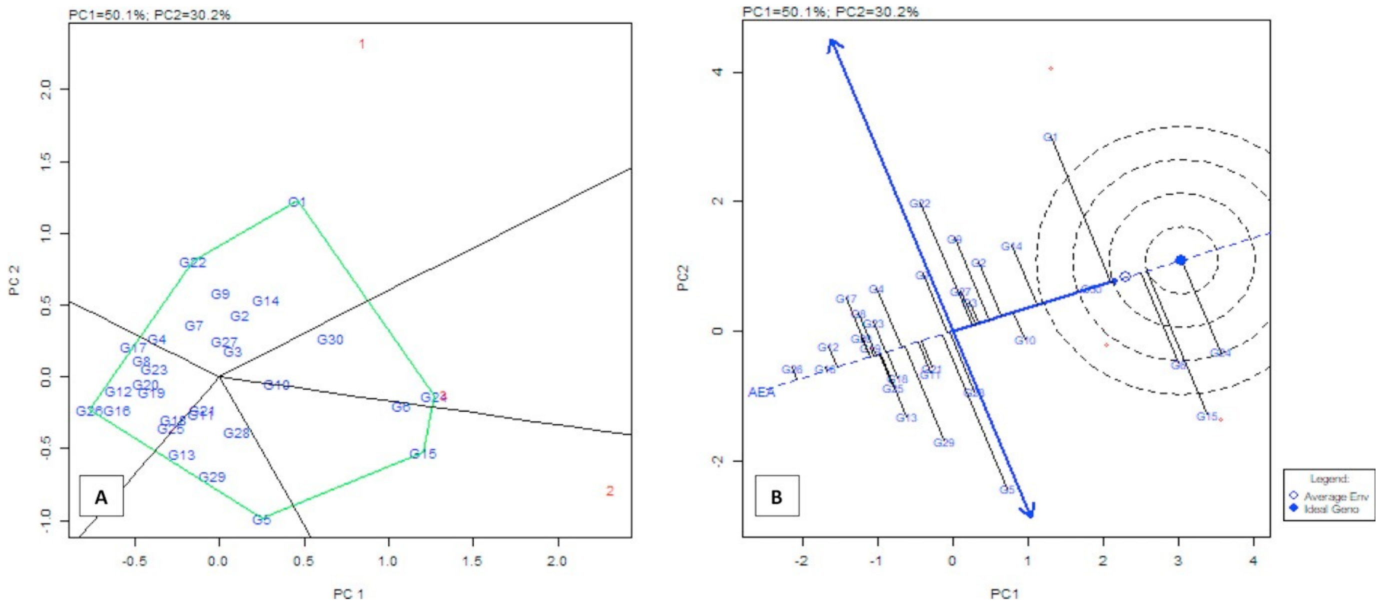

Fig. 5. Which-won-where view of the genotype **1** genotype × environment (GGE) interaction biplot (Yan et al. 2000) for total relative vitamin content (A) and stability analysis for this trait (B) presented in the average-environment coordination (AEC) view. G1 to G30 corresponds with lettuce accessions listed in Table 1 and Expt. 1 (E1) to Expt. 3 (E3) corresponds with experiments in the nutrient film technique hydroponic system listed in Table 2. Black lines drawn on the which-won-where GGE biplots (A–C) group environments into mega-environments where similar performance of the respective traits was observed, and the genotypes with the most extreme position within each mega-environment have the highest trait value. The genotype and environment positioned closest to the bullseye in the AEC view (D–F) are considered the most ideal for each respective trait, and genotypes positioned closest to the x-axis are considered most stable.

similar positive relationship among vitamin contents was found between ascorbic acid and b-carotene in several baby leaf lettuce accessions (Simko 2019).

The identification of lettuce with higher vitamin content suggests that nutritious lettuce is being commercialized, but the improvement of nutritional compounds such as vitamins was not an explicitly targeted trait for breeding. Some of these cultivars—for example, Rex—are used for production in hydroponics and have proven to exhibit excellent horticultural characteristics including less tipburn, good head formation, uniformity, and so on (Hochmuth et al. 2012, 2017). In addition, several breeding lines from the UF-IFAS lettuce breeding program including 43007 (romaine) and B1196 (Boston) for g-tocopherol, BG18-0558 (romaine) for a-tocopherol, and 45060 (Latin) had higher content of these vitamins. The identified variation for vitamin content is useful to improve nutrition in future lettuce cultivars, which could be further adapted to maintain their productivity and health benefits in different hydroponic environments. However, it has yet to be determined if the content of tocopherols, phylloquinone, and ascorbic acid is heritable to design breeding strategies that will increase these compounds. There are few examples of crops improved for higher vitamin content or their precursors using conventional breeding methods.

No reports were available at the start of this investigation regarding accessions with the lowest or highest content of the vitamins studied in this research when lettuce is cultivated in hydroponics. Therefore, there is a possibility that germplasm with even higher content of these vitamins could be available but remains unidentified. For instance, wildtype accessions in the genus Lactuca have proved to possess more ascorbic acid than traditional and modern lettuce cultivars when grown in nonhydroponic systems (Medina-Lozano et al. 2021; van Treuren et al. 2018).

G × E INTERACTION FOR NUTRITIONAL COMPOUNDS IN HYDROPONIC LETTUCE. It is believed that variations in growing conditions may significantly influence the nutritional content of lettuce (Mou 2008). In fact, vitamin levels were experiment dependent in this research, and the interaction with the environment seems to be complex for most of the studied compounds. Tocopherols, phylloquinone, and ascorbic acid might be complex traits highly influenced by the environment, and therefore, this may require specific considerations for breeding purposes.

On the basis of the results of this study, it may be possible to identify stable accessions with high contents of g-tocopherol and phylloquinone when conducting selection in environments such as Expts. 1, 2, and 3. This perhaps limits the variation in environmental conditions required to identify genetically superior breeding material for these vitamins. Conversely, the crossover G × E interaction and lack of ideal selection environment detected for a-tocopherol and ascorbic acid may indicate that the selection of lettuce lines with higher contents of these vitamins should be evaluated using multiple environments or planting times while breeding for hydroponic production. Moreover, the calculation of the TRVC for the vitamins studied in this research indicated that a single environment, Expt. 3, was the most representative environment to identify lettuce with the overall highest and most stable content of these four compounds collectively; yet there was a crossover G × E interaction that confirms that evaluation for selecting more than one vitamin should be conducted in multitrials. Expt. 3 was within the range of temperatures that are most suited for lettuce cultivation such as 18 to 25 °C day and 10 to 15 °C night (Hayes 2018), which perhaps is optimal for selection for higher vitamin content. Because of the limited environmental data collected during this research, further research could specifically elucidate the ideal selection environment, or most relevant environmental and genotypic factors, influencing the selection of hydroponic lettuce with higher vitamins.

It is unknown if additional testing will be required for these vitamins and germplasm if planted in commercial fields, as research suggests that different nutrients, such as nutritional traits measured as a proxy of b-carotene, have demonstrated variable responses between greenhouse and field cultivation (Hayashi et al. 2012; Volpe et al. 2020). Nevertheless, certain environmental factors, such as optimal temperatures, may be critical to consider regardless of the production system. For example, lettuce grown in Expts. 2 or 3 of this study generally resulted in higher content of these vitamins when lettuce was grown below the upper 28 °C threshold (Hayes 2018). Nevertheless, breeders will likely contend with complex G × E interactions when breeding for higher vitamins, as they do when selecting for other horticultural traits highly influenced by the environment, such as tipburn, bolting, reduced head weight, and other heat stress disorders (Jenni and Hayes 2010; Jenni and Yan 2009; Lafta et al. 2017, 2021).

Conclusions

Differences in vitamin content, moisture, soluble solids, and sugars was detected among the accessions tested in this research indicating genetic variation in lettuce for the measured traits. Therefore, breeding lettuce for higher content in tocopherol, phylloquinone, and ascorbic acid as well as with higher soluble solids could be possible, especially for the use in hydroponic systems. Measuring soluble solids cannot be used as indication of levels of fructose in lettuce. These results suggest the potential for identifying accessions high in multiple vitamins for better breeding efficiency; however, environmental and G × E factors differ among vitamins and further analysis is warranted to determine the ideal selection environments for breeding-lettuce with enhanced vitamin contents.

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