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High-throughput Characterization of Fruit Phenotypic Diversity among New Mexican Chile Pepper (*Capsicum* spp.) Using the Tomato Analyzer Software

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Abstract. **Fruit architecture and morphology-related traits are among the determinants of fruit diversity and are major contributors to yield and yield potential in chile pepper (*Capsicum* spp.). This study aimed to characterize 105 genotypes of a *Capsicum* diversity panel consisting of cultivars, breeding lines, landrace, and wild species belonging to twelve different pod (fruit) types, for 32 morphometric Tomato Analyzer (TA) descriptors. Hierarchical cluster analysis grouped the genotypes into eight clusters based on the TA descriptors. A multivariate principal component analysis yielded two principal components, PC1 and PC2, which explained 53.24% and 10.11% of the variation in fruit diversity, respectively. The basic measurements—namely, perimeter, area, width midheight, maximum width, height midwidth, maximum height, and curved height were the most discriminating descriptors with a maximum contribution to the overall fruit shape. There was a strong, positive correlation for basic measurements and fruit shape index, whereas blockiness was negatively correlated with distal angle macro. Additive genetic effects and high heritability for the fruit traits were observed. Results of this study will provide valuable information to breed high-yielding chile pepper cultivars based on fruit morphology traits.**

The diversity of fruit morphology within the genus *Capsicum* is one of its distinguishing features and is a consequence of domestication (Perry et al., 2007). *Capsicum annuum* L. is believed to have been domesticated thousands of years ago in Mexico or North Central America. Previous analyses dated wild *Capsicum* harvesting to ∼8000 years ago, followed by the cultivation and domestication of the *C. annuum* ∼6000 years ago (Byers, 1967; Perry et al., 2007). The shape of the wild chile pepper fruit can be oval, circular, or elongated; continuous domestication, breeding, and selection has caused significant variation in shape, size, and color in the *Capsicum* (Borovsky and Paran, 2011). Early stages of domestication involved key traits such as nondeciduous fruits and fruit orientation from erect to pendant (Colonna et al., 2019). Since domestication until the modern era of cultivation, significant diversity has been lost due to the genetic bottlenecks (Wouw et al., 2010). Researchers have recently paid attention to this critical issue and have started to characterize the genetic diversity of different crops, including potato (*Solanum tuberosum*) (Machida-Hirano and Niino, 2017), eggplant (*S. melongena*) (Oladosu et al., 2021), tomato (*S. lycopersicum* L.) (Figàs et al., 2018), and chile pepper (Ortiz et al., 2010).

Extensive diversity in fruit-related traits has been observed in different pepper-growing regions of the world. Nankar et al. (2020c) reported variability in fruit shape, ranging from elongated, conical, bell and round, to pumpkin-shaped in chile pepper genotypes from the Balkan region of Europe. In another study, fruits of *C. chinense* genotypes from Brazil were described to be elongated, blocky, triangular, campanulate, and almost round (Moreira et al., 2018). Arain and Sial (2022) reported highly significant, positive correlation between fruit yield per plant, number of fruits per plant, dry weight, and single fruit weight and recommended that these traits for indirect selection for the improvement of yield in the chile pepper (*C. annuum* L.). A strong, positive correlation between total yield and yield components (red yield, green yield, and 10 pod weight) was also observed in a diverse panel of chile pepper evaluated in New Mexico growing conditions (Lozada et al., 2022a). Continued breeding and selection has resulted in varieties with increased fruit size, greater variation in shape, and improved fruit mass (Hill et al., 2017). In New Mexico, the earliest cultivated chile pepper types were smaller than the current New Mexican pod-types (Bosland, 2015). Continuous breeding and selection in the New Mexico State University (NMSU) Chile Pepper Breeding and Genetics program have led to the development of varieties that have increased fruit size and improved fruit morphology, flavor, and yield, such as 'NuMex Heritage 6-4' (Bosland, 2012), 'NuMex Heritage Big Jim', (Bosland and Coon, 2013) and 'NuMex Sandia Select' (Bosland and Coon, 2014).

In recent years, more high-throughput phenotyping tools have been developed for breeding and selection toward genetic improvement of important traits in chile pepper (Lozada et al., 2022b). Tomato Analyzer (TA) is a morphometric and colorimetric tool that has been developed for the phenotypic characterization of traits related to fruit architecture and morphology (Brewer et al., 2006; Gonzalo et al., 2009; Rodríguez et al., 2010). The TA was initially developed for phenotypic characterization of tomato fruit samples (Figàs et al., 2015; Nankar et al., 2020b); it has now been extensively used to evaluate the fruit diversity in chile pepper (Nankar et al., 2020a; Nimmakayala et al., 2021; Pereira-Dias et al., 2020) and eggplant (Plazas et al., 2014; Kaushik et al., 2016). TA allows the high-throughput characterization of at least 30 attributes related to fruit morphology from eight categories including basic measurements, fruit shape index, blockiness, homogeneity, proximal fruit end shape, distal fruit end shape, asymmetry, and internal eccentricity, among others (Rodríguez et al., 2010). TA basic measurements consist of seven parameters—perimeter, area, width mid-height, max width, height midwidth, maximum height, and curved height—and are the major determinants of fruit morphology (Table 1). Indirect selection of these parameters can contribute to improved fruit size that can then contribute to the yield potential of chile pepper. Highly significant positive phenotypic and genetic correlations was previously observed between yield per plant and fruit diversity traits (fruit length and fruit width) in a tomato diversity panel, a close relative of chile pepper, and can be used as main criteria for yield improvement (de Souza et al., 2012).

New Mexico is one of the largest producers of chile pepper in the United States, with 51,000 tons of production in 2021 from an area of 8500 acres with average productivity of 6 tons/acres (U.S. Department of Agriculture National Agriculture Statistics Service [USDA-NASS], 2021). The average productivity decreased by 25% as the area planted to chile pepper production remained the same. This resulted in a reduction in economic activity of almost 10% in 2020 compared with the previous year, from $50.1 million to $44.9 million. Ninety-one percent of the 2021 chile crop was sold for processing, with 8.9% of the crop sold as fresh market (USDA-NASS, 2021). This significant decline in the total production of chile pepper in the state indicates a strong need to improve productivity by evaluating different traits related to yield and yield potential. Fruit morphology is one of the main drivers of yield in chile pepper; therefore, understanding phenotypic diversity using novel phenomics tools would be a key to genetic improvement. In the current study, a high-throughput image analyzer was used to collect data on a set of fruit attributes including fruit perimeter, shape indices, size, and asymmetry in a collection of diverse chile pepper lines evaluated under New Mexico growing conditions.

The objectives of this study were to 1) measure fruit-related traits in New Mexican chile pepper using TA, 2) assess the correlation between fruit traits, and 3) identify genotypes that could be used as potential parents to improve yield and yield potential in a chile pepper breeding program. Information from this study will be relevant for future genomics-assisted breeding for the genetic improvement of fruit morphology, architecture, and yield in *Capsicum*.

Materials and Methods

Plant material. A *Capsicum* diversity panel (CDP) consisting of 105 chile pepper genotypes was used in the current study. The CDP was represented by cultivars (62 genotypes; 59%), breeding lines (22; 21%), wild (20; 19%), and a landrace. Entries of the CDP belonged to round, conical, elongated, and bell fruit shapes. Seeds were initially sown at the Fabián García Science Center, Las Cruces, NM (32°16′46.7″N, 106°46′24.7″W), under standard greenhouse conditions for chile pepper (Sharma et al., 2017). Seedlings with eight to 10 true leaves were transplanted into the field in an augmented block design at the Leyendecker Plant Science Research Center, Las Cruces, NM (32°11′58.1″N 106°44′30.5″W) for the 2021 growing season. Plants were transplanted ~25 cm (10 inches) apart in 4.5 m (15 feet) plots with 1 m (~3 feet) between plots to maintain at least 15 plants per plot and per genotype. The check varieties used were 'Charger' (New Mexican pod type) and 'Centella' (jalapeño type). Standard cultural and management practices for growing chile pepper in Southern New Mexico were used (Bosland and Walker, 2004). Transplanting was conducted in April and mature pepper fruits were harvested from September to November.

The CDP was planted in an augmented design consisting of unreplicated test genotypes and replicated checks per block. Test genotypes and check treatments are considered as random and fixed effects, respectively (Federer, 1961). In augmented types, test genotypes are not replicated because of the large numbers of entries and the availability of seeds for each genotype; however, check treatments are replicated in each block to be used as a reference to calculate error and blocking effects (Federer et al., 2001). Random effects for new genotypes were used to calculate various sources of variation. Test genotypes and checks were randomly distributed in each block. Augmented design can accommodate unequal number of entries in each block making it a flexible experimental design to evaluate large number of entries in unreplicated scheme, saving time and resources without comprising the critical differences between the tested treatments. The CDP consisted of seven blocks, with Blocks 2, 3, and 5 comprising 15 genotypes each. Blocks 1 and 4 consisted of 16 genotypes each and blocks 6 and 7 consisted of 17 and 11 genotypes, respectively.

Collection of phenotypic data. Fruits at maturity stage (>150 d after transplanting, DAT) were chosen randomly, where two fruits per plant from up to four individual plants per genotype were collected and bulked for processing. The mature chile pepper fruit samples were collected between 158 and 207 DAT. TA-derived scanned images were processed following the protocol described by Rodríguez et al. (2010); main protocol was divided into a series of steps which included selection and preparation of fruit samples, image collection and analysis, manual adjustment of attributes, and data analysis. A total of eight fruits per genotype were collected, washed, cleaned, blot-dried, and cut through the center with a serrated knife. For each genotype, the samples were cut longitudinally. The fruit samples were scanned with the cut side down using an Epson V19® scanner (Epson, Inc., Los Alamitos, CA, USA). Image analysis was conducted using Tomato Analyzer v. 4.0 software. Large fruit samples (e.g., New Mexican, cayenne pod types) were scanned at a lower resolution [(150 dots per inch (dpi)], whereas smaller fruits (e.g., jalapeño, ornamental types) were processed at a higher resolution (400 dpi). Data were recorded for 32 fruit morphometric descriptors from eight trait categories: basic measurements (seven traits), fruit shape index (three traits), blockiness (three traits), homogeneity (three traits), proximal fruit end shape (four traits), distal fruit end shape (three traits), asymmetry (three traits), and internal eccentricity (six traits). The morphometric descriptors were assessed based on the definitions in Table 1 (Ramos et al., 2018). A detailed explanation and characterization for these morphometric descriptors were given by Gonzalo et al. (2009) and Hurtado et al. (2013). The pepper population was further regularly and consistently monitored for maturity in the field. Flowering time represented the number of days when the flowers start to develop from the day of transplanting (Lozada et al., 2022a). Flowering time was classified as early (<46 d to flowering), medium (46–50 d), and late (>50 d).

Statistical analysis. Data recorded from the processed images were analyzed using R and XLSTAT software. Analysis of variance (ANOVA) for an augmented block design determined statistical differences among means for morphometric traits using the augmented randomized complete block design function in the 'augmentedRCBD' package (Aravind et al., 2021) in R 4.1.2, as described by Federer (1961), where the replicated checks were considered as fixed and the unreplicated genotypes as random effects to estimate the reliable residual variance (mean squared error) and block effect adjustments. Mean squares (error variance) of unreplicated genotypes were estimated using replicated controls (checks) according to the Delta method and subsequently the genetic parameters were estimated. Overall, usefulness of

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Table 1. Morphometric fruit descriptors for the *Capsicum* diversity panel collected using the Tomato Analyzer software. Definitions adapted from Ramos et al. (2018).

Attribute	Abbreviation	Definition
Basic measurement		
Perimeter (mm)	P	
Area (mm)	A	
Width mid-height (mm^2)	WMH	Width measured at 1/2 of the fruit's height
Max width (mm)	MW	Maximum horizontal distance of the fruit
Height mid-width (mm)	HMW	Height measured at 1/2 of the fruit's width
Maximum height (mm)	MH	Maximum vertical distance of the fruit
Curved height (mm)	CH	Height measured along a curved line through the fruit
Fruit shape index		
Fruit shape index external I	FSIE1	Ratio of MH to MW
Fruit shape index external II	FSIE2	Ratio of HMW to width midheight
Curved fruit shape index	CFSI	Ratio of the curved height to the width of the fruit at midcurved height; measured perpendicular to the curved height line
Blockiness		
Proximal fruit blockiness	PFB	The ratio of the width at the upper blockiness position (U) to WMH
Distal fruit blockiness	DFB	The ratio of the width at the lower blockiness position (L) to WMH
Fruit shape triangle	FST	The ratio of the width at U to the width at L
Fruit shape triangle	PFB	The ratio of the width at U to the width at L
Homogeneity		
Circular	C	The ratio of the error resulting from a best-fit circle to the area of the fruit
Rectangular	R	The ratio of the area of the rectangle bounding the fruit to the area of the rectangle bounded by the fruit
Ellipsoid	ED	The ratio of the error resulting from a best-fit ellipse to the area of the fruit
Proximal fruit end shape		
Shoulder height	SH	The ratio of the average height of the shoulder points above the proximal endpoint (P) to maximum height (H)
Proximal angle micro (degrees)	PAMi	The angle between best-fit lines drawn through the fruit perimeter on either side of the proximal endpoint
Proximal angle macro (degrees)	PAMa	The angle between best-fit lines drawn through the fruit perimeter on either side of the proximal endpoint
Proximal indentation area	PIA	The ratio of the area of the proximal indentation to the total area of the fruit, multiplied by 10
Distal fruit end shape		
Distal angle micro (degrees)	DAMi	The angle between best-fit lines drawn through the fruit perimeter on either side of the distal endpoint
Distal angle macro (degrees)	DAMa	The angle between best-fit lines drawn through the fruit perimeter on either side of the distal endpoint
Distal indentation area	DEA	The ratio of the area of the distal indentation to the total area of the fruit, multiplied by 10
Asymmetry		
Ovoid	OV	Ovoid is calculated from the maximum width, the height at which the maximum width occurs, the average width above that height, and the average width below that height
H. Asymmetry.ob	HAOV	If the area of the fruit is greater below midheight than above it, H. Asymmetry.ob is the average distance between a horizontal line through the fruit at midheight and the midpoint of the fruit's height at each width
V. Asymmetry	VA	The average distance between a vertical line through the fruit at midwidth and the midpoint of the fruit's width at each height
Internal eccentricity		
Eccentricity	EY	The ratio of the height of the internal ellipse to the maximum height
Fruit shape index internal	FSII	The ratio of the internal ellipse's height to its width
Eccentricity area index	EAI	The ratio of the area of the fruit outside the ellipse to the total area of the fruit
Distal eccentricity	DC	The ratio of the height of the internal ellipse to the distance between the top of the ellipse and the bottom of the fruit
Proximal eccentricity	PC	The ratio of the height of the internal ellipse to the distance between the bottom of the ellipse and the top of the fruit
Width widest pos	WWP	

the Delta method has been reported to be feasible to estimate the genetic parameters based on their positive association with trait heritability (You et al., 2016).

Means, standard deviations, minimum/maximum values, and coefficients of variation were used for descriptive analysis of traits. The genotypic (σ_g^2), phenotypic (σ_p^2), and environmental (σ_e^2) variances were obtained from the ANOVA table according to the expected value of the mean square described by Federer as follows: $\sigma_g^2 = \sigma_p^2 - \sigma_e^2$. Phenotypic and genotypic coefficients of variation (PCV and GCV, respectively) were estimated according to Burton (1951), as follows: $GCV = \frac{\sigma_g^2}{\sqrt{\bar{x}}} \times 100$ and $PCV = \frac{\sigma_p^2}{\sqrt{\bar{x}}} \times 100$ where $\bar{x}$ = mean. Broad-sense heritability (H^2) was calculated according to the method of Lush (1940), as $H^2 = \frac{\sigma_g^2}{\sigma_p^2}$, and the adjusted means as suggested by Darlington and Hayes (2017). Genetic advance (GA) and genetic advance percent mean (GAM) were estimated according to Johnson et al. (1955), as follows: $GA = k \times \sigma_g \times \frac{H^2}{100}$, where k = standardize selection differential and $GAM = \frac{\mathrm{GA}}{\bar{x}} \times 100$, where $\bar{x}$ = mean. The scattergrams were built for TA descriptors using the XLSTAT software version 15.

The Spearman rank correlation coefficient (r_s) was calculated as follows: $R = 1 - \frac{6\Sigma d^2}{n \times (n^2 - n)}$. Contribution of morphometric TA descriptors to the fruit diversity of the CDP were computed using the correlation coefficient heat map and correlation network analyses, which were produced with the 'ggcorplot' and 'qgraph' functions in R, respectively. A total of 32 TA descriptors were used to establish clusters based on fruit shape for the CDP using Ward's coefficient by agglomerative hierarchical clustering in R. The 'circlize' package in the R program was used for circular implementation of the dendrogram. Multivariate principal component analysis (PCA) was performed as described by Jolliffe (2002) to understand and exploit the relationship among the morphometric traits and their contribution to the diversity in fruit trait data. Different PCA parameters such as eigenvalues and percent variance accounted for by different components were computed using 'ggplot2', 'missMDA', 'FactoMineR', and 'Factoextra' packages in R.

Results

ANOVA for the TA descriptors showed significant differences ($P \leq 0.01$) between the blocks, genotypes, checks, and genotype vs. checks for most of the morphometric TA

Table 2. Summary statistics and genetic variability components of Tomato Analyzer descriptors.

Trait	Attribute[i]	Mean n = 8	Min	Max	Skewness	PV	GV	GCV %	PCV %	hBS %	GA	GAM %	CV %
Color	Color	1.8	0.9	4.5	1.81**	1.0			55.3				
Basic measurements													
Perimeter	P	17.4	0.0	48.4	0.73**	373.7	365.7	109.7	110.85	97.9	39.03	223.83	15.51
Area	A	12.9	0.0	43.5	0.9**	395.7	385.9	152.1	154.02	97.5	40.02	309.87	22.12
Width midheight	WMH	1.7	0.3	6.0	1.05**	2.0	1.9	80.5	82.31	95.7	2.8	162.45	16.27
Maximum height	MH	6.4	0.0	6.6	0.6**	52.0	50.8	110.8	101.92	97.6	5.06	201.62	19.41
Max width	MW	2.5	0.0	15.1	0.66**	6.5	6.3	99.8	110.65	95.9	12.93	220.73	19.13
Height midwidth	HMW	5.9	0.0	17.1	0.74**	42.0	40.6	108.8	112.17	96.7	14.52	225.91	16.58
Curved height	CH	6.9	0.0	18.1	0.59*	54.3	52.5	105.6	107.43	96.7	14.7	214.27	19.03
Fruit shape index													
Fruit shape index external I	FSIE1	2.4	0.4	4.7	–0.14 NS	1.9	1.7	54.3	57.05	90.5	2.58	106.52	17.36
Fruit shape index external II	FSIE2	3.4	0.0	7.4	0.23 NS	7.1	6.6	75.8	79.01	92.0	5.06	149.9	22.71
Curved fruit shape index	CFSI	4.0	0.0	9.4	0.26 NS	9.8	9.0	75.5	78.8	91.9	5.92	149.4	23.01
Blockiness													
Proximal Fruit Blockiness	PFB	0.9	0.3	1.7	0.18 NS	0.2	0.2	44.6	46.96	90.2	0.82	87.38	14.37
Distal Fruit Blockiness	DFB	0.7	0.1	1.8	1**	0.1	0.1	47.0	53.68	76.7	0.56	84.98	25.34
Fruit Shape Triangle	FST	1.6	0.9	3.9	1.45**	0.6	0.4	36.8	47.34	60.3	0.96	58.93	29.56
Homogeneity													
Ellipsoid	ED	0.1	0.0	0.4	0.23 NS	0.0	0.0	59.0	61.53	91.8	0.12	116.56	17.27
Circular	C	0.3	0.3	0.6	–0.32 NS	0.0	0.0	59.5	60.32	97.5	0.33	121.26	9.4
Rectangular	R	0.4	0.0	0.2	0.13 NS	0.0	0.0	17.8	19.46	83.6	0.14	33.56	7.87
Proximal fruit end shape													
Proximal angle micro	PAMi	127.3	0.0	0.5	–0.39 NS	1541.6	683.0	20.5	40.61	44.3	35.89	28.19	30.67
Proximal angle macro	PAMa	98.2	71.9	201.1	0.15 NS	2254.5	1623.4	41.0	30.84	72.0	70.53	71.82	22.97
Shoulder height	SH	0.3	17.5	226.2	0.26 NS	0.0	0.0	26.8	48.35	43.5	0.11	36.47	25.69
Proximal indentation area	PIA	0.9	0.0	4.6	1.73**	2.5	2.0	159.9	176.88	81.8	2.66	298.34	89.99
Distal fruit end shape													
Distal angle micro	DAMi	96.9	17.6	198.3	0.42 NS	2992.9	2391.8	50.5	56.46	79.9	90.2	93.09	26.28
Distal angle macro	DAMa	69.6	0.0	151.6	0.29 NS	2770.8	2318.2	69.2	75.62	83.7	90.85	130.53	31.58
Distal indentation area	DEA	0.1	0.0	0.4	0.95**	0.0	0.0	92.2	110.45	69.7	0.19	158.89	65.86
Asymmetry													
V. Asymmetry	VA	0.2	0.0	1.1	0.34 NS	0.1	0.1	127.5	134.6	89.7	0.56	249.09	42.33
Ovoid	OV	0.3	0.0	0.7	1.32**	0.0	0.0	68.0	73.37	86.0	0.37	130.16	26.72
H. Asymmetry.ob	HAOV	0.5	0.0	2.2	1.16**	0.5	0.4	118.4	125.25	89.4	1.26	230.98	39.36
Internal eccentricity													
Eccentricity	EY	0.7	0.6	0.8	–0.26 NS	0.0	0.0	4.5	6.78	43.7	0.05	6.11	5.09
Fruit shape index internal	FSII	3.4	0.0	7.5	–0.97**	7.3	6.7	76.2	79.67	91.5	5.11	150.43	23.49
Eccentricity area index	EAI	0.5	0.3	0.6	3.21**	0.0	0.0	21.2	22.45	88.9	0.19	41.15	7.42
Distal eccentricity	DC	0.9	0.5	1.3	–0.62**	0.0			14.76				21.77
Proximal eccentricity	PC	0.9	0.6	1.5	0.25 NS	0.0	0.0	7.0	12.56	31.0	0.07	8.04	10.42
Width widest pos	WWP	0.4	0.1	0.6	0.02 NS	0.0	0.0	40.2	46.27	75.4	0.26	71.93	23.25

[i] Each abbreviated attribute is explained in Table 1.

NS, *, ** Not significant or significant at $P < 0.05$ or $P \leq 0.01$, respectively.

H = horizontal; V = vertical.

descriptors, indicating the existence of variability (Supplemental Table 1). Mean squares for different sources of variation mentioned in the ANOVA table were higher compared with the residuals. The coefficient of variation (*CV*) ranged between 5.09 (eccentricity) and 89.99 (proximal indentation area). The maximum range of variation was explained for proximal/distal fruit end shape and asymmetry (Table 2). Morphometric TA descriptors related to basic measurements were the least variable traits. Fruit data showed a unimodal asymmetrical distribution for all fruit diversity traits (Fig. 1A–H). Morphometric TA descriptors including the basic measurements (0.5–1.05), fruit shape index (0.23–0.26), blockiness (0.18–1.5), homogeneity (0.13–0.23), proximal/distal fruit end shape (0.15–1.73), and asymmetry (0.34–1.32) were positively skewed, whereas fruit shape index external I (–0.14), circular (–0.32), and proximal angle micro (–0.39) were negatively skewed. Three out of four descriptors for internal eccentricity—namely, eccentricity, distal eccentricity, and fruit shape index internal, were negatively skewed (–0.26 to –0.96) (Table 2).

Genetic variability, heritability, and genetic advance. Various parameters including genotypic variation (GV), phenotypic variation (PV), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad-sense heritability (H^2), genetic advance (GA), and genetic advance percent mean (GAM) for all morphometric TA descriptors were calculated. The GCV estimates ranged from 4.5 for eccentricity to 159.9 for proximal indentation area. PCV was slightly higher than the GCV for all traits except maximum height (MH) and proximal angle macro (PAMa). The heritability (H^2) estimates ranged from 0.31 (proximal eccentricity) to 0.98 (perimeter). Most of the fruit diversity traits showed high H^2 (>0.70). Among the traits, the basic measurements and fruit shape index descriptors showed the highest H^2 (>0.95), whereas the lowest H^2 values were recorded for proximal eccentricity (0.31) and shoulder height (0.43). The estimated genetic advance ranged from 0.07 (proximal eccentricity) to 90.85 (distal angle macro). Unlike heritability, there was no pattern observed for GA and GAM estimates. Morphometric descriptors for basic measurements showed maximum GAM (162.45–309.87) with the highest heritability estimates (0.95–0.96). Most of the proximal fruit end shape descriptors had low GAM (28.19%–71.82%), except the proximal indentation area, which had a high value (298.84). The descriptors for proximal fruit end shape also showed lower heritability (0.43–0.72), which was expected due to lower GA and GAM.

Hierarchical cluster analysis. Initial visual assessment categorized the CDP into four different clusters (Fig. 2): conical (59%), elongated (24%), bell (7%), and round (10%). Hierarchical cluster analysis (HCA) based on the 32 morphometric TA descriptors revealed eight clusters. Grouping of genotypes based on fruit shape was observed in most clusters; however, some of the groups displayed variability, where genotypes with different fruit shapes were grouped in the same cluster. Only Clusters 2 (red), 3 (dark violet), and 7 (green) exclusively grouped the genotypes based on fruit shape and consisted of elongated, conical, and round genotypes, respectively (Fig. 3; Supplemental Table 2). All genotypes except '21C573'

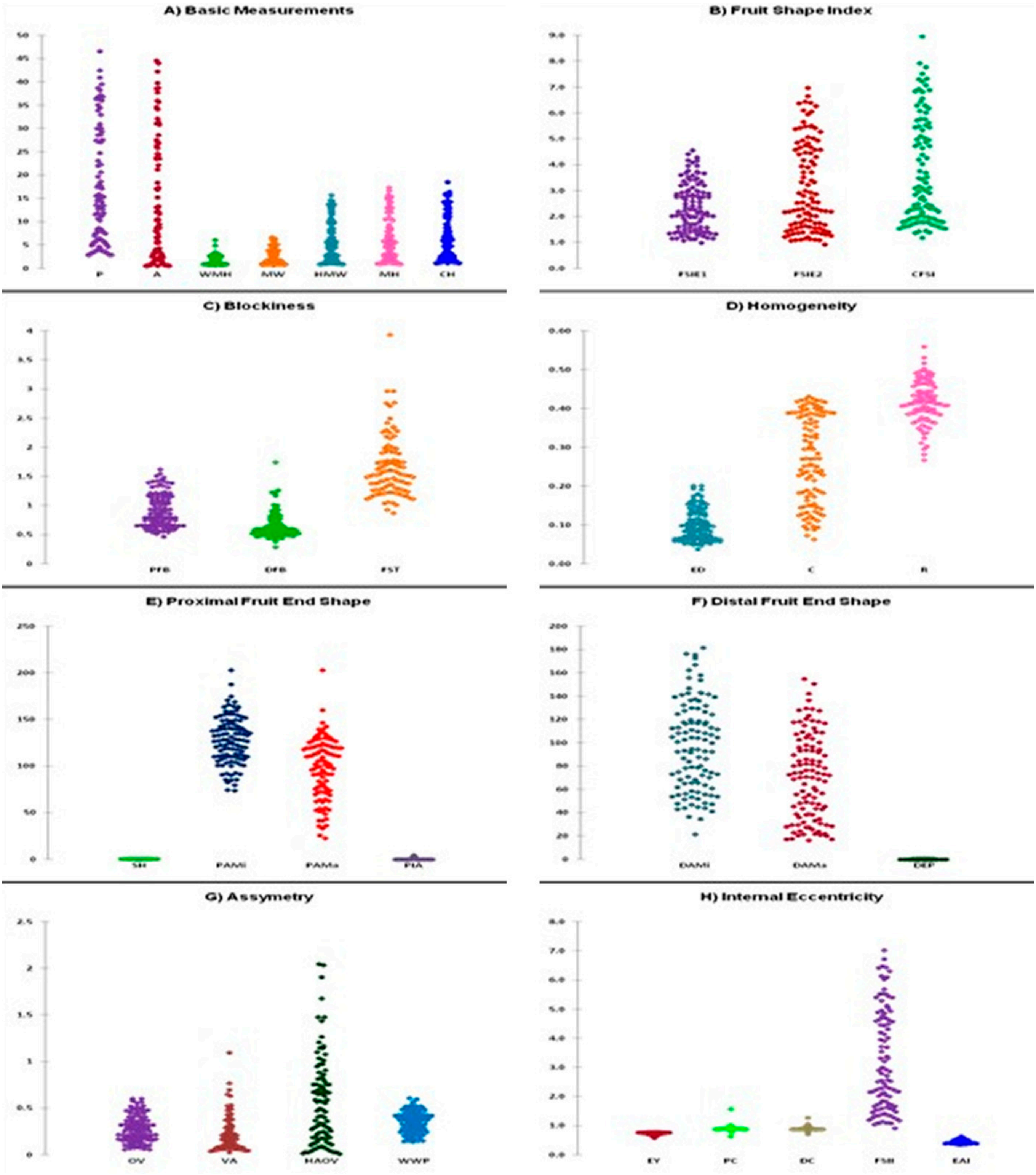

Fig. 1. Scattergrams displaying distribution of different fruit morphometric parameters measured using the Tomato Analyzer. The external fruit features from the longitudinal section were measured by basic measurements: (**A**) fruit size, (**B**) fruit shape index, (**C**) blockiness, (**D**) homogeneity, (**E**) proximal fruit end shape, (**F**) distal fruit end shape, (**G**) asymmetry, and (**H**) internal eccentricity. NM = New Mexican pod type.

('17W18') in Cluster 1 were cultivars from the elongated and conical varietal group with medium to large fruit sizes. Cluster 7 only had a single genotype which belongs to the round varietal group with a small fruit size. Cluster 4 consisted of genotypes from the conical varietal group with the exception of '21C512' ('Tipo Ancho'). Cluster 6 had the majority of bell pepper genotypes (6) with larger fruit size; the cluster, however, was predominantly composed of genotypes with conical fruits (69%). There were 15 genotypes in Cluster 5, seven (46%) of which were elongated, and the remaining genotypes were conical.

Cluster 8 was the most diverse group, with 71% conical and 26% round, and included 17 wild genotypes, 13 breeding lines, and 8 cultivars. HCA was mainly based on morphometric TA descriptors; however, a relationship between maturity group and pod type were further observed in the current

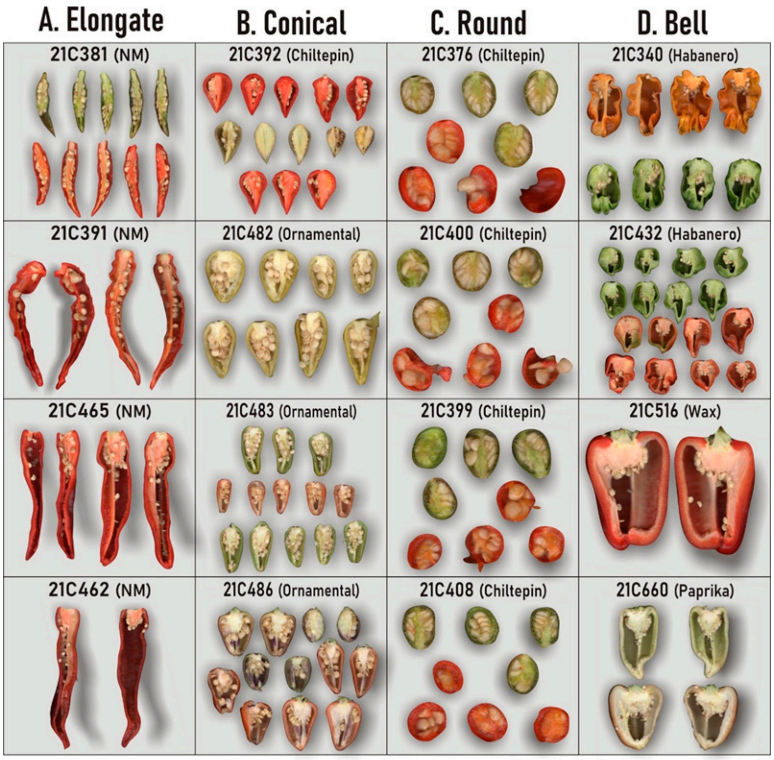

Fig. 2. Representative genotypes for different fruit shapes of the *Capsicum* diversity panel. NM = New Mexican pod type.

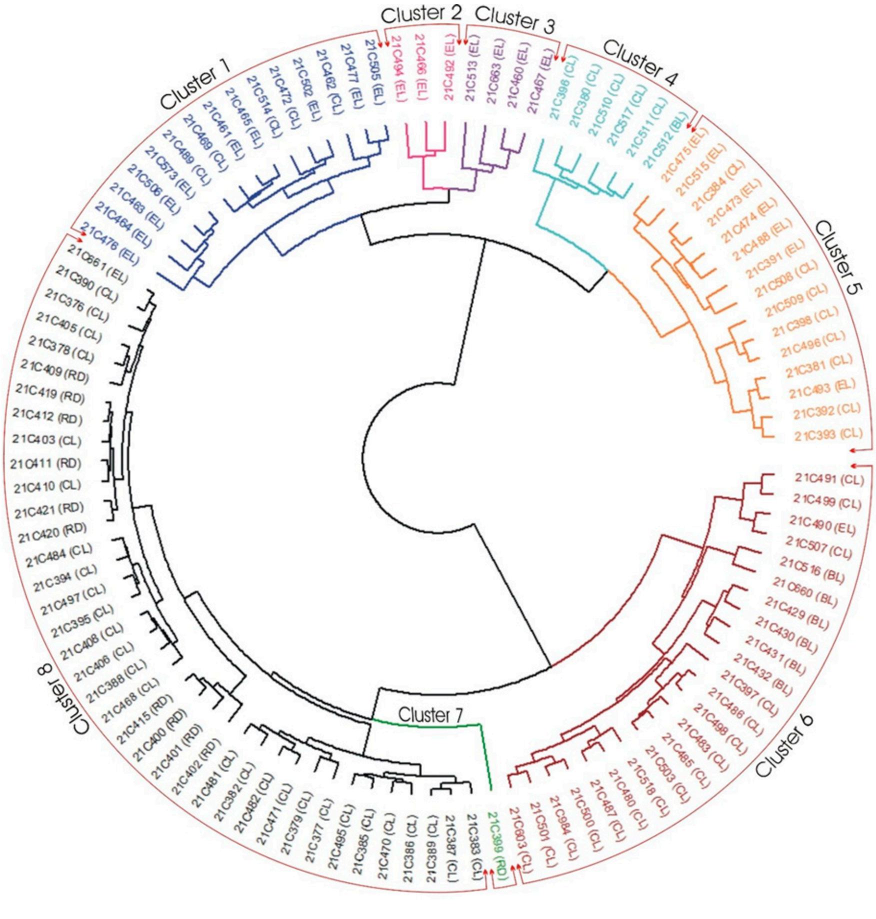

Fig. 3. Hierarchical cluster analysis–derived dendrogram constructed using 32 morphometric Tomato Analyzer (TA) descriptors showing eight clusters. Conical (CL), bell (BL), round (RD), and elongated (EL) group. Cluster 1 (C1; blue), C2 (red), C3 (dark violet), C4 (deep pink), C5 (orange), C6 (brown), C7 (green), and C8 (black).

study (Supplemental Table 2). Clusters 1, 2, and 3 consisted of New Mexican pod type genotypes with medium maturity. All the chiltepins were found in clusters 5, 7, and 8 with late maturity groups. Clusters 3, 4, and 7 were predominantly genotypes belonging to the medium and late maturity group. Cluster 6 was the most diverse group with ornamental, jalapeño, paprika, serrano, habanero, tabasco, and wax pod type genotypes belonging to medium and late maturity groups.

Principal component analysis. Multivariate PCA was used to identify the contribution of the linear combinations among different morphometric TA descriptors to the phenotypic diversity of the fruit trait data. The first two principal components, PC1 and PC2, explained 52.5% and 10.3% of the variation, respectively (Table 3; Fig. 4). The first four principal components accounted for almost 80% of the variation for different TA descriptors. The variance for PC1 was explained by 25 positively correlated TA descriptors related to basic measurements (seven traits), fruit shape index (three traits), blockiness (three traits), homogeneity (two traits), proximal fruit end shape (two traits), distal fruit end shape (one trait), asymmetry (one trait), and internal eccentricity (four traits).

Descriptors related to basic measurements had a positive contribution toward the variance of PC1 and PC2 (Table 3). None of the other descriptors had shown any positive effects that had contributed to the variance for PC1 and PC2. The basic measurements were considered the most discriminating descriptors that had significantly contributed to and explained the variation for the CDP (Table 3). The genotype by descriptor (G × D) interaction with ellipses in the biplot explained the distribution of genotypes and the traits that had a positive relationship with a particular varietal group. Each ellipse depicted a unique number of genotypes for each varietal group based on fruit shape and was given a particular color (Fig. 5). The ellipses also showed the presence of overlaps between groups based on fruit shape. Genotypes belonging to the elongated group were present in the positive quadrants of the biplot. Majority of the morphometric TA descriptors that had contributed to the positive quadrant of PC1 and PC2 were related to basic measurement, fruit shape index, blockiness, homogeneity, proximal fruit end shape, distal fruit end shape, asymmetry, and internal eccentricity. The genotypes from the conical group were distributed in all four quadrants indicating high genetic diversity for the CDP.

Basic measurements were the major contributors to the variation for the elongated group. The genotypes from the bell group clustered in the second and third quadrants of the biplot, which comprised the positive and negative quadrants of PC2 (Fig. 5). These genotypes were more associated with the proximal fruit end shape, distal fruit end shape, and internal eccentricity. Genotypes associated with the round group were distributed into the third and fourth quadrants of the biplot. None of the TA descriptors had a

Table 3. Principal component analysis of Tomato Analyzer descriptors: PCA descriptor trait contribution, correlation coefficient (R^2), eigenvector, and eigenvalues for principal components 1 (PC1), 2 (PC2), and 3 (PC3).

	Principal components			Correlation coefficient (R^2)			Eigenvector					
Trait	PC1	PC2	PC3	PC1	PC2	PC3	1	2	3	Eigenvalue	Variance (%)	Cumulative variance (%)
Basic measurements												
P	4.80	4.80	0.07	0.91	0.39	0.05	−0.213	−0.180	0.094	17.04	53.24	53.24
A	3.67	8.83	0.66	0.79	0.53	0.14	−0.200	−0.252	0.097	3.24	10.11	63.35
WMH	1.22	14.29	6.25	0.46	0.68	0.43	−0.137	−0.421	0.084	2.95	9.21	72.57
MW	3.45	10.91	0.69	0.77	0.59	0.14	−0.187	−0.291	0.084	2.13	6.66	79.23
HMW	4.79	3.39	0.15	0.90	0.33	0.07	−0.215	−0.161	0.105	1.21	3.78	83.00
MH	4.95	3.09	0.06	0.92	0.32	0.04	−0.217	−0.149	0.093	0.97	3.02	86.02
CH	4.93	3.40	0.05	0.92	0.33	0.04	−0.217	−0.152	0.086	0.76	2.36	88.38
Fruit shape index												
FSIE1	3.50	6.12	0.01	0.77	−0.45	−0.02	−0.192	0.196	0.019	0.70	2.20	90.58
FSIE2	5.02	2.01	0.53	0.93	−0.26	−0.13	−0.212	0.158	0.038	0.58	1.81	92.39
CFSI	5.02	1.79	1.53	0.92	−0.24	−0.21	−0.208	0.194	0.029	0.51	1.59	93.98
Blockiness												
PFB	4.59	0.02	3.08	0.88	−0.03	0.30	−0.209	−0.094	−0.119	0.42	1.31	95.29
DFB	2.97	3.47	6.72	0.71	0.34	−0.45	−0.159	−0.046	0.278	0.27	0.86	96.15
FST	0.14	1.18	10.38	0.15	−0.20	0.55	−0.076	−0.066	−0.514	0.25	0.78	96.93
Homogeneity												
ED	4.75	0.03	1.13	0.90	0.03	−0.18	−0.198	0.080	−0.020	0.21	0.67	97.60
C	4.53	3.67	0.08	0.88	−0.34	−0.05	−0.207	0.190	0.000	0.17	0.54	98.13
R	1.77	6.02	3.35	−0.55	0.44	0.31	0.118	−0.348	0.144	0.15	0.48	98.61
Proximal fruit end shape												
SH	0.68	0.31	5.81	0.34	−0.10	−0.41	−0.082	0.153	0.239	0.14	0.42	99.03
PAMi	0.17	0.16	2.85	−0.17	0.07	0.29	0.035	−0.134	−0.176	0.09	0.27	99.31
PAMa	2.66	2.57	7.39	−0.67	0.29	0.47	0.154	−0.286	−0.163	0.06	0.19	99.50
PIA	3.30	2.34	3.04	0.75	0.28	−0.30	−0.184	−0.049	0.075	0.05	0.15	99.64
Distal fruit end shape												
DAMi	2.80	4.92	1.73	−0.69	0.40	−0.23	0.172	−0.134	0.154	0.04	0.12	99.76
DAMa	3.63	4.05	0.82	−0.79	0.36	−0.16	0.194	−0.136	0.080	0.03	0.08	99.84
DEP	1.78	0.04	1.51	0.55	0.03	−0.21	−0.117	0.107	−0.026	0.02	0.05	99.89
Asymmetry												
OV	2.74	2.05	13.76	0.68	−0.26	0.64	−0.178	−0.099	−0.330	0.01	0.03	99.92
VA	3.96	0.38	1.62	0.82	0.11	−0.22	−0.208	−0.007	0.005	0.01	0.03	99.95
HAOV	3.49	1.85	4.27	0.77	−0.25	0.36	−0.210	−0.032	−0.188	0.01	0.03	99.97
Internal eccentricity												
EY	1.60	1.67	1.85	−0.52	0.23	0.23	0.114	−0.231	0.129	0.00	0.01	99.99
PC	0.04	0.94	1.66	0.08	0.17	−0.22	−0.083	0.081	0.330	0.00	0.00	100.00
DC	1.65	0.93	0.07	0.53	0.17	−0.04	−0.172	−0.087	0.065	0.00	0.00	100.00
FSII	5.02	2.02	0.55	0.92	−0.26	−0.13	−0.212	0.159	0.038	0.00	0.00	100.00
EAI	4.85	0.10	0.02	0.91	−0.06	0.02	−0.206	0.008	−0.025	0.00	0.00	100.00
WWP	1.54	2.69	18.34	−0.51	0.30	−0.74	0.148	0.120	0.367	0.00	0.01	99.99

NS, *, ** Not significant or significant at $P < 0.05$ or $P \leq 0.01$, respectively.

significant association with the round genotypes. However, the round group was more closely associated with the bell group as some of the corresponding genotypes overlapped.

Correlation matrix and correlation network. Correlation matrix showed a positive and significant correlation between different morphometric TA descriptors (Supplemental Table 3). The basic measurements were highly correlated, with values between 0.79 and 0.99. Morphometric TA descriptors for fruit shape index, blockiness, and homogeneity also showed positive correlation. The TA descriptors for proximal fruit end shape and distal fruit end shape were negatively correlated with exception of SH, DAMa, and VA. The relationships among the traits were further explained through a correlation network where only traits with correlation >0.70 were considered for the 32 morphometric traits (Fig. 6). Descriptors related to basic measurements showed the strongest correlation, followed by fruit shape index and internal eccentricity. A TA descriptor, circular (C; homogeneity), showed a strong correlation with fruit shape index and internal eccentricity.

Discussion

One of the major objectives of crop improvement programs is to improve yield and yield potential by targeting yield-contributing traits such as fruit architecture and morphology. Plant breeders can use highly positive correlated conventional fruit attributes such as fruit length and width as indirect selection to enhance yield. Recent advances in phenomics accelerate the collection of fruit attributes for the characterization of diverse chile pepper germplasm. A high-throughput digital tool such as the TA is a novel platform for the fast and accurate measurement of morphometric fruit traits (Ramos et al., 2018). In the current study, a CDP was used to evaluate 32 morphometric TA descriptors for New Mexican chile pepper.

The study confirmed the presence of sufficient genetic diversity for the morphometric TA descriptors for the CDP consisted of 105 genotypes, in agreement with previous studies that had used these traits to explain the diversity in pepper (Moreira et al., 2018) and tomato (Figàs et al., 2015; Nankar et al., 2020b). A higher PCV than GCV for all the traits was observed in the current study, and the minimal difference between the genotypic and phenotypic CV indicated little environmental variance. The morphometric TA descriptors showed a considerable amount of genetic variation confirming the predominance of additive genetic effects. Effective phenotypic selection depends on the contribution of the additive genetic effects for a particular trait (Praksh et al., 2017). Early selection for genetic improvement can be implemented for traits controlled by additive effects (Naegele et al., 2016; Praksh et al., 2017); therefore, these effects can be further exploited for breeding desired fruit morphology traits in chile pepper.

Hierarchical clustering and correlation network were previously used to assess the

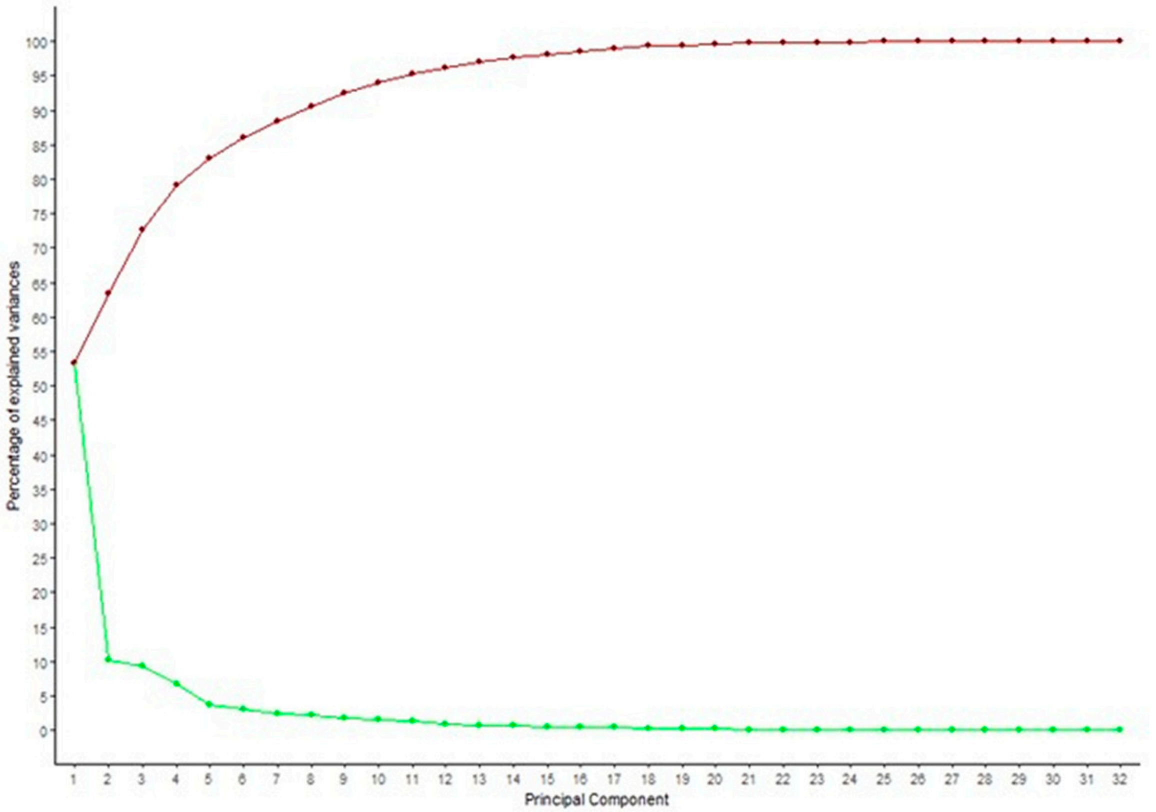

Fig. 4. Variance plot displaying variation explained by each principal component. The red line explains the cumulative variation contributed by 32 principal components and the green line indicates variation contributed by an individual component.

relationship among different germplasm collections based on morphometric TA descriptors in peppers (Nankar et al., 2020a, 2020c; Zhigila et al., 2014) and tomato (Gonzalo et al., 2009; Grozeva et al., 2021). The distinctness of the germplasm used in the study, as reported by the hierarchical clustering and correlation network, explained the overall impact of TA descriptors on fruit shape and size. Cluster analysis divided the CDP into eight groups based on their fruit shape attributes related to morphometric TA descriptors. The genotypes identified through cluster analysis will allow the NMSU Chile breeding program to use these genetic resources to improve yield and yield-contributing traits by employing TA descriptors. For example, the genotypes ‘21C643’ (‘NuMex Sunset’), ‘21C464’ (‘NuMex Sunrise’), ‘21C477’ (‘NuMex Joe E. Parker’), ‘21C489’ (‘NuMex Heritage Big Jim’), and ‘21C490’ (‘NuMex Heritage 6-4’) from Cluster 1 could be used as potential parental lines in a breeding program to improve fruit morphology-related traits.

Breeding lines, wild genotypes, and cultivars were clustered into different groups. Clusters 2, 3, and 7 were the most discriminating groups, exclusively comprising elongated, conical, and round genotypes, respectively. We anticipated distinct clusters based on the fruit diversity traits; however, some of the genotypes overlapped. All groups except Clusters 2, 3, and 7 had genotypes with at least two fruit shapes. Cluster 8 was the most diverse group that included genotypes from wild, breeding lines, and cultivars. The overlapping of the genotypes seems to be associated with different morphometric TA descriptors that contributed to the diversity of fruit shapes. This observation was in contrast with the findings of Figàs et al. (2015) but agreed with those of Grozeva et al. (2021) and Cebolla-Cornejo et al. (2013). Figàs et al. (2015) demonstrated the characterization of the tomato diversity panel into five distinct groups based on conventional and TA descriptors; however, two groups from the panel showed a continuous variation for the TA morphometric traits, resulting in overlapping of the genotypes due to closely related fruit morphology. Despite high phenotypic correlations, no consistent clustering of the same genotypes was observed, in agreement with Cebolla-Cornejo et al. (2013). In another study, cluster analysis did not identify differences between the local varieties and breeding lines and grouped them into the same cluster due to closely related TA descriptors (Grozeva et al., 2021). Grouping of the genotypes based on fruit morphology and not on population type suggested that fruit traits are the major determinants of the different clusters and that population type had little to no contribution to the clustering of the diversity panel. Our results agree with other studies in which a similar approach was employed for the characterization and varietal identification of multiple horticultural crops based on fruit diversity traits in pepper (Nankar et al., 2020a), tomato (Khameneh et al., 2021), and eggplant (Hurtado et al., 2013).

Multivariate PCA was employed to study the divergence of NMSU chile pepper germplasm for morphometric fruit diversity descriptors and how these traits had contributed to the fruit shape and size. The PCA findings further confirmed the presence of genetic variability for all TA descriptors reported by ANOVA. Eigenvectors derived from the principal components indicated that TA descriptors such as basic measurements (perimeter, area, width midheight, maximum width, height midwidth, maximum height, and curved height) and fruit shape index (FSIE1, FSIE2, and CFSI) were the most discriminant parameters that had contributed to the overall fruit shape and size. Other studies (Bozokalfa et al., 2009; Danojevic et al., 2017; Tripodi and Greco 2018; Tsonev et al., 2017) have recorded similar observations, where they explained variability and contribution of the TA descriptors to the characterization of a diversity panel through genotype × descriptor PCA biplot. The contribution of basic measurements and fruit shape index in the divergence of the genotypes was recorded in our study and was consistent with observations made on chile pepper germplasm collections from Turkey (Bozokalfa et al., 2009), Uganda (Nsabiyera et al., 2013), Serbia (Danojevic et al., 2017), Bulgaria (Tsonev et al., 2017), and Brazil (Bianchi et al., 2020).

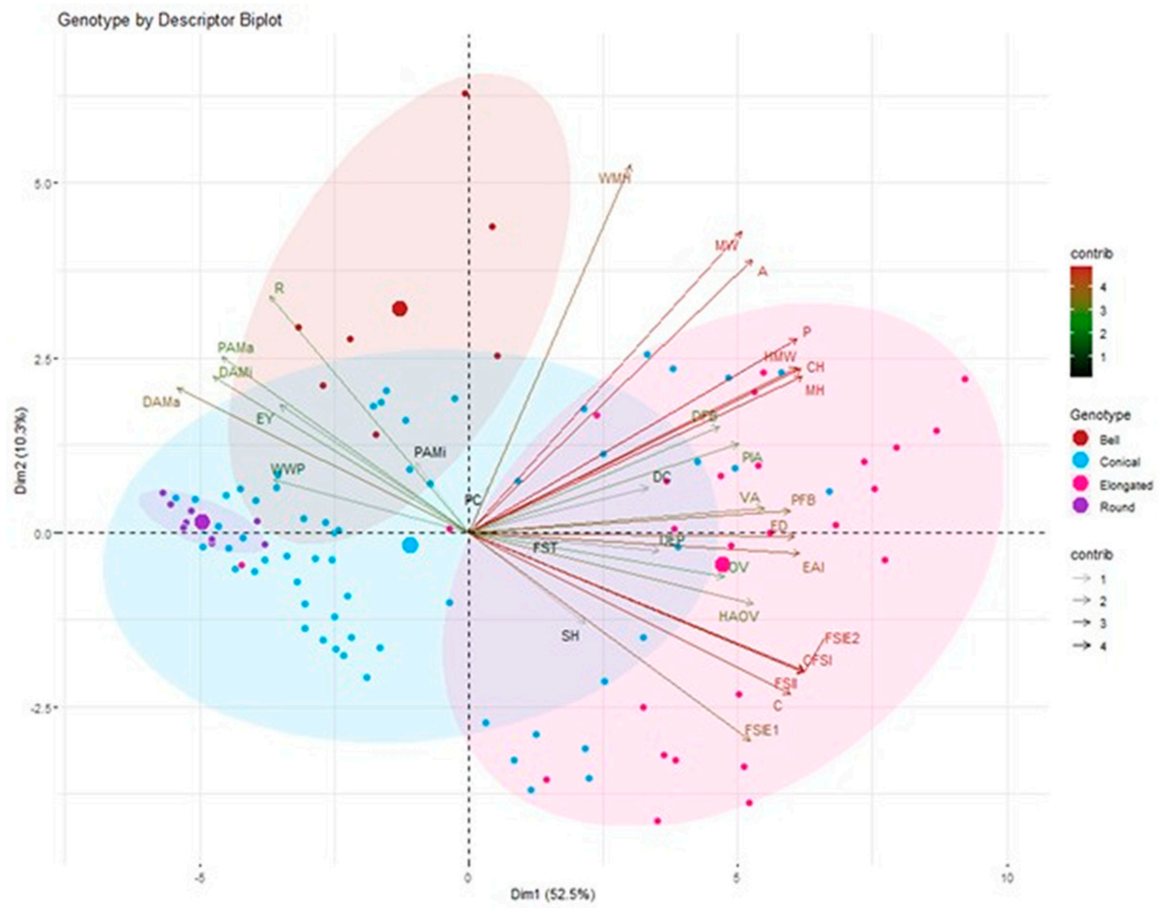

Fig. 5. Genotype by descriptor ellipse biplot displaying genotypes categorized based on the fruit shape revealed by principal component (PC) analysis. Accessions belonging to bell, conical, elongated, round shapes are shown in red, light blue, pink, and purple, respectively. Traits contributing to principal components PC1 and PC2 are also assigned different gradient colors. Color intensities and lengths of the arrows represents the contribution of the traits to the first two principal components. Dark red color and longer arrows indicate a higher contribution of the response variables. Perimeter (P), area (A), width mid-height (WMH), max width (MH), height mid-width (HMW), maximum height (MH), curved height (CH), fruit shape index external I (FSIE I), fruit shape index external II (FSIE II), curved fruit shape index (CFSI), proximal fruit blockiness (PFB), distal fruit blockiness (DFB), fruit shape triangle (FST), circular (C), rectangular (R), ellipsoid (ED), shoulder height (SH), proximal angle micro (PAMi), proximal angle macro (PAMa), proximal indentation area (PIA), distal angle micro (DAMi), distal angle macro (DAMa), distal indentation area (DEA), ovoid (OV), H. asymmetry.ob (HOB), V. asymmetry (VA), eccentricity (EY), fruit shape index internal (FSII), eccentricity area index (EAI), distal eccentricity (DC), proximal eccentricity (PC), and width widest pos (WWP).

In addition to clustering based on morphometric parameters, the CDP showed diversity based on pod type and maturity group. It was observed that all New Mexican genotypes from three different clusters had the same maturity group (medium). Likewise, chiltepins with a late maturity group were found in three clusters. Ornamental, jalapeño, and paprika types were grouped in the same cluster representing the late and medium maturing genotypes. This unique pattern of the clustering based on maturity group indicated some kind of relatedness or correlation of the fruit diversity traits to flowering time. However, a comprehensive study is recommended to further reveal the genetic basis of this observation.

Conclusions

The current study is part of the modernization efforts aimed at accelerating the genetic gain in the NMSU Chile Pepper Breeding and Genetics program through the use of high-throughput phenotyping tools such as the TA. The usefulness of the morphometric TA descriptors, which are difficult to measure through conventional phenotyping, was demonstrated. Genetic variability parameters, ANOVA, and correlation network analyses provided valuable information about the traits of interest, interaction among traits, and overall contribution of TA descriptors to fruit shape and size that could be used to make informed decisions for breeding and selection. Strong, positive relationships among the morphometric TA descriptors were observed. Several cultivars with desired fruit shape and morphology based on cluster analyses were recommended as parental lines for hybridization to improve yield in chile pepper breeding programs. Results using the TA for measuring fruit-related traits will provide a basis for genome-wide association study and genomic selection for yield and yield-contributing traits in New Mexican chile pepper.

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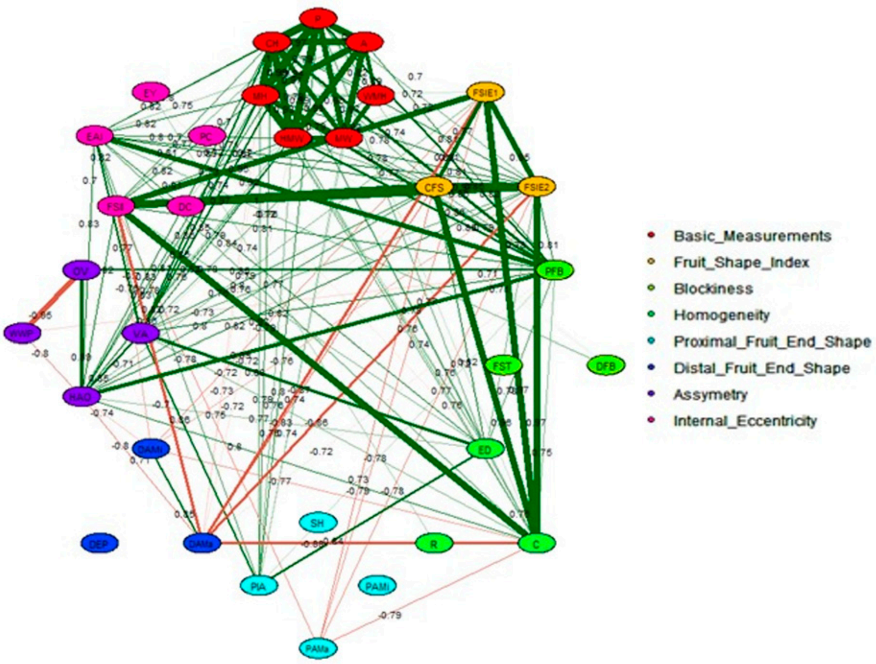

Fig. 6. Correlation network, constructed using 32 morphometric Tomato Analyzer descriptors, illustrates the relationships between eight fruit diversity traits (basic measurements, fruit shape index, blockiness, homogeneity, proximal fruit end shape, distal fruit end shape, asymmetry, internal eccentricity). The width of each band represents the strength of the correlation. Positive correlations are shown by green color bands whereas negative correlations are displayed by red color bands.

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Supplemental Table 1. Analysis of Variance of the Tomato Analyzer descriptors. Refer to Table 1 for the abbreviations.

Source	Treatment (ignoring blocks)	Treatment: check	Treatment: test vs. check	Treatment: test	Block (eliminating treatments)	Residuals
df	107	1	1	105	6	148
			Mean sum of squares			
Color	1.03 NS	0.02 NS	2.59 NS	1.03 NS	0.01 NS	1.18
Basic measurements						
A	499.84**	3799.93**	8135.83**	395.68**	87.63**	9.8
P	436.08**	1810.85**	5616.27**	373.65**	105.89**	7.93
WMH	2.21**	0.06 NS	26.07**	2.01**	0.41**	0.09
MH	62.8**	353.59**	905.9**	52**	11.87**	1.24
MW	7.23**	17.88**	69.03**	6.54**	3.21**	0.27
HMW	51.28**	284.14**	795.24**	41.98**	8.57**	1.39
CH	64.86**	325.47**	914.93**	54.29**	20.74**	1.8
Fruit shape index						
FSIE1	2**	3.37**	10.68**	1.91**	0.95**	0.18
FSIE2	7.63**	27.94**	40.05**	7.12**	6.47**	0.57
CFSI	10.28**	35.8**	39.03**	9.76**	11.35**	0.79
Blockiness						
PFB	0.21**	0.92**	1.02**	0.19**	0.08**	0.02
DFB	0.13**	0.28**	0.32**	0.12**	0.16**	0.03
FST	0.59**	0.15 NS	0.04 NS	0.6**	0.04 NS	0.24
Homogeneity						
R	0.01**	0.05**	0.01**	0.01**	0.0041**	0.0011
C	0.03**	0.07**	0.16**	0.03**	0.0024**	0.00071
ED	0.0044**	0.02**	0.01**	0.0041**	0.0032**	0.00034
Proximal fruit end shape						
PAMa	2336.12**	13,182.01**	57.15 NS	2254.53**	1041.77 NS	631.15
PAMi	1554.12**	193.15 NS	4234.57*	1541.55**	1214.07 NS	858.59
SH	0.02**	0.03*	0.0011 NS	0.02**	0.01 NS	0.01
PIA	2.49**	1.05 NS	4.7**	2.48**	3.99**	0.45
Distal fruit end shape						
DAMa	2936.23**	9807.76**	13433.59**	2770.81**	959.49 NS	452.6
DAMi	3157.09**	10,352.78**	13204.09**	2992.87**	364.61 NS	601.05
DEP	0.02**	0.01 NS	0.0018 NS	0.02**	0.01*	0.01
VA	0.09**	0.16**	0.21**	0.09**	0.09**	0.01
Asymmetry						
OV	0.05**	0.22**	0.24**	0.04**	0.01 NS	0.01
HAOV	0.48**	0.53**	2.13**	0.46**	0.05 NS	0.05
Internal eccentricity						
EY	0.0025**	0.0034 NS	0.00071 NS	0.0025**	0.01**	0.0014
FSII	7.79**	27.02**	39.03**	7.31**	6.07**	0.62
EAI	0.01**	0.04**	0.03**	0.01**	0.01**	0.0012
DC	0.02 NS	0.04 NS	0.03 NS	0.02 NS	0.17**	0.04
PC	0.01*	0.001 NS	0.0016 NS	0.01*	0.01 NS	0.01
WWP	0.03**	0.12**	0.18**	0.03**	0.02*	0.01

NS, *, ** Not significant or significant at $P < 0.05$ or $P \leq 0.01$, respectively.

df = degrees of freedom.

Supplemental Table 2. Maturity group, pod type, fruit shape, and clustering of the *Capsicum* diversity panel.

Genotype	Accession name	Seed sources	Maturity group	Population type (cultivar/breeding line/ land race/wild)	Pod type	Fruit shape	Cluster
21C460	NuMex Big Jim	NMSU	Medium	Cultivar	New Mexican	Elongated	3
21C461	NuMex Sandia	NMSU	Early	Cultivar	New Mexican	Elongated	1
21C462	NuMex Eclipse	NMSU	Medium	Cultivar	New Mexican	Elongated	1
21C463	NuMex Sunset	NMSU	Medium	Cultivar	New Mexican	Elongated	1
21C464	NuMex Sunrise	NMSU	Medium	Cultivar	New Mexican	Elongated	1
21C465	NuMex Conquistador	NMSU	Medium	Cultivar	New Mexican	Elongated	1
21C466	NuMex R Naky	NMSU	Medium	Cultivar	New Mexican	Conical	2
21C467	NM 6–4	NMSU	Medium	Cultivar	New Mexican	Elongated	3
21C468	NuMex Centennial	NMSU	Medium	Cultivar	Ornamental	Conical	8
21C469	NuMex Espanola Improved	NMSU	Medium	Cultivar	New Mexican	Conical	1
21C470	NuMex Twilight	NMSU	Medium	Cultivar	Ornamental	Conical	8
21C471	NuMex Bailey Piquin	NMSU	Medium	Cultivar	Piquin	Conical	8
21C472	NuMex Rio Grande 21	NMSU	Medium	Cultivar	New Mexican	Conical	1
21C473	NuMex SunBurst	NMSU	Early	Cultivar	de arbol	Elongated	5
21C474	NuMex Sunglo	NMSU	Medium	Cultivar	de arbol	Elongated	5
21C475	NuMex Sunflare	NMSU	Medium	Cultivar	de arbol	Elongated	5
21C476	NuMex Sweet	NMSU	Medium	Cultivar	Paprika	Elongated	1
21C477	NuMex Joe E Parker	NMSU	Medium	Cultivar	New Mexican	Elongated	1
21C984	NuMex Primavera	NMSU	Medium	Cultivar	Jalapeno	Conical	6
21C480	NuMex Pinata	NMSU	Medium	Cultivar	Jalapeno	Conical	6
21C663	NuMex Nematador	NMSU	Medium	Cultivar	Cayenne	Elongated	3
21C481	NuMex Christmas	NMSU	Medium	Cultivar	Ornamental	Conical	8
21C482	NuMex Memorial Day	NMSU	Medium	Cultivar	Ornamental	Conical	8
21C483	NuMex St Patrick’s Day	NMSU	Medium	Cultivar	Ornamental	Conical	6
21C484	NuMex Halloween	NMSU	Medium	Cultivar	Ornamental	Conical	8
21C485	NuMex Thanksgiving	NMSU	Medium	Cultivar	Ornamental	Conical	6
21C486	NuMex Valentines Day	NMSU	Medium	Cultivar	Ornamental	Conical	6
21C487	NuMex Jalmundo	NMSU	Medium	Cultivar	Jalapeno	Conical	6
21C488	NuMex Garnet	NMSU	Medium	Cultivar	Paprika	Elongated	5
21C489	NuMex Heritage Big Jim	NMSU	Medium	Cultivar	New Mexican	Conical	1
21C490	NuMex Heritage 6-4	NMSU	Medium	Cultivar	New Mexican	Elongated	6
21C491	NuMex Vaquero	NMSU	Medium	Cultivar	Jalapeno	Conical	6
21C492	NuMex Las Cruces	NMSU	Medium	Cultivar	Cayenne	Elongated	2
21C493	NuMex Cinco de Mayo	NMSU	Early	Cultivar	Ornamental	Elongated	5
21C494	NuMex April Fools Day	NMSU	Early	Cultivar	Ornamental	Conical	2
21C495	NuMex Easter	NMSU	Early	Cultivar	Ornamental	Conical	8
21C496	NuMex Chinense New Year	NMSU	Medium	Cultivar	Ornamental	Conical	5
21C497	NuMex Earth Day	NMSU	Medium	Cultivar	Ornamental	Conical	8
21C498	NuMex Veterans Day	NMSU	Medium	Cultivar	Ornamental	Conical	6
21C499	NuMex Orange Spice	NMSU	Medium	Cultivar	Jalapeno	Conical	6
21C500	NuMex Lemon Spice	NMSU	Medium	Cultivar	Jalapeno	Conical	6
21C501	NuMex Pumpkin Spice	NMSU	Medium	Cultivar	Jalapeno	Conical	6
21C502	NuMex Sandia Select	NMSU	Medium	Cultivar	New Mexican	Elongated	1
21C503	NuMex Summer Solstice	NMSU	Medium	Cultivar	Ornamental	Conical	6
21C505	NuMex Vince Hernandez	NMSU	Medium	Cultivar	Paprika	Elongated	1
21C506	NuMex Sabroso	NMSU	Medium	Cultivar	New Mexican	Elongated	1
21C507	NuMex Cajohns	NMSU	Medium	Cultivar	Serrano	Conical	6
21C508	NuMex Lotalutein	NMSU	Medium	Cultivar	Serrano	Conical	5
21C429	NuMex Suave Red	NMSU	Late	Cultivar	Habanero	Bell	6
21C430	NuMex Suave Orange	NMSU	Late	Cultivar	Habanero	Bell	6
21C431	NuMex Farolitos	NMSU	Late	Cultivar	Habanero	Bell	6
21C432	NuMex Trick or Treat	NMSU	Late	Cultivar	Habanero	Bell	6
21C509	NuMex Nobasco	NMSU	Late	Cultivar	Tabasco	Conical	5
21C510	Floral Gem	P G Smith	Early	Cultivar	Wax	Conical	4
21C511	Chilehuacle Orange	J Salinas (INIA)	Late	Cultivar	Chihuacle	Conical	4
21C512	Tipo Ancho	J Salinas (INIA)	Late	Cultivar	Poblano	Bell	4
21C513	Pasilla Salvaterra	J Salinas (INIA)	Medium	Cultivar	Pasilla	Elongated	3
21C514	Mulato	J Laborde	Medium	Cultivar	Poblano	Conical	1
21C515	CM-334	J Salinas (INIA)	Late	Landrace	Wild	Elongated	5
21C516	Giant Szegedi	Stokes Seeds	Medium	Cultivar	Wax	Bell	6
21C517	Esmerelda Ancho	J Salinas (INIA)	Medium	Cultivar	Poblano	Conical	4
21C518	Navojoa	P G Smith	Late	Cultivar	Piquin	Conical	6
21C660	Feher Ozon	Seed Savers Exchange	Medium	Cultivar	Paprika	Bell	6
21C661	NMCA 10101	Oaxaca, Mexico	Late	Wild	Chiltepin	Elongated	8
21C376	NMSU-18C5	C. Hayano	Medium	Breeding line	Chiltepin	Conical	8
21C377	NMSU-18C7	C. Hayano	Late	Breeding line	Chiltepin	Conical	8
21C378	NMSU-18C8	C. Hayano	Late	Breeding line	Chiltepin	Conical	8
21C379	NMSU-18C9	C. Hayano	Late	Breeding line	Chiltepin	Conical	8
21C380	NMSU-18C10	C. Hayano	Late	Breeding line	Chiltepin	Conical	4
21C381	NMSU-18C11	C. Hayano	Late	Breeding line	Chiltepin	Conical	5
21C382	NMSU-18C12	C. Hayano	Late	Breeding line	Chiltepin	Conical	8

(Continued on next page)

Supplemental Table 2. (*Continued*)

Genotype	Accession name	Seed sources	Maturity group	Population type (cultivar/breeding line/ land race/wild)	Pod type	Fruit shape	Cluster
21C383	NMSU-18C13	C. Hayano	Late	Breeding line	Chiltepin	Conical	8
21C384	NMSU-18C14	C. Hayano	Late	Breeding line	Chiltepin	Conical	5
21C385	NMSU-18C18	C. Hayano	Late	Breeding line	Chiltepin	Conical	8
21C386	NMSU-18C19	C. Hayano	Late	Breeding line	Chiltepin	Conical	8
21C387	NMSU-18C22	C. Hayano	Late	Breeding line	Chiltepin	Conical	8
21C388	Chiltepin	C. Hayano	Late	Wild	Chiltepin	Conical	8
21C389	NMSU-18C24	C. Hayano	Late	Breeding line	Chiltepin	Conical	8
21C390	NMSU-18C25	C. Hayano	Late	Breeding line	Chiltepin	Conical	8
21C391	NMSU-18C26	C. Hayano	Late	Breeding line	Chiltepin	Elongated	5
21C392	Chiltepin	C. Hayano	Late	Wild	Chiltepin	Conical	5
21C393	NMSU-18C28	C. Hayano	Late	Breeding line	Chiltepin	Conical	5
21C394	NMSU-18C29	C. Hayano	Late	Breeding line	Chiltepin	Conical	8
21C395	NMSU-18C30	C. Hayano	Late	Breeding line	Chiltepin	Conical	8
21C396	18C32	C. Hayano	Late	Wild	Chiltepin	Conical	4
21C397	NMSU-18C33	C. Hayano	Late	Breeding line	Chiltepin	Conical	6
21C398	NMSU-18C34	C. Hayano	Late	Breeding line	Chiltepin	Conical	5
21C399	Chiltepin	C. Hayano	Late	Wild	Chiltepin	Round	7
21C400	Chiltepin	C. Hayano	Late	Wild	Chiltepin	Round	8
21C401	Chiltepin	C. Hayano	Late	Wild	Chiltepin	Round	8
21C402	Chiltepin	C. Hayano	Late	Wild	Chiltepin	Round	8
21C403	Chiltepin	C. Hayano	Late	Wild	Chiltepin	Conical	8
21C405	Chiltepin	C. Hayano	Late	Wild	Chiltepin	Conical	8
21C406	Chiltepin	C. Hayano	Late	Wild	Chiltepin	Conical	8
21C408	Chiltepin	C. Hayano	Late	Wild	Chiltepin	Conical	8
21C409	Chiltepin	C. Hayano	Late	Wild	Chiltepin	Round	8
21C410	Chiltepin	C. Hayano	Late	Wild	Chiltepin	Conical	8
21C411	Chiltepin	C. Hayano	Late	Wild	Chiltepin	Round	8
21C412	Chiltepin	C. Hayano	Late	Wild	Chiltepin	Round	8
21C415	Chiltepin	NMSU-18C1139	Late	Wild	Chiltepin	Round	8
21C419	Chiltepin	NMSU-18C1139	Late	Wild	Chiltepin	Round	8
21C420	Chiltepin	C. Hayano	Late	Wild	Chiltepin	Round	8
21C421	Chiltepin	C. Hayano	Late	Wild	Chiltepin	Round	8
21C573	17W18	S Walker	Late	Breeding line	New Mexican	Elongated	1
21C603	NMSU-20C342	J Laborde	Medium	Breeding line	Jalapeno	Conical	6

Supplemental Table 3. Correlation matrix for the Tomato Analyzer descriptors. Please refer to Table 1 for the abbreviations.

	Basic Measurements							Fruit Shape Index			Blockiness			Homogeneity			Proximal Fruit End Shape				Distal Fruit End Shape				Asymmetry			Internal Ecentricity				
	P	A	WMH	MW	HMW	MH	CH	FSIE1	FSIE2	CFSI	PFB	DFB	FST	ED	C	R	SH	PAMi	PAMa	PIA	DAMi	DAMa	DEP	OV	VA	HAOV	WWP	EY	PC	DC	FSII	EAI
P		0.9833	0.8249	0.9556	0.9883	0.9912	0.9954	0.656	0.769	0.7425	0.8502	0.6861	0.2507	0.7731	0.7249	−0.3009	0.3102	−0.1037	−0.5234	0.7996	−0.5639	−0.6779	0.4205	0.6756	0.8516	0.8134	−0.5453	−0.346	0.34	0.7125	0.7668	0.7991
A	<.0001		0.8885	0.9741	0.9764	0.9749	0.9743	0.5832	0.6863	0.6403	0.8171	0.6471	0.231	0.6828	0.6374	−0.1893	0.2786	−0.0838	−0.4245	0.737	−0.5203	−0.6194	0.368	0.6614	0.7869	0.7628	−0.5492	−0.2495	0.3033	0.7009	0.6841	0.7453
WMH	<.0001	<.0001		0.9167	0.795	0.7863	0.7922	0.245	0.3302	0.2767	0.5766	0.4027	0.1932	0.3994	0.2869	0.1014	0.0922	0.0114	−0.1032	0.5525	−0.2728	−0.3477	0.2392	0.4836	0.58	0.547	−0.4111	−0.0337	0.1497	0.5614	0.3274	0.4432
MW	<.0001	<.0001	<.0001		0.9253	0.9282	0.936	0.4483	0.5891	0.5549	0.7878	0.6002	0.2486	0.667	0.5265	−0.1915	0.2202	−0.053	−0.3482	0.7391	−0.4363	−0.5388	0.3736	0.6148	0.7806	0.718	−0.491	−0.2691	0.2632	0.6571	0.5863	0.7029
HMW	<.0001	<.0001	<.0001	<.0001		0.9976	0.9928	0.7194	0.8102	0.7684	0.8522	0.7076	0.2221	0.7407	0.7645	−0.2828	0.3365	−0.1226	−0.5499	0.7583	−0.6064	−0.7195	0.3907	0.6892	0.8244	0.817	−0.5716	−0.2913	0.3643	0.7351	0.8083	0.7998
MH	<.0001	<.0001	<.0001	<.0001	<.0001		0.9962	0.7222	0.8175	0.7819	0.8618	0.7075	0.2383	0.7643	0.7747	−0.3169	0.3367	−0.1164	−0.5618	0.7731	−0.6159	−0.7286	0.41	0.6932	0.8453	0.8287	−0.571	−0.3343	0.3534	0.7316	0.8157	0.817
CH	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.7033	0.8074	0.7816	0.8632	0.6977	0.2553	0.7838	0.7648	−0.3235	0.3226	−0.1114	−0.5578	0.7881	−0.6028	−0.7158	0.4263	0.6911	0.8566	0.8314	−0.5622	−0.3556	0.3428	0.716	0.8055	0.8155
FSIE1	<.0001	<.0001	0.0106	<.0001	<.0001	<.0001	<.0001		0.9516	0.9111	0.681	0.5433	0.1743	0.6252	0.9672	−0.4684	0.4597	−0.2104	−0.7794	0.5022	−0.7876	−0.8687	0.3492	0.6403	0.6468	0.7575	−0.5572	−0.3092	0.3321	0.6048	0.9525	0.7041
FSIE2	<.0001	<.0001	0.0005	<.0001	<.0001	<.0001	<.0001	<.0001		0.9706	0.8088	0.6666	0.1988	0.7608	0.9742	−0.56	0.432	−0.2137	−0.7812	0.6391	−0.7612	−0.8622	0.4397	0.6822	0.7728	0.8249	−0.5645	−0.4056	0.3852	0.6465	0.9999	0.8232
CFSI	<.0001	<.0001	0.0037	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.7773	0.6623	0.2108	0.8169	0.9638	−0.6166	0.4322	−0.1797	−0.7939	0.6792	−0.7208	−0.833	0.4782	0.6236	0.7986	0.8005	−0.4958	−0.5142	0.3222	0.5819	0.9703	0.8275
PFB	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.6628	0.4545	0.7837	0.7546	−0.388	0.2113	−0.0449	−0.4345	0.7305	−0.6402	−0.7162	0.4424	0.8527	0.8167	0.8877	−0.7296	−0.3947	0.2243	0.6092	0.8075	0.9214
DFB	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		−0.187	0.6124	0.6014	−0.1483	0.2643	−0.0916	−0.466	0.6217	−0.3664	−0.455	0.2593	0.3458	0.6562	0.5163	−0.2564	−0.2471	0.3626	0.5608	0.6651	0.7625
FST	0.0089	0.0162	0.0451	0.0095	0.0209	0.013	0.0076	0.0712	0.0391	0.0285	<.0001	0.0527		0.3871	0.2395	−0.2895	−0.1328	0.134	0.0053	0.2711	−0.3263	−0.2791	0.2335	0.6291	0.3147	0.5066	−0.5862	−0.3363	−0.1605	0.1807	0.1975	0.2777
ED	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.7649	−0.6238	0.2881	−0.1205	−0.6204	0.8438	−0.56	−0.6455	0.5686	0.5886	0.8782	0.7472	−0.421	−0.7186	0.3239	0.569	0.7586	0.8362
C	<.0001	<.0001	0.0026	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0126	<.0001		−0.5692	0.4384	−0.1841	−0.7932	0.6165	−0.7706	−0.8755	0.4455	0.6687	0.7582	0.8048	−0.5593	−0.4519	0.3175	0.628	0.9744	0.791
R	0.0016	0.0498	0.2963	0.0471	0.003	0.0008	0.0006	<.0001	<.0001	<.0001	<.0001	0.1256	0.0024	<.0001	<.0001		−0.2265	0.1815	0.5556	−0.4259	0.5057	0.5369	−0.4607	−0.3404	−0.553	−0.4827	0.223	0.7393	−0.1776	−0.2142	−0.5601	−0.4591
SH	0.0011	0.0035	0.3427	0.0221	0.0004	0.0004	0.0007	<.0001	<.0001	<.0001	0.0282	0.0057	0.1707	0.0025	<.0001	0.0184		−0.1247	−0.3866	0.2446	−0.3044	−0.3549	0.1311	0.1062	0.2587	0.1633	−0.0619	−0.0846	0.2571	0.1936	0.4331	0.2463
PAMi	0.2855	0.3886	0.907	0.5861	0.2064	0.2303	0.2511	0.0288	0.0264	0.0628	0.6448	0.3456	0.1667	0.214	0.0565	0.0602	0.1986		0.2348	−0.1178	0.03	0.1499	−0.0436	−0.0311	−0.1407	−0.1543	−0.0146	0.0629	−0.2306	−0.1467	−0.2129	−0.104
PAMa	<.0001	<.0001	0.2876	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.957	<.0001	<.0001	<.0001	<.0001	0.0144		−0.4781	0.5617	0.6621	−0.3784	−0.2986	−0.5556	−0.5031	0.1769	0.4411	−0.3789	−0.4923	−0.7815	−0.5217
PIA	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0045	<.0001	<.0001	<.0001	0.0107	0.2246	<.0001		−0.4367	−0.5276	0.5338	0.4894	0.859	0.706	−0.3304	−0.5938	0.362	0.5667	0.6366	0.7816
DAMi	<.0001	<.0001	0.0043	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0006	<.0001	<.0001	<.0001	0.0014	0.7576	<.0001	<.0001		0.8506	−0.3149	−0.6991	−0.6146	−0.7443	0.6342	0.3675	−0.2523	−0.616	−0.7612	−0.5874
DAMa	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0034	<.0001	<.0001	<.0001	0.0002	0.1215	<.0001	<.0001	<.0001		−0.3811	−0.7092	−0.7012	−0.8014	0.6213	0.4157	−0.2885	−0.6459	−0.8623	−0.7045
DEP	<.0001	<.0001	0.0126	<.0001	<.0001	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	0.0067	0.015	<.0001	<.0001	<.0001	0.1763	0.6545	<.0001	<.0001	0.0009	<.0001		0.2727	0.5535	0.4097	−0.1386	−0.5525	0.1903	0.3158	0.4392	0.4827
OV	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	0.0003	0.2739	0.7494	0.0017	<.0001	<.0001	<.0001	0.0043		0.6128	0.8876	−0.9545	−0.249	0.0625	0.5924	0.681	0.7047
VA	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0009	<.0001	<.0001	<.0001	0.0069	0.1464	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.8479	−0.4603	−0.6494	0.2872	0.6511	0.7709	0.8501
HAOV	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0913	0.1107	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		−0.7973	−0.4658	0.1946	0.6779	0.8237	0.8288
WWP	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0074	<.0001	<.0001	<.0001	0.0204	0.5244	0.8807	0.067	0.0005	<.0001	<.0001	0.1525	<.0001	<.0001	<.0001		0.1329	0.0313	−0.5447	−0.5637	−0.5741
EY	0.0002	0.0092	0.7291	0.0049	0.0022	0.0004	0.0002	0.0011	<.0001	<.0001	<.0001	0.0099	0.0004	<.0001	<.0001	<.0001	0.3839	0.518	<.0001	<.0001	<.0001	<.0001	<.0001	0.0094	<.0001	<.0001	0.1703		−0.1197	−0.2362	−0.4048	−0.5563
PC	0.0003	0.0014	0.122	0.0059	0.0001	0.0002	0.0003	0.0004	<.0001	0.0007	0.0196	0.0001	0.0971	0.0006	0.0008	0.0659	0.0072	0.0164	<.0001	0.0001	0.0084	0.0025	0.0485	0.5206	0.0026	0.0436	0.7478	0.2172		0.438	0.3838	0.2553
DC	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0613	<.0001	<.0001	0.026	0.0447	0.1298	<.0001	<.0001	<.0001	<.0001	0.0009	<.0001	<.0001	<.0001	<.0001	0.0138	<.0001		0.6441	0.5848
FSII	<.0001	<.0001	0.0005	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0405	<.0001	<.0001	<.0001	<.0001	0.0269	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.822
EAI	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0036	<.0001	<.0001	<.0001	0.0102	0.2841	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0077	<.0001	<.0001	