Evaluation of a hand-held spectrophotometer as an in-field phenotyping tool for tomato and pepper fruit quality

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Abstract
Phenotyping for vegetable fruit quality traits can involve laborious postharvest and biochemical assays, decreasing efficiency of data collection. Portable devices that are easy to use and withstand in-field conditions to non-destructively and accurately quantify internal fruit quality traits would greatly enhance efficiency in breeding programs. We evaluated a hand-held quality spectrophotometer, the Felix-750, as an in-field tomato (Solanum lycopersicum L.) and pepper (Capsicum annuum) high-throughput phenotyping tool. Fruit quality traits included pH, soluble solids, carotenoids, and shriveling germplasm grown in replicated split-plot field trials. Germplasm included elite inbred cultivars and introgression lines of tomato, and diverse hybrid and open-pollinated cultivars of pepper. Our study employed a multi-faceted approach to evaluate the use of the Felix-750 in a plant breeding program. Our approach included chemometrics and trait-based partial least squares regression modeling, and examination of patterns in the λ-specific spectroscopy data based on variables relevant to genetic, fruit, and environmental factors using principal component analysis and biplots. Results of our study revealed: (a) the scope and limitations of the Felix-750 in fruit quality trait assessment based on the range of predictive power of partial least squares models; (b) insights into the complex relationships of spectroscopy data with genetic diversity, fruit biology and biochemistry, and factors related to environment. Additional research on the Felix-750 is needed to determine its potential applications at early and later stages of a breeding pipeline. We also suggest researchers explore more advanced chemometric tools and 3-D fruit hyperspectral imaging approaches.

Abbreviations: HTPP, high-throughput plant phenotyping; ILs, introgression lines; NIRS, near-infrared spectroscopy; PCA, principal component analysis; PLSR, partial least squares regression; RMSECV, root-mean square error of cross-validation; RPD, residual predictive deviation; SEC, standard error of calibration; SEP, standard error of prediction; UC, University of California; vis/NIRS, visible/near-infrared spectroscopy.

1 | INTRODUCTION

The world population is predicted to reach 9.9 billion by 2050 and consequent global rises in food crop demands are projected (Thomas & Dunston, 2019; Tilman, Balzer, Hill, & Befort, 2011). Improvements in food crop productivity are essential to support the nutritional needs of a growing population.
world population. The decrease in supplies of fresh water and its impact on limiting crop yields is also of importance. Water is central in crop production and irrigated agriculture accounts for ~90% of harvested land area in California (Pathak et al., 2018). Limited water and water stress results in growth reductions and yields below optimum levels if plants are unable to adapt (Cramer, Urano, Delrot, Pezzotti, & Shinozaki, 2011). Breeding crops that will deliver yield and quality under abiotic stresses including limited water is becoming a global priority.

Tomato (Solanum lycopersicum L.) and pepper (Capsicum annuum) are two economically valuable vegetable crops in the United States. Both crops belong to the Solanaceae family: the Solanum genus includes the cultivated tomato with processing and fresh-market tomato, and the Capsicum genus includes the domesticated pepper with hot chile and sweet peppers. Processing tomatoes are made into various processed products including soup, sauce, salsa, and prepared food. Fresh-market tomatoes are used fresh in salads and sandwiches. Likewise, pepper fruits are consumed as a fresh vegetable, processed for sauce and canning, dried as a spice, and used for industrial extracts. The United States ranked first globally for both vegetable commodities in 2017–2018 with California producing 47% of the U.S. pepper production (44 and 60% with bells and chile, respectively) and 63% of the tomato production (93 and 27% processing and fresh, respectively) (California Agricultural Statistics Review, 2017–2018). Tomato and pepper contribute significant vitamins, minerals, and antioxidants to human nutrition (Baenas, Belovic; Ilic, Moreno, & García-Viguera, 2018; Khachik et al., 2002; Olatunji & Afolayan, 2018; Rao & Rao, 2007; Rodriguez-Concepcion et al., 2018).

Genetic diversity is a prerequisite for breeding improved crops and attaining genetic gain. Plant breeders may introduce novel germplasm to generate and maintain ample variability and promote new recombinant genotypes to select for plants expressing desired target traits. Due to genetic bottlenecks that occurred during domestication of wild to cultivated species, and subsequent declines in genetic variation, breeders frequently explore wild relatives of crop plants (Dempewolf et al., 2017). Wild species retain variation at morphological, physiological, and biochemical levels, and in reproductive biology while thriving in their natural habitats (Bauchet & Causse, 2012). For example, tomato cultivars are estimated to contain less than 5% of the genetic diversity of their wild species’ relatives as measured using restriction fragment length polymorphic DNA markers (Bai & Lindhout, 2007; Peralta & Spooner, 2005; Miller & Tanksley, 1990). Wild tomato relatives contain agriculturally valuable traits that can be introgressed into cultivated tomato. These traits range from resistances to multiple pathogens and tolerances to drought, heat, and salinity (Labate et al., 2007) to fruit quality characteristics important in both the fresh and processing market classes (Blanca et al., 2015).

Plant breeders evaluate genetic material grown across replicated field trials to assess genotype × environment interactions and determine the best performing genotypes that meet breeding objectives. To achieve this goal, breeders must rapidly and accurately phenotype large numbers of field-grown plants to identify superior plants or populations and make selections. Trait phenotyping and the associated labor, time, and resources required is a major bottleneck in the breeding pipeline and one of the primary constraints in plant breeding (Araus & Carins, 2014; Li, Zhang, & Huang, 2014; Singh, Ganapathysubramanian, Singh, & Sarkar, 2016; Tanger et al., 2017; Zhang & Zhang, 2018; Andrade-Sanchez et al., 2013; White et al., 2012). The phenotyping bottleneck further intensifies for data collection of fruit quality traits, which typically involve laborious postharvest processing and biochemical assays with expensive chemicals and specialized lab equipment. High-throughput plant phenotyping (HTPP) methods are being explored actively to help increase and achieve breeding efficiency (Furbank & Tester, 2011; Granier & Vile, 2014; Rebetzke, Jimenez-Berni, Fischer, Deery, & Smith, 2019; Singh et al., 2016).

Relatively little research has been done to date on HTPP for fruit quality trait breeding with portable hand-held instruments. Such tools should be easy to use, accurate, and robust to withstand real-time and in-field conditions. For in-field fruit phenotyping for fruit quality traits, HTPP tools should tolerate variations due to fruit flesh color, maturity, genotype, and environment, and must be competent to nondestructively measure (or quantify) internal fruit quality attributes. Visible (vis)/near-infrared spectroscopy (NIRS) (380–2,500 nm) is being investigated for postharvest fruit quality evaluations (Samamad, Ribeiro, de Almeida Lopes, Puschmann, & de Oliveira Silva, 2018 in cashew; Huang, Lu, & Chen, 2018 in tomato; Viegas, Mata, Duarte, & Lima, 2016 in wax jambu; Kumar, McGlone,
Fruits can be evaluated with spectroscopic techniques. These techniques study the vis (380–720 nm) and NIR (780–2,500 nm) wavelengths (λ) of the electromagnetic spectrum based on absorption of energy from molecules or chemical constituents in these regions. Signals of almost all major structures and functional groups of organic compounds (e.g., O–H, C–H, and N–H) are detected within this λ range (Wang et al., 2015). When incident light contacts the surface of a sample (e.g., a fruit), the light undergoes spectral changes as it interacts with the fruit at a molecular level (at a depth of ~1–2 cm below the peel) per a sample’s chemical composition and internal organic matter (Slaughter & Donis-Gonzalez, unpublished data, 2019). Molecules are excited and experience shifts in their energy levels or states. Consequently, the light is reflected, transmitted, or absorbed. For instance, if the energy level of the light matches the energy level required to excite the molecule to a higher vibrational energy level from the ground state, absorption of light occurs (Pasquini, 2003). A specific molecule, such as a water molecule, can partially or fully absorb the light at a given λ or λ range resulting in the absorption spectrum.

The resulting vis/NIR spectrum of a biological sample is frequently convoluted due to several interfering factors: water highly absorbs NIR radiation; low signal/noise ratio (i.e., measure of the quality or resolution of a peak); high overlap of combination bands (i.e., simultaneous stretching and excitation bands) and overtones bands (i.e., bands due to transitions of molecules from ground to higher energy levels); scattering of light (e.g., specular or mirror-like reflectance); instrumental noise; and complex constitution of the biological sample such as a fruit (e.g., tissue heterogeneities and internal phase changes) (Jaleh & Fakhri, 2016; Nicolaï et al., 2007; Rinnan, van den Berg, & Engelsen, 2009; Viegas et al., 2016; Wang et al., 2015). Chemometrics, or computational chemistry, and mathematical and multivariate statistical tools (e.g., partial least squares regression) and spectral preprocessing treatments (see below) are essential to extract the fruit quality relevant information from the convoluted vis/NIR spectrum (Abdi, 2010; Dale et al., 2012; Rinnan, 2014; Rinnan et al., 2009). Preprocessing treatments include two types, scatter-correction (e.g., standard normal variate) and spectral derivative (e.g., Savitzky-Golay derivations) methods. Preprocessing treatments are comprehensively reviewed in Rinnan et al., 2009, Wang et al., 2015, and Nicolaï et al., 2007.

The goal of our study was to evaluate the potential of the Felix-750, a vis/NIRS post-harvest quality handheld spectrophotometer, as an in-field HTPP tool for fruit quality trait breeding in tomato and pepper. The Felix-750 is a programmable hand-held spectrometer designed for post-harvest quality inspection and preharvest maturity assessment. It uses interactance to capture data in reflectance, raw absorbance, or preprocessed modes of first- and second-derivatives. Wavelength spectral range of the Felix-750 is 285–1200 nm (±10 nm) with λ resolution (or spectral sample size) of 3 nm and spectral resolution (or resolving power) of 8–13 nm.

Our first objective was to collect fruit data and build and validate trait-based partial least squares regression models using vis/NIRS data from the Felix-750 on tomato and pepper fruit samples. Our second objective was to assess patterns in the spectroscopy data based on grouping variables of in-field irrigation treatments (full or reduced) in both crops, genetic material (inbred or introgression line) and market types (processing or fresh-market) in tomato, and fruit flesh color and maturity (early, marketable, or late-season genotype groups) in pepper using principal component analyses and biplots. We studied fruit quality traits of pH, soluble solids, and carotenoids concentration in the tomato introgression lines and inbred cultivars, and shrink, and carotenoids concentration in the pepper breeding lines, hybrid, and open-pollinated cultivars across replicated field trials.

2 | MATERIALS AND METHODS

2.1 | Plant materials

2.1.1 | Tomato

Introgression lines (ILs) that each contain a specific chromosome introgression derived from a wild tomato S. habrochaites accession LA1777 in a S. lycopersicum inbred processing tomato cultivar E6203 background were used in this study (Table 1). Fifteen and 25 diverse ILs were employed in 2017 and 2018, respectively. Cultivar E6203 was used as a control both years since it is the recurrent parent of all the ILs. In 2018, we included five additional California inbred processing tomato cultivars to expand phenotypic diversity: Hunt 100, UC204B, Peto 95-43, and Orion. The University of California (UC) Davis Tomato Genetics Resource Center (tgrc.ucdavis.edu) provided seed of the tomato ILs, and the St. Clair lab provided seed of the inbred cultivars. All lines used in this study were grown as potted plants in a UC Davis greenhouse to provide sufficient seed for replicated field experiments.
TABLE 1  Processing tomato introgression lines (ILs) and inbred cultivars used in the study. Chromosome number of *S. habrochaites* acc. LA1777 introgression in the background of cultivar E6203 is also included.

| Entry in the study | Genotype  | Chromosome introgression | Type of genetic material |
|--------------------|-----------|--------------------------|--------------------------|
| B1                 | LA3913    | 1                        | IL                       |
| B2                 | LA3918    | 1                        | IL                       |
| B3                 | LA3921    | 2                        | IL                       |
| B4                 | LA3922    | 2                        | IL                       |
| B5                 | LA3926    | 3                        | IL                       |
| B6                 | LA3927    | 3                        | IL                       |
| B7                 | LA3930    | 4                        | IL                       |
| B8                 | LA3933    | 4                        | IL                       |
| B9                 | LA3938    | 5                        | IL                       |
| B10                | LA3939    | 5                        | IL                       |
| B11                | LA3943    | 5                        | IL                       |
| B13                | LA3948    | 7                        | IL                       |
| B15                | LA3951    | 7                        | IL                       |
| B16                | LA3953    | 8                        | IL                       |
| B17                | LA3955    | 8                        | IL                       |
| B18                | LA3956    | 9                        | IL                       |
| B19                | LA3957    | 9                        | IL                       |
| B20                | LA3958    | 9                        | IL                       |
| B21                | LA3960    | 9 and 10                 | IL                       |
| B22                | LA3963    | 10                       | IL                       |
| B23                | LA3965    | 2, 10, and 11            | IL                       |
| B24                | LA3967    | 11                       | IL                       |
| B25                | LA3968    | 12                       | IL                       |
| B26                | LA3969    | 12                       | IL                       |
| B27                | LA3975    | 3                        | IL                       |
| B28                | E6203     | –                        | Inbred cultivar; control |
| B33                | Hunt 100  | –                        | Inbred cultivar          |
| B34                | UC204B    | –                        | Inbred cultivar          |
| B35                | Peto 95–43| –                        | Inbred cultivar          |
| B37                | Orion     | –                        | Inbred cultivar          |

To expand phenotypic diversity of the tomato material evaluated in this study, fresh-market heirloom-type tomato cultivars and lines with diverse genetic backgrounds were also evaluated in 2018 (Table 2). These tomatoes were grown in an independent experiment at the Student Farm as part of the UC Davis Student Collaborative Organic Plant Breeding Education (SCOPE) tomato project (https://plantbreeding.ucdavis.edu/scope-project).

2.1.2  |  Pepper

Pepper cultivars and lines that represented different pod types, shapes, pungency levels, and varying tolerances to water and temperature stresses were included in this study. Ten and 15 diverse hybrid and open-pollinated (OP) cultivars were employed in 2017 and 2018, respectively (Table 3). Pepper types included New Mexican chile peppers, yellow wax peppers, and sweet bell peppers. Two early maturing California sweet bell pepper cultivars, Doblon and Hybrid UGI1208, were included as controls and deficit irrigation treatment was initiated when these controls had immature fruit set. Santa Fe Grande, an early hybrid yellow wax pepper, was also added as a control in 2018. All pepper seeds were provided by Dr. Paul Bosland, Chile Pepper Institute, New Mexico State University, Las Cruces.

2.2  |  Field experimental design, layout, and methods

All field experiments were conducted during summers of 2017 and 2018. In the spring of each year, seeds of tomato
TABLE 2 University of California-Davis Student Collaborative Organic Plant Breeding Education (SCOPE) project fresh-market heirloom-type tomato material used in the study. Genotype details are included where “×” refers to a cross between two (or more) fresh-market parents that are either inbred or hybrid cultivars.

| Entry in the study | Genotype details |
|--------------------|-------------------|
| SF1                | 1730 (Momotaro × Black Magic) |
| SF2                | 1602-2-30 (Cherokee Purple × Green Zebra) |
| SF3                | 1603-2 (Marvel Stripe × Green Zebra) |
| SF4                | 1603-1 (Marvel Stripe × Green Zebra) |
| SF5                | 1601-4-14 (Brandywine × Green Zebra) |
| SF6                | 1601-4-21 (Brandywine × Green Zebra) |
| SF7                | Beauty King |
| SF8                | 1705 (Marvel Stripe × Vintage Wine) |
| SF9                | Lemon Ice |
| SF10               | Summer Sunrise |
| SF11               | 1602-2-24 (Cherokee Purple × Green Zebra) |
| SF12               | 1763 (Cherokee Purple × Green Zebra × New Girl) |
| SF13               | 1736-1 (Early Girl × Cherokee Purple) |
| SF14               | Green Zebra |
| SF15               | 1704 (Cherokee Purple × Vintage Wine) |
| SF16               | 1733-1 (New Girl × Cherokee Purple) |
| SF17               | 1730 (Momotaro × Black Magic) |
| SF18               | JZ3-2 |
| SF19               | 1733-2 (New Girl × Cherokee Purple) |
| SF20               | 1733-3 (New Girl × Cherokee Purple) |
| SF21               | Momotaro |
| SF22               | 1736-2 (Early Girl × Cherokee Purple) |
| SF23               | New Girl |
| SF24               | 1602-3-5 (Cherokee Purple × Green Zebra) |
| SF25               | Damsel |
| SF26               | JU |

and pepper cultivars, breeding lines, and controls were seeded into plastic 73-cell flats filled with soil media in a UC Davis Plant Sciences greenhouse. Flats were watered daily and fertilized weekly with a 10–30–20 N–P–K solution. Prior to field transplanting, when tomatoes were ~5 wk old and peppers ~7 wk old, flats were placed in a lath house for 1 wk to harden off seedlings. Subsequently, seedlings were hand-transplanted into field locations at the UC Davis Plant Sciences Field Facility and sprinkle irrigated for ~2 wk before switching over to drip irrigation (see below).

For both crops in each year, a split-plot experimental design was employed separately. The mainplots were the drip-irrigation treatment (full and reduced water) and the subplots were the genotypes (cultivars, breeding lines, or controls). Beds were spaced 1.5 m apart and the eight plants within each plot were spaced 0.3 m apart in a row with 1.2 m blank space (alleys) between the plots within a row (similar to Lounsbery, Arms, Bloom, & St. Clair, 2016). Border rows surrounded each experiment at each location to minimize edge effects. Double-border rows between mainplots were included to provide physical separation of soil moisture profiles. The number of blocks per mainplot were as follows: three (in 2017) or four (in 2018) for tomatoes, and three (in 2017) or four (in 2018) for peppers. Blocks were perpendicular to the water pressure gradient, starting from the pressurized water source. The tomato split-plot experiment was repeated once at each of two locations (2017 and 2018). The pepper split-plot experiment was repeated once at each of two locations (in 2017) or twice at one location (in 2018).

The two water treatments were full water (normal) crop evapotranspiration rate (ETc) and reduced water (40% ETc for tomato and 85% ETc for pepper). The reduced water treatment for tomato started when 51% of control plots reached days to first ripe fruit and in pepper when there was 51% immature fruit set in control plots. Water was applied three times per week. To calculate the normal...
TABLE 3 Pepper open-pollinated (OP) and hybrid cultivars used in the study. Pod type and colors for each group of pepper genotypes are also included. Three groups are early and late-season, and marketable pepper genotypes.

| Entry in the study | Genotype                  | Cultivar type | Pod type | Early mature color | Late ripe color | Marketable color |
|--------------------|---------------------------|---------------|----------|--------------------|-----------------|------------------|
| K1                 | Santa Fe Grande           | OP            | Wax      | Yellow             | Red             | Mature yellow    |
| K3                 | NuMex Orange Spice        | OP            | Jalapeno | Green              | Orange          | Ripe orange      |
| K4                 | NuMex Lemon Spice         | OP            | Jalapeno | Green              | Yellow          | Ripe yellow      |
| K5                 | NuMex Pumpkin Spice       | OP            | Jalapeno | Green              | Orange          | Ripe orange      |
| K6                 | Green Bell                | Hybrid        | Bell     | Green              | Red             | Mature green     |
| K7                 | Orange Bell               | Hybrid        | Bell     | Green              | Orange          | Ripe orange      |
| K9                 | NuMex Eclipse             | OP            | New Mexican | Green        | Brown          | Ripe brown       |
| K10                | NuMex Sunrise             | OP            | New Mexican | Green        | Yellow          | Ripe yellow      |
| K11                | NuMex Heritage 6-4        | OP            | New Mexican | Green        | Red             | Mature green     |
| K12                | NuMex Sandia Select       | OP            | New Mexican | Green        | Red             | Mature green     |
| K13                | Pepperoncini              | OP            | Italian  | Pale Green        | Red             | Mature Pale green|
| K15                | Poblano                   | Hybrid        | Poblano  | Green              | Red             | Mature green     |
| K16                | NuMex Las Cruces Cayenne  | OP            | Cayenne  | Green              | Red             | Ripe red         |
| K17                | Doblon                    | Hybrid        | Sweet banana | Yellow     | Red             | Mature yellow    |
| K18                | Hybrid UG111208           | Hybrid        | Bell     | Green              | Red             | Mature green     |

ET$_c$ for both crops, the daily reference ET$_o$ data from the California Irrigation Management Information System (CIMIS) local weather station in Davis was used. Weekly measurements of crop canopy were used to calculate crop coefficients ($K_c$) to determine each crop’s ET$_c$. Each tomato field was fertigated starting ∼5 wk post-transplant with 26.5 L of 32–0–0 urea ammonium nitrate (UAN32) weekly for 4 wk. Each pepper field was fertigated starting ∼6 wk post-transplant with 26.5 L of UAN32 weekly for 4 wk. Separate standard management practices specific for each crop, including pesticide applications and weed management, were used.

2.3 Trait phenotyping in the field, fruit quality and laboratory assays

The fruit were sampled for data collection from either the first two plants per subplot in 2017 or all plants per subplot in 2018. Five tomato fruits per subplot were randomly sampled and harvested. Sampling number of pepper fruits varied from three to five, depending on the size and variety. Samples (per subplot) were scanned in the field using the Felix-750 Produce Quality Meter. Briefly, an individual fruit in-field was placed and scanned on the lens ensuring contact with the perimeter of the bracket around the lens. Samples within a subplot were consecutively scanned. Adaptor accessories were selected per compatibility with the size of the fruits, such as a 19-mm reflector cone was used for rather uniformly sized tomato fruits, whereas an 11-mm reflector cone and a small fruit adaptor were more appropriate for variably sized pepper fruits. More details about the Felix-750 and its operations are provided in the F-750 Instruction Manual, https://felixinstruments.com/food-science-instruments/portable-nir-analyzers/f-750-produce-quality-meter/.

Maturity data, including day to first green fruit and ripe fruit, was collected for tomato as described in Lounsbery et al., 2016. All tomato genotypes were uniformly harvested for sampling at mature fruit size and color when 95% of cultivar E6203 control plots had reached fruit maturity. For peppers, harvest continued throughout the season depending on fruit color changes, so maturity was individually monitored per genotype. There were three random and user-defined groups of genotypes for pepper data collection (Table 2). For the early season group (entries K1, K6, K11, K12, K13, K15, K16, and K17), pepper genotypes were harvested at either their mature green or yellow stages. For the late-season group (entries K1, K6, K11, K12, K13, and K16), pepper genotypes were harvested at their fully ripe color stage. For marketable group (applied to all entries), pepper genotypes were harvested at their respective marketable mature color stage.

After harvest, tomato fruit subsamples were homogenized together per sample in a blender and the puree was stored in 2-ml tubes at −80 °C. Fruit puree total soluble solids concentration (SSC) was measured as °Brix with a digital pocket refractometer PAL-1 (ATAGO Co.) with accuracy ±0.2%. Fruit puree pH was measured with a standard pH meter.
For peppers, dry-down (or shrink) was obtained for both early and late-season genotype groups. Pepper samples were cut into slices, weighed (excluding stem or diseased tissue, if any), and dried for 24 h in an oven chamber at 51.7 °C. Dried peppers were then weighed and ground into fine powder using coffee grinders before storing 2-ml aliquots of each sample at –80 °C as described in Guzman, Hamby, Romero, & Bosland, 2010. For shrink, the wet-based moisture content was calculated as shown below (adapted from Rice Knowledge Bank, International Rice Research Institute).

\[
\text{Shrink (\%)} = \frac{(\text{fresh wt. (g)} - \text{oven dried wt. (g)}) \times 100}{\text{fresh wt. (g)}}
\]

To prepare tissue for biochemical assays, pepper fruits were cut and pericarp was trimmed to remove septum, placenta, and seeds before submerging the trimmed peppers into liquid N and storing in 2-ml tubes at –80 °C. Pepper fruits were subsequently freeze-dried using a lyophilizer. Lyophilized pepper fruit samples were then ground into a fine powder and stored in 2-ml aliquots at –80 °C until use.

To obtain tomato fruit carotenoids, the solvent system for extraction was adapted from Laur & Tian, 2011 and Guzman et al., 2010, with following modifications. For fast simultaneous extraction of both polar and non-polar carotenoids, hexane, acetone, and ethyl-acetate solvents were combined to make a 2:1:1 (v/v/v) extraction mixture. Tomato homogenate was exposed to sonication in a water bath for 10 min to enhance dissolution followed by centrifugation at 13,000 rpm for 3 min. The extract was measured at 450 nm using a UV Visible Spectrophotometer (Shimadzu) with a UV quartz cuvette to quantify the total tomato fruit carotenoid content (de Carvalho et al., 2012; Rivera & Canela, 2012).

For light-sensitive oven-dried and lyophilized pepper samples, the carotenoid extraction and saponification protocol was adapted from Rodriguez-Uribe, Guzman, Rajapakse, Richins, and O’Connell (2012) and Norris, Terrence, and Dean (1995). Briefly, 22 mg of a pepper ground sample was extracted using a solvent mixture of 3:2 (v/v) acetone and ethyl-acetate at room temperature followed by 30 min in the dark. Subsequently, 400 µl of water was added and the mixture was centrifuged at 17,000 rpm for 10 min to separate the upper organic phase layer with carotenoids and non-polar ethyl-acetate from the bottom non-organic layer of water and acetone in the supernatant. A centrifugal rotary evaporator was used for 25 min to evaporate the ethyl-acetate from the extracted phase. Dried extract was re-suspended in 100% ethanol followed by a sonication water bath treatment same as the tomato extractions. Prior to quantification of the total fruit carotenoid content with a spectrophotometer at 450 nm, either 10- or 100-fold dilutions were performed, based on sample’s pigmentation intensity.

For each tomato and pepper fruit sample, two technical replicates were analyzed to obtain an average absorbance value per sample. The formula for estimating the concentration of total fruit carotenoids in both crops with a spectrophotometer was adapted from Rivera and Canela (2012), Rodriguez-Amaya (2001), and Šaponjac, Ćanadanović-Brunet, Četković, and Djilas (2016). Modifications to the formula included multiplication by the protocol’s correction factor of 2 in both tomato and pepper and a diluting factor of either 10 or 102 in pepper, as shown below.

\[
X \text{ (mg kg}^{-1}\text{)} = \frac{A \times Y \text{ (ml)} \times 10^6 \times C \times D}{(A_{1cm}^{1\%} \times 100) \times \text{sample wt. (g)}}
\]

In the above equation, \(X\) is concentration (mg kg\(^{-1}\)) of the carotenoid in a given sample, \(A\) is absorbance measured at 450 nm, \(Y\) is volume of the solution that gives an absorbance of \(A\) at 450 nm (1 ml), \(10^6/100\) are conversion factors to calculate the concentration in units of mg kg\(^{-1}\), \(C\) is the correction factor, and \(D\) is the diluting factor. Next, \(A_{1cm}^{1\%}\) is the average absorption coefficient defined as the theoretical absorbance of a solution of 1% (w/v) concentration in a cuvette with a path length of 1 cm (2,500 is the value typically used to quantify total carotenoid content from a sample containing mixture of pigments; Batra et al., 2017; Mínguez-Mosquera, Honero-Mendez, & Pérez-Gálvez, 2002), and \(g\) is the weight of fruit sample.

### 2.4 Statistical analyses

Statistical analyses performed included independent and combined spectroscopy and fruit quality trait data analyses. See Figure 1 for a schematic outlining the statistical analyses.

#### 2.4.1 Analysis of trait data (independent of spectroscopy data)

Each trait for tomato and pepper was analyzed separately using R (version 3.5.2, 2018 R Core Team). The ggplot2 package was used to investigate outliers and/or missing data for each trait. Information on removed technical outliers was incorporated into modeling of spectroscopy data (see later sections). Analysis of variance (ANOVA) Type III with Satterthwaite approximations was performed (see below) for each trait dataset pooled across both locations because there were no significant genotype × location and water treatment × location interactions.
In the model above, fixed-effects were selected to be Genotype = tomato and pepper lines and varieties; Treatment = full or reduced water; Genotype × Treatment = genotype × treatment interaction; Location = location 1 or 2; and Genotype × Location = genotype × location interaction. Random-effects were selected to be Rep = water treatment and location as nested factors for traits with data collected from both locations; Rep = main-plot (or experimental unit of water treatment) for traits with data collected from only one location; Block = blocks and reps nested; Genotype × Rep = genotype × rep interaction. Two factors were considered to be nested when all levels of one factor occurred with only one level of another factor.

Tukey-Kramer tests were used to perform pairwise mean comparisons and mean separations when a significant effect \( (P \leq .05) \) was detected for any main effect (data not shown).

### 2.4.2 Analysis of spectroscopy and trait data

The F-750 model builder software (https://felixinstruments.com/support/F-750/software/) was used to access the spectroscopy data from the Felix-750 for fruits of both crops and transform it into absorbance format using the internal calculation, Absorption = log(1/Reflectance).

For both crops, \( n \times N \) mean spectral matrices and \( n \times 1 \) trait column-vectors, where \( n = \) samples and \( N = \lambda \), were concatenated using MATLAB R2017b (MathWorks). Spectral data for subsamples was averaged to obtain a one row-vector of spectral data for a given sample, known as the mean spectrum. Spectral information from fruit subsamples was averaged per sample to synchronize with the sample trait value, which was attained from combined puree or fine powder of subsamples. Samples with missing or outlier trait data (see above, Analysis of Trait Data) were excluded from the spectral matrix to maintain a 1:1 relationship between the mean spectrum and sample trait value. Descriptive summary statistics including sample size (\( n \)), mean, max, min, and standard deviation using each trait vector were calculated (Tables 4 and 5).

The resolving power of the Felix-750 is confined within 306–1137 nm relative to the total \( \lambda \) spectral range of 285–1200 nm; therefore, all spectra using the Felix-750 were collected for 306–1137 nm. Figures feature of MATLAB was used to visualize the mean spectra by plotting mean spectral matrices for each spectral dataset against the 306–1137 nm \( \lambda \) region. Outlier spectra were removed from each spectral dataset similar to outlier trait removal from trait datasets (see above, Analysis of Trait Data). Teflon, standard white spheres with 25.30-mm diameter were used...
TABLE 4  Descriptive statistics for tomato traits of pH, °Brix, and carotenoids concentration (mg kg⁻¹). Trait subsamples were averaged per sample and samples were pooled across genotypes and locations

| Trait                  | Sample size (n) | Mean   | Max value | Min value | Standard deviation |
|------------------------|-----------------|--------|-----------|-----------|--------------------|
| pH                     | 473             | 4.38   | 4.77      | 3.34      | 0.11               |
| °Brix                  | 473             | 5.08   | 7.1       | 3.6       | 0.56               |
| Carotenoids concentration | 474             | 32.97  | 87.66     | 1.04      | 17.92              |

TABLE 5  Descriptive statistics for pepper traits of shrink and carotenoids concentration (mg kg⁻¹). Trait subsamples were averaged per sample and samples were pooled across genotypes and locations for each genotypes group (early and late-season and marketable genotypes)

| Trait                  | Pepper genotypes group | Sample size (n) | Mean   | Max value | Min value | Standard deviation |
|------------------------|------------------------|-----------------|--------|-----------|-----------|--------------------|
| Shrink                 | Early season           | 123             | 91.59  | 95.78     | 84.90     | 1.83               |
|                        | Late season            | 77              | 85.77  | 92.22     | 70.27     | 3.75               |
| Carotenoids concentration | Early season         | 123             | 768.13 | 3,587.27  | 49.09     | 769.91             |
|                        | Late season            | 78              | 6,798.37 | 14,436.36 | 1,363.64  | 3,193.10           |
|                        | Marketable            | 228             | 3,375.09 | 185,090.90 | 78.18     | 12,730.37          |

as reference material to determine irrelevant and noisy λ (regions associated with instrumental variation) and achieve the complete region of λ selection (WS) for calibration model development (see later sections). Teflon spheres were also used to assess the calibration performance of the Felix-750 under varying temperatures of three fruit postharvest storage rooms, 0, 10, and 20 °C. Geometry of Teflon sphere references represented an average fruit. Five repeated spectra of Teflon subsamples were averaged to have a mean spectrum per Teflon sphere. Five replicated spectra of Teflon spheres were averaged to have a mean spectrum per temperature treatment. See Supplemental Figure S1 for an image of Teflon spheres as well as spectral λ plots for the WS process.

The complete post-processed (post WS process with Teflon spheres reference) spectroscopy region for fruits of both crops was established as 402–1137 nm. Multiple λ ranges within the post-processed λ region were selected for regression modeling (see later sections) based on literature for each trait in both crops (Table 6).

2.5  Preprocessing treatments

Several preprocessing treatments in addition to raw (or no preprocessing) using the PLS-Toolbox 7.0 (Eigenvector Research, Inc.) with MATLAB were applied as appropriate for each trait dataset in both crops to improve calibration models: standard normal variate (SNV), second derivative (SD) with Savitzky-Golay (SG), orthogonal signal correction (OSC) (Barnes, Dhanoa, & Lister, 1989; Boulet & Roger, 2012; Dale et al., 2012; Rinnan et al., 2009; Savitzky & Golay, 1964; Sjoblom, Svensson, Josefson, Kullberg, & Wold, 1998; Zimmermann & Kohler, 2013). Algorithms for preprocessing treatments were integrated into partial least squares (PLS) analyses (see next section) using in-house scripts. Along with every preprocessing treatment, each trait vector was auto-scaled for normalization and transformed trait data (see above, Analysis of Trait Data) was used when appropriate.

2.5.1  Modeling the relationship between spectroscopy and trait data

Partial Least Squares Regression (PLSR) was employed to develop relationship models between the spectral and trait data of both crops using the PLS-Toolbox 7.0 with MATLAB. Partial least squares essentially predicts Y from X by modeling the shared structure between the two matrices by extracting a group of orthogonal latent variables (LVs) by running a simultaneous decomposition of X and Y (Abdi, 2010). For the regression then the focus is concentrated on these orthogonal factors to model the relationship.

Data were categorized into spectra and trait matrices for each trait dataset using a four-fold cross-validation technique repeated four times with 75% of the samples allocated to the calibration set and 25% of the samples to the validation set. When predicting trait values for the validation set, the PLSR algorithm calculates new LV estimates as linear combinations of the original variables from the spectra matrix and uses these estimates as predictors. Minimum value for the root-mean square error of cross-validation (RMSECV) is used to select the optimum number of latent variables (nLV) (max was fixed as 20) used to calculate internally the best model for each constituent. The RMSECV is obtained by comparing the predicted trait value with its reference value. In addition to nLV, PLSR statistical parameters to estimate performance of the selected model included coefficient of determination.
### TABLE 6 Vis-NIR spectral wavelength (λ) ranges (nm) tested for tomato and pepper modeling. (A) Trait-specific λ ranges, and (B) λ ranges explained based on literature review

(A)

| Crop                              | Trait                   | Wavelength (λ) ranges tested                                                                 |
|-----------------------------------|-------------------------|---------------------------------------------------------------------------------------------|
| Tomato                            | pH                      | 402–1,137, 700–1,100, 800–1,100, 939–1,026                                                  |
|                                   | °Brix                   | 402–1,137, 700–1,100, 741–1,071, 800–1,100, 939–1,026                                      |
|                                   | Carotenoids concentration | 402–1,137, 402–502                                                                           |
| Early season pepper genotypes     | Shrink                  | 402–1,137, 600–1,100, 800–1,100                                                             |
|                                   | Carotenoids concentration | 402–1,137, 402–502                                                                           |
| Late-season pepper genotypes      | Shrink                  | 402–1,137, 600–1,100, 800–1,100                                                             |
|                                   | Carotenoids concentration | 402–1,137, 402–502                                                                           |
| Marketable pepper genotypes       | Carotenoids concentration | 402–1,137, 402–502                                                                           |

(B)

| Wavelength (λ) range | Trait-based reasoning                                                                 | Literature                                                                                 |
|----------------------|--------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| 402–1,137            | This was the complete post-processed spectroscopy region for both crops in our study. | NA                                                                                         |
| 402–502              | The absorption maxima for the visible light spectrum of commonly quantified food carotenoids (in variety of solvents) locate within this region. | Van Meulebroek, Bussche, Steppe, and Vanhaecke, 2014; Garzón et al., 2012; Laur and Tian, 2011; Mínguez-Mosquera et al., 2002; Rodríguez-Amaya, 2001 |
| 600–1,100            | Water content using this region in apples was predicted with $R^2 = .96$             | Qing, Ji, and Zude, 2007                                                                     |
| 700–1,100            | °Brix using this region in pineapple was predicted with $R^2 = .72$. Ranges within this region in mandarin fruits, including 720–950 nm with $R^2 = .70-.90$, and 700–930 nm with $R^2 = .93$ were used to predict °Brix. Similarly, °Brix was predicted using 735–930 nm^2 in peach, nectarine and plum fruits with $R^2 = .90$. Wavelengths of 768 and 986 nm were significant for pH predictions as associated with C–H stretch fourth and O–H stretch second overtones, respectively | Guthrie, Wedding, and Walsh, 1998; Guthrie, Walsh, Reid, and Liebenberg, 2005; McGlone, Fraser, Jordan, and Künnemeyer, 2003; Golic and Walsh, 2006; Osborne, Fearn, and Hindle, 1993; González-Caballero et al., 2010 |
| 741–1,071            | This region includes the λ identified as relevant to sugar and water for SSC distribution in melons, including, 830, 850, 870, 905, and 930 nm. Similarly, this region also includes the identified λ of sugar spectra, including 838, 888, 913, 978, and 1,005 nm. Wavelengths of 910 and 963 nm were relevant for °Brix predictions as associated with C–H stretches, 960 nm with O–H stretch, and 950–1075 nm with both chemical groups | Long, Walsh, and Greensill, 2005; Williams and Norris, 1987; Wang et al., 2015 |
| 800–1,100            | °Brix using this region in kiwifruit was predicted with $R^2 = .90$. Water content using 900–1700 nm in date fruit was predicted with $R^2 = .90$ | McGlone and Kawano, 1998; Mireei, Mohtasebi, Massudi, Rafiee, and Arabanian, 2010 |
| 939–1,026            | For sugar content, 948 and 982 nm λ were important within this region. Absorptions at 975 and 976 nm, respectively, were related with soluble solid content. Wavelength of 986 nm was significant for pH prediction. | González-Caballero et al., 2010; Shao and He, 2007; Osborne et al., 1993; González-Caballero et al., 2010 |

Note. $R^2$ refers to the coefficient of determination for prediction (see Modeling the relationship between spectroscopy and trait data in statistical analyses section of Materials and Methods). Letters a–f show that the data/stats in the Trait-based reasoning column correspond with the references in the Literature column.
for calibration ($R^2_{\text{cal}}$) and coefficient of determination for prediction ($R^2_{\text{pred}}$), standard error of calibration (SEC), standard error of prediction (SEP), residual predictive deviation (RPD), and average difference between predicted and actual values (Bias). Partial least squares models with maximum $R^2$ and RPD, and lowest SEC, SEP, and Bias values, were selected as the optimum preprocessing method and best regression calibration models.

### 2.5.2 Classification methods (or pattern recognition) and analyses

Principal component analysis allows data compression (Dale et al., 2012), which is useful with large numerical data of spectroscopy. Principal components are non-correlated linear combinations that describe the covariance structure (or maximal variation) of the multivariate data (Tzeng & Berns, 2005). Principal component analysis (PCA) in our study was explored to detect spectral patterns based on water treatment (normal or reduced) within both crops, genetic material type (IL or inbred) in tomato, and fruit flesh color (pale green, green, and yellow in early season; red in late-season; and pale green, green, yellow, orange, red, and brown in marketable) in peppers. R scripts were written in-house for PCA analysis of spectroscopy data of both crops. For pepper PCs, the R package factoextra (developed by Dr. Alboukadel Kassambara, HalioDx) was used for flesh color ellipses.

Biplot analysis, a PCA variant, is an instructive graphical representation of a multivariate numerical dataset (Jolliffe & Cadima, 2016; Lipkovich & Smith, 2002). Using biplots, information on both $n$ and $N$ are represented simultaneously in two or more dimensions because algorithms define and run the two matrices together. Values of $n$ are characterized by points on the plots and $N$ via calibrated vectors onto the two (or more) PCs of the spectral data. Wavelength vectors convey the direction along which the spectroscopy data vary the most. Enhanced algorithms using ggbiplot package in R (developed by Dr. Vince Q. Vu, Ohio State University) allow further groupings on biplots based on external factors using multivariate normal data ellipses. In our study, we used biplots with $\lambda$ vectors to assess and analyze spectral patterns after: dividing the data based on both water treatments and genetic material type as grouping variables in tomato, and both water treatments and genotype groups (early, late, and marketable) as grouping variables in pepper; combining the spectroscopy data of processing and fresh-market heirloom-type tomato with market class as a grouping variable; combining the spectroscopy data of all pepper genotype groups and using both water treatment and maturity as grouping variables; and using fruit flesh color and genotype as grouping variables within each maturity genotype group in pepper. R scripts were written in-house and adapted from algorithms developed by Dr. V.Q. Vu.

### 3 RESULTS AND DISCUSSION

#### 3.1 Trait data visualization and statistical analyses using linear mixed-effects models

Tomato genotypes were evaluated under full (normal ET$_c$) and reduced (40% ET$_c$) irrigation treatments across replicated field experiments for the fruit quality traits of pH, °Brix (soluble solids), and carotenoids concentration. In tomato trait ggplots, the reduced water treatment was associated with lower values for °Brix, and higher values for both carotenoids concentration and pH (Supplemental Figure S2). The reduced water treatment used for tomato was relatively severe (60% reduction) because the ILs used in our study contained chromosome introgressions from a water stress-tolerant wild tomato, *S. habrochaites* (Arms, Bloom, & St. Clair, 2015; Lounsbery et al., 2016), with habitat in dry slopes of Andean Ecuador and Peru (Knapp & Peralta, 2016).

Pepper genotypes were evaluated under full (normal ET$_c$) and reduced (85% ET$_c$) irrigation treatments for the fruit traits of shrink and carotenoids concentration. Trait ggplots exhibited a general reduction in shrink in late-season relative to early season peppers, but a broader range (i.e., the difference between max and min) was evident (Figures S3A and S4A). There was an unclear pattern for shrink within both early and late-season pepper genotype groups as this trait was genotype-specific (Supplemental Figures S3A and S4A). Patterns of carotenoids concentration under water treatments were genotype-group specific, generally higher in genotype groups of early season (Supplemental Figure S3B) and marketable (Supplemental Figure S5), and lower in late-season (Supplemental Figure S4B) genotype group under reduced water. Reduced water treatment used for peppers was mild (15% reduction) because the genotypes used in our study were not selected for water-stress tolerance during breeding.

In general, the results from ANOVA were both crop- and genotype-group specific (Table 7). There were no significant interactions detected for genotype × location and water × location effects; hence, data was pooled across locations for each trait in each crop for analysis. Genotypes were significantly different for all traits in both crops. Water treatment had a significant effect on °Brix in tomatoes, with an average °Brix of 4.9 ± 0.03 under reduced water relative to 5.3 ± 0.04 under normal water treatment. Previous studies showed that tomato plants under
TABLE 7 Summary of ANOVAs performed on tomato and pepper trait data. F-test statistics for genotype, water treatment, and genotype × water treatment effects are presented for both crops (and genotype groups for peppers)

| Trait                  | Crop                  | Field location | F-test statistic | Genotype | Water treatment | Genotype × water treatment |
|------------------------|-----------------------|----------------|------------------|----------|----------------|---------------------------|
| pH                     | Tomato                | 1, 2           | 15.39***         | 3.43     | 0.91           |                           |
| °Brix                  |                       |                | 12.26***         | 30.55*** | 1.64           |                           |
| Carotenoids concentration | Pepper early season | 1              | 3.94**           | 1.28     | 0.58           |                           |
| Carotenoids concentration | Pepper late season  |                | 139.30***        | 6.75*    | 3.72*          |                           |
| Carotenoids concentration | Pepper marketable  |                | 18.82***         | 3.54     | 0.53           |                           |
| Shrink                 | Pepper early season  |                | 30.23***         | 2.18     | 0.96           |                           |
| Shrink                 | Pepper late season   |                | 18.84***         | 0.58     | 0.9            |                           |
| Pericarp thickness     | Pepper marketable    |                | 7.99*            | 0.6      | 0.75           |                           |
| Pericarp thickness     | Pepper marketable    |                | 25.77***         | 0.02     | 0.99           |                           |

Note. Within columns, F-test values without asterisks are insignificant at the .05 probability level.
°F-test values for tomatoes are presented for each analysis by combination of locations (see Materials and Methods).
*Significant at the .05 probability level.
**Significant at the .01 probability level.
***Significant at the .001 probability level.

Water-deficit increase their accumulation of fruit soluble solids (Nuruddin, Madramootoo, & Dodds, 2003; Yin et al., 2010). However, this was not observed in our study except in one IL, B5. Fruit pH was not significantly affected by water treatment, in agreement with studies by Klunklin and Savage (2017) and Alvino, d’Andria, and Zerbi (1988). In addition, tomato carotenoids were also not significantly affected by the water treatment, as was also observed by Krumbein, Schwarz, and Klarling (2006).

Effects of water stress on carotenoids concentration in fruits reported in the literature range from negative to non-significant to positive (Ripoll et al., 2014). In peppers, the only significant effect of water treatment on carotenoids was detected in early season peppers (Table 7), with an average carotenoids concentration of 816.36 ± 100.94 mg kg⁻¹ under reduced water relative to 720.67 ± 95.85 mg kg⁻¹ under normal. Additionally, genotype × water treatment was also significant for this trait. The mild water reduction treatment in peppers could account for the lack of significant effects of water treatment on other pepper traits. The significant effect observed for carotenoids in the early season genotype group suggests that water stress influenced the accumulation of carotenoids as fruits matured early in the season, but the effect was insignificant later as fruits matured into horticulturally marketable and late stages. It is possible that pepper genotypes adapted to the mild water stress as the season progressed for carotenoids accumulation. Katerji, Mastorrelli, and Hamdy (1993) found the highest sensitivity to water stress in peppers at early fruit setting, as measured with fresh and dry weight, fruit size, and number under greenhouse conditions.

3.2 Characterization and pre-processing of spectroscopy data of the Felix-750

Spectroscopy data was transformed from the Felix-750 reflectance mode to absorbance mode when exported from the Felix-750 model builder software (see Materials and Methods). Reflection only provides information about the surface, whereas absorption contains information on the chemical composition of the fruit (Slaughter & Donis-Gonzalez, unpublished data, 2019; Nicolaï et al., 2007).

Mean absorbance spectral matrices for each spectral dataset from the Felix-750 were visualized in MATLAB in raw, pre- and post-preprocessing treatments modes against λ variables in tomato and pepper (Figure 2; Supplemental Figure S6). For tomato fruits (Figure 2), which are comprised of 92–95% water (Davies, Hobson, & McGlasson, 1981; Wilkerson et al., 2013), a water-related absorption band occurred around 950 nm in the NIR region associated with the third overtone of O–H bonds, as is typically observed for most fruits and vegetables (González-Caballero, Sánchez, López, & Pérez-Marín, 2010; Huang et al., 2018; McGlone & Kawano, 1998). Water highly absorbs NIR, convoluting spectral data of fruits, or in other words making the spectrum complex with supplemental variability. However, water-related absorption bands associated with overtone bands of the O–H bonds, such as at 1,450 nm and a combination band at 1,940 nm (Huang et al., 2018, Nicolaï et al., 2007; Samamad et al., 2018) were not an issue with the Felix-750 post-processed spectroscopy region of 402–1137 nm in our study. Absorption bands in the visible region between 400–500 nm in tomato (Figure 2) and 400–750 nm in pepper (Supplemental
Figure S6) fruits were most likely due to the presence of fruit pigments (e.g., carotenoids, anthocyanins) and chlorophyll a and b (González-Caballero et al., 2010; McGlone et al., 2002). Overtone band of the O–H bonds at 760 nm may also be dominating these absorption bands due to pigments in the visible region (Nicolaï et al., 2007).

### 3.3 Partial least squares models using raw and preprocessed spectroscopy data

Although other PLSR statistical parameters played a role in estimating model performance (see Materials and Methods), we succinctly focus the discussion on $R^2$ and RPD values (see below). Tomato fruit models for all $\lambda$ ranges and preprocessing treatments resulted in poor fit, defined as low $R^2_{\text{cal}}$ and $R^2_{\text{pred}}$ values for pH, °Brix, and carotenoids, suggesting low calibration and predictive power of trait models (Supplemental Table S1A). Chang, Laird, Mausbach, and Hurburgh (2001) established the three quality categories of models based on the RPD values as excellent (RPD > 2), moderate (1.4 < RPD < 2), and unreliable (RPD < 1.4). The RPD values of all tomato models in our study were less than 1.4, indicating a lack of reliability. de Oliveira et al., 2014, Chen, 2008, and Walsh, Golic, & Greensill, 2004 also observed poor prediction models for tomato internal quality evaluation using NIRS technology.
Subsequently, models were built after dividing the spectroscopy data into normal and reduced water treatments. The $R^2_{\text{cal}}$ and $R^2_{\text{pred}}$ values of models improved modestly for each tomato trait (Supplemental Table S1B).

Performance of pepper fruit models for $\lambda$ ranges and pre-processing treatments tested were genotype group-specific (Supplemental Table S2A). Robust models with excellent RPD values (1.61–3.07) and high $R^2_{\text{cal}}$ (0.76–0.92) and $R^2_{\text{pred}}$ values (0.73–0.91) were achieved for carotenoids in early season genotype group. When the early season genotype group was modeled for each water treatment, the predictive power of carotenoid models remained moderate to high (Supplemental Table S2B). Correspondingly, in the pepper ANOVA analysis (see Analysis of trait data section in Results and Discussion), the trait of carotenoids in the early season genotype group was significant for water treatment.

Pepper models, on average, had better predictive capacity than models for tomato. For shrink, $R^2_{\text{cal}}$ and $R^2_{\text{pred}}$ values across all $\lambda$ ranges tested were low-moderate in the early and late-season genotype groups (Supplemental Tables S2A and S3A). For normal water treatment samples within both genotype groups, $R^2_{\text{cal}}$ but not $R^2_{\text{pred}}$ values improved (Supplemental Tables S2B and S3B). For reduced water treatment samples within early season peppers, $R^2_{\text{cal}}$ values were high and $R^2_{\text{pred}}$ values were moderate (Supplemental Table S2B). In late-season peppers, $R^2_{\text{cal}}$ and $R^2_{\text{pred}}$ values did not improve after dividing the data based on water treatment for modeling (Supplemental Table S3B).

The $R^2_{\text{cal}}$ and $R^2_{\text{pred}}$ values for carotenoid concentration in both late-season and marketable genotype groups were low to moderate, with higher $R^2$ values for late-season peppers (Supplemental Tables S3A, S4A). Dividing the marketable genotypes data by water treatment for carotenoids concentration only improved fitting of the models in reduced relative to normal water treatment (Supplemental Table S4B). In contrast, dividing the data for late-season genotype group by water treatment highly improved the models for the normal water treatment with $R^2_{\text{cal}}$ and $R^2_{\text{pred}}$ values of $\sim 0.75$. However, for the reduced water treatment, only the $R^2_{\text{cal}}$ values ($\sim 0.98$) were improved (Supplemental Table S3B). To our knowledge, there is insufficient literature on the use of NIRS in combined trait datasets of chile and sweet pepper fruits for fruit quality traits of shrink and carotenoids concentration. However, for bell pepper fruits, Sánchez et al. (2019) and Penchaiya, Bobelyn, Verlinden, Nicolaï, and Saeyes (2009) both observed moderate-to-high $R^2_{\text{cal}}$ and $R^2_{\text{pred}}$ values for firmness and soluble solids, and dry matter and soluble solids, respectively.

Tomato models were poor in fit for all traits and when data was divided by water treatment. Performance of pepper models was trait-, genotype group- and water treatment-specific. A number of factors may contribute to the low calibration and predictive power of majority of the models. Some of these factors are discussed in the following paragraphs.

### 3.4 Fruit and horticultural factors

A random location on each tomato and pepper fruit was scanned with the Felix-750 when collecting spectroscopy data on fruits in the field. Tomato is a heterogeneous fleshy fruit, comprised of different tissue and cell types, including epidermis, pericarp, septa, gelatinous locules, vascular bundles, and seeds (Rost, 1996). Each internal biological component has its own biochemistry and corresponding biochemical reactions and products. With phase changes inside non-uniform biological material such as a tomato fruit, scattering results from multiple refractions adding noise to the captured spectroscopy data (Nicolaï et al., 2007). Cell wall interfaces (McGlone & Kawano, 1997), suspended particles such as starch granules (Il’yasov & Krasnikov, 1991) and structures such as pores and cell membranes introduce scattering elements influencing the intensity of scattered light that is emitted (McGlone & Kawano, 1997) and the reflected spectrum of the fruit (Nicolaï et al., 2007). In our study, spectra scans of many fruits were averaged, and many fruits were blended together to obtain a tomato puree for fruit quality assays, contributing to possible sources of variation in the data.

In contrast to tomato, pepper is a hollow fruit with no gel or fleshy tissue. Nonetheless, pepper fruit is also comprised of diverse fruit tissues including pericarp, placenta, locules, capsicum glands, and seeds (Rizzi & Tebon, 2019). Pepper fruit shape, size, and market type (e.g., bell, jalapeno, chili) add to pepper fruit’s variability and the interaction between light and internal fruit molecules. Similar to tomato, spectral scans of several pepper fruits were averaged to obtain one scan per sample in our study. Both oven- and freeze-dried pepper subsamples were combined, ground, and averaged for trait assays. These sources of potential variation and noise due to fruit biology and biochemistry may be contributing agents to the reduced fitting of both tomato and pepper models (de Oliveira et al., 2014). With the hundreds to thousands of plants grown in typical breeding field trials, scanning multiple sections on one fruit is impractical on a large-scale. Likewise, blending separately each fruit subsample per sample and conducting independent postharvest assays is neither feasible nor applicable to large-scale processing breeding programs.

Fruit pericarp thickness in pepper fruits also varies depending on the distance from the stem end, potentially
influencing the amount of light that is internally absorbed relative to diffused (e.g., light reflected in all directions) (Slaughter and Donis-Gonzalez, unpublished data, 2019). Penetration power of light is expected to be lower with thicker pericarp, thus affecting the interface between light and fruit. Although processing tomatoes generally have thicker pericarps to withstand the rigors of mechanical harvest, differences in the thickness exist with the distance from the blossom end. Furthermore, some pepper genotypes in our study were translucent (e.g., K13, pepperoncini), making it difficult to exclude the soil surface in the Felix-750 scans. With fruits of such genotypes, the proportion of light transmitted (which is not of interest) becomes high compared with other fruits. Sorting materials based on fruit size, pericarp thickness, and translucency is inefficient and impractical when working with large numbers of diverse genetic material typically used in plant breeding programs.

3.5 Environmental factors

Ambient light and in-field fluctuating temperatures play a role in accurate spectral data acquisition (Nicolaï et al., 2007; Walsh, Guthrie, & Burney, 2000; Wang et al., 2015) while using an instrument such as the Felix-750. Golic and Walsh (2006) observed that temperature differences can cause bias between calibration and validation sets. In our study, a Teflon reference experiment confirmed no apparent differences in calibration spectra collected by the Felix-750 under 0, 10, and 20 °C (see Materials and Methods and Supplemental Figure S1). During the warm months of June through September in Davis, CA, daily air temperatures naturally vary and can easily reach 40 °C (California Irrigation Management Information System, 2019) exceeding this tested range of capacity of the Felix-750 to endure temperature differences. Additionally, tomato and pepper canopies can be dense and spatial fruit surface temperature effects can be present due to plant conditions (e.g., leaf cover, transpiration, scanned fruit’s height from the ground, distance between neighboring fruits on the same plant) and environmental conditions (e.g., wind, relative humidity, and solar radiation). The Felix-750 fruit scans were obtained a few hours apart and, due to large sample numbers, sometimes over several days. It is unrealistic to scan in-field and on-plant all fruits within all blocks, mainplots, and locations at one time point due to severe time and labor constraints. When incorporating variable horticultural maturity timelines of fruits based on genotype, the process becomes even more complex. Nonetheless, the Felix-750 is designed to internally compensate for temperature-induced spectra variation if a user builds calibration models under three arbitrary temperature ranges. These temperature ranges are specified by a user based on predicted theoretical lower and upper threshold environmental temperature values. By doing this, robust predictions can be made when fruits are scanned at actual and variable temperatures in the field for validation as the Felix-750 can calibrate for the temperature-induced fluctuations. However, scanning in-field individual fruits, each at multiple temperatures, in a breeding trial for calibration is impractical and too laborious. Alternatively, temperature variable-eliminating preprocessing treatments (Chauchard, Roger, & Bellon-Maurel, 2004; Roger, Chauchard, & Bellon-Maurel, 2003) and global robust calibration models (Peirs, Scheerlinck, Touchant, & Nicolaï, 2003; Wang, Pan, Li, & Han, 2009) could be possible solutions to compensate for effects of temperature fluctuations on the predictive power of models. Additionally, in future studies, extraction of chemical information with the Felix-750 could potentially be coupled with imaging-based technologies to enable high-throughput of the process and possibly enhance efficiency (Wang et al., 2015; Zhang & Zhang, 2018).

3.6 Pattern recognitions in the spectroscopy data using principal component analysis and biplots

After inconclusive PLS results with several of the trait regression models (see previous section), the Felix-750 spectroscopy data was also analyzed for pattern recognitions using PCA and biplots.

Principal component analysis of the complete tomato post-processed and SNV-treated spectroscopy data from the Felix-750 did not exhibit any clear groupings (plot not shown). All the data points were intermixed onto the plane projected on PCI1 and PC2. Subsequently, PCA was conducted using the genetic material type (inbred or IL) and water treatment (normal or reduced) as the grouping variables (Figure 3). Spectroscopy data points were labeled per their genetic material type and water treatment; ILs were more dispersed relative to inbreds. The ILs are more genetically diverse than inbred cultivars in our study, in agreement with the observed trend; ILs contained different introgressions from a water-stress tolerant wild tomato, S. habrochaites (Lounsbery et al., 2016; Monforte & Tanksley, 2000). In addition to containing genes for agriculturally useful traits, deleterious genes are also present in introgressions from a wild species (Bai & Lindhout, 2007), resulting in additional diversity in the ILs. In contrast, the inbred cultivars used in our study shared parentage and were selected for and adapted to California (Park, West, & St. Clair, 2004). The inbred cultivars often belong to commercially elite germplasm and are homozygous at many
Principal component analysis (PCA) of post-processed (402–1137 nm) and standard normal variate (SNV)-treated tomato spectroscopy data. Water treatment (normal and reduced) is used as the grouping variable and spectroscopy data points are labeled per their genetic material type (introgression line [IL] and inbred). See Table 1 for a list of tomato ILs and inbreds.

There was not a distinct difference in the spread of spectroscopy data samples from either of the water treatments, suggesting that samples did not behave differently under the water stress treatment. Statistical analyses of the trait data are congruous with this observed trend (see Analysis of trait data section in Results and Discussion). Other than for °Brix, no significant water treatment effect was detected in tomato trait data. This result suggests that either the tomato genotypes in our study largely expressed water-stress tolerance, or the majority of the IL genotypes exhibited tolerance masking the effect of inbreds. When spectroscopy data was divided based on water treatment and analyzed independently per water treatment using biplots, the spread of IL samples remained greater relative to inbreds (data not shown). However, no clear separation of the two groups within either of the two treatment biplots was observed.

In the biplots, red λ vectors with in-between gaps represent the λ with variable spectral patterns. Vectors with collapsed alignment indicate lack of variability in spectral patterns of the specified λ. However, this alignment could also potentially suggest an occurrence of an underlying spectral pattern in higher dimensions, which we are unable to perceive with the 2-D vectors. We can view the spectral sample points in relative terms such that sample points that plot in the same general direction as a particular λ vector are positively correlated, while ones on opposite sides from the origin are negatively correlated. Spectral patterns of inbreds and ILs in the biplots were variable for the same λ regions further confirming the lack of separation of ILs and inbreds under both treatments. Therefore, as expected, separate PLS modeling within normal and reduced water treatment datasets did not improve fitting of the models (see PLS modeling section in Results and Discussion).

Spectroscopy data from both processing (ILs and inbreds) and fresh-market heirloom-type tomatoes were combined and analyzed together using biplots (Figure 4). Samples of processing tomato coalesced into a defined region within the grouping ellipse relative to distributed samples of fresh-market tomato. The ILs in our study were less diverse compared with our fresh-market tomato samples because all ILs shared the same genetic background of a processing tomato inbred cultivar E6203 (see Materials and Methods). Additionally, fresh-market tomatoes in our study encompassed fruit samples from single- and multi-way hybrids, advanced families, single plant selections, and elite cultivars from diverse sources.

Processing tomato is selected for uniformity in shape, size, flesh color, firmness, ripening, and fruit quality processing characteristics such as pH and soluble solids as required by tomato processors (Anthon, LeStrange, & Barrett, 2010; Rick, 1978). In contrast, fresh-market tomato is selected for diversity in flesh color, size and shape, flavor, and shelf-life (Bai & Lindhout, 2007). Processing and fresh-market tomatoes are essentially treated as two separate market classes by plant breeders because of their distinctive production systems, end uses, and target traits (Bauchet & Causse, 2012). In our study, the Felix-750 was successfully able to differentiate between the two tomato market classes, indicative of its discerning ability between diverse genetic materials. To our knowledge, no similar study for processing and fresh-market tomato has been reported in the literature.

Biplot of combined spectroscopy data of all pepper genotype groups (early and late-season, and marketable) with water treatment as the grouping variable failed to show a clear separation among samples of either water treatment (Supplemental Figure S7). Spectral groupings of data were present, but samples from both water treatments overlapped in these groupings. Wavelengths with variable spectral patterns for the two groups were at the end of vis and NIR spectrums. Carotenoids in the early season genotype group was the only significant trait for water treatment in
the pepper data as detected by ANOVA (see Analysis of trait data section in Results and Discussion), agreeing with the observed biplot trend. The water stress treatment was mild for peppers, which likely contributed to the absence of a distinct pattern in the data based on the normal treatment. Severity and duration of water stress impacts trade-offs in fruit productivity and quality (Ripoll et al., 2014). Jeeatid et al. (2018) observed the accumulation of capsaicinoid (alkaloids related to pungency in pepper fruits) yield with onset of specific levels of water stress in hot pepper cultivars. In a future study, it would be useful to conduct a preliminary water-stress treatment gradient experiment and employ the threshold level that reveals discernable fruit phenotypic differences compared with a normal water treatment.

Pepper maturity genotype group was also used as a grouping variable (Supplemental Figure S8). Wavelengths of the end of the vis spectrum were variable in spectral patterns for discriminating among the maturity groups. The spectral patterns showed that peppers appeared approximately uniform early in their maturity as sample points overall were positioned around the origin. Peppers became different from each other as they approached marketable stages as sample points were distributed across the 2-D plane in addition to situating around the origin. As peppers approached ripe stages, they appeared more similar as suggested by the proximity of the sample points to each other and the origin. Nonetheless, most of the spectroscopy data points within the maturity group ellipses overlapped. Pepper material used in our study was a mixture of diverse genotypes with overlapping market classes. Domesticated C. annuum species consist of pungent and non-pungent accessions within five broad categories: fresh-market, processing, dried spice, industrial extracts, and ornamental types (Poulos, 1994; Srivastava & Mangal, 2019). Each market class is bred with a specific focus on horticultural and biochemical traits to meet consumer end uses of the fruits. In our study, the majority of the genetic material is used for multiple market classes, agreeing with the observed biplot trend. If the study was repeated with genotypes from independent pepper market classes (similar to processing and fresh-market tomato, see previous section), ability of the Felix-750 to differentiate among the pepper market classes could be tested.

Other factors (e.g., flesh color and genotype) were evaluated as grouping variables among the pepper genotype groups because no clear separation of the data was observed with water treatments (see below). The early season and marketable pepper genotype groups in our study were comprised of various fruit flesh colors. Principal component analysis of early season (Figure 5a) and marketable (Figure 5b) genotype groups expressed discrete spectral groupings associated with flesh color. Spectral clusters of early season pepper genotypes consisted of pale green, green, and yellow peppers. Similarly, clusters of
FIGURE 5  Principal component analysis (PCA) of post-processed (402–1137 nm) and standard normal variate (SNV)-treated pepper spectroscopy data. Fruit flesh color for (a) early season and (b) marketable genotype groups was used as the grouping variable with special features using the R package factoextra for flesh color ellipses. (c) Spectroscopy data points of late-season pepper genotype group were labeled per their genotype and no grouping variables were used. See Table 3 for a list of pepper genotypes and corresponding colors within all genotype groups.
marketable pepper genotypes consisted of pale green, green, yellow, orange, red, and brown peppers. Late-season genotypes only contained red peppers, so genotype was used as the grouping variable (Figure 5c). Kumar et al. (2015) observed that genotypes were the major source of variability in a PCA of heterogeneous apple seedlings from two seasons. We also observed separation based on genotypes in the red peppers of the late-season genotype group. Fruit flesh color and genotype greatly contributed to the groupings of the Felix-750 spectroscopy data in our pepper study.

The hue and intensity of pepper fruit color are associated with the visual appeal of the fruit and consumer’s perception of ripeness, freshness, and nutritional value (O’Donoghue, Brummell, McKenzie, Hunter, & Lill, 2018). Variation in the carotenoid biosynthetic pathway is due to multiple underlying loci controlling the biochemical variation in pepper fruit colors (Guzman et al., 2010; Paran & van der Knaap, 2007; Srivastava & Mangal, 2019). In our study, we had a diverse collection of pepper genotypes representing biochemical and phenotypic diversity. The Felix-750 was successfully able to distinguish fruit color groups due to their unique spectral patterns. In a future study, it would be helpful to use larger sample sizes in each maturity group and subdivide the dataset based on fruit color diversity for PLS calibration models. It would also be insightful to conduct association analyses with a mixed modeling approach in the future to detect for associations between plant factors and the λ-specific spectroscopy data of a large sample size of each flesh color sub-group within each maturity genotype group.

4 | CONCLUSIONS AND FUTURE DIRECTIONS

We evaluated the potential ability of the Felix-750 postharvest quality spectrophotometer as a HTPP tool in replicated field trials for assessing fruit quality traits in tomato and pepper for breeding. We determined that tomato PLSR models in our study were poor fitting for each trait tested (pH, °Brix, and carotenoids concentration) under the chemometric approaches used (λ ranges and preprocessing treatments). Fitting of pepper models was trait-specific (shrink and carotenoids concentration) and maturity genotype-group specific (early and late-season and marketable) for all λ ranges and treatments. Model fitting was improved in some pepper maturity genotype groups by dividing the data based on water treatment, whereas no such improvement was observed in tomatoes.

A closer examination of the λ-specific tomato vis/NIRS data (402–1137 nm) using PCA and biplots modeling provided insights into patterns based on irrigation treatments, genetic material type and market class in tomato. Based on the tomato results, we conclude that the genetically diverse material tested, and in some cases exceeded, the ability of the Felix-750 to provide spectroscopy data for predictive regression models. Pepper spectroscopy data was evaluated for patterns based on irrigation treatments, fruit flesh color and maturity. Based on the pepper results, we conclude that sub-grouping of diverse germplasm is likely necessary to use the Felix-750 effectively and reliably in plant breeding programs.

The results of our study suggest that additional testing of ability of the Felix-750 to serve as a HTPP tool in a vegetable breeding pipeline is warranted. We suggest testing Felix at early stages of evaluating divergent germplasm in a breeding program to make informed decisions about selection of materials for breeding, and at later stages of selection between- or within-line and/or family based on fruit quality trait performance. Moreover, breeding researchers should consider assessing the Felix-750 further by exploring temperature-eliminating calibration models and investigating more advanced multivariate statistical tools beyond regression and combining spectroscopy-extracted chemical data with in-field 3-D fruit hyperspectral imaging techniques. These strategies may provide reliability in fruit data collection with the Felix-750 in a breeding program and improve accuracy of spectroscopy data that may be less affected by the confounding factors of temperature fluctuations and sample specific heterogeneities.

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AUTHOR CONTRIBUTIONS

A.K., conceptualization of study, experimental design and methodologies, data collection, data analysis and visualization, data interpretation, manuscript writing, and revisions; I.R.D.G., conceptualization of study, input on methodologies, provider of specialized software and the Felix-750, input on data analyses and interpretation, and input on manuscript revisions; D.A.S.C., conceptualization of study, provider of plant materials, guidance on experimental design and methodologies, guidance on data
analyses, data interpretation, manuscript writing and revisions, and project supervision.

**DATA AVAILABILITY**

The authors declare that datasets supporting the conclusions of the study are included in this article (or in supplemental material).

**CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interest.

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