



RESEARCH ARTICLE

Diversity analysis of select tomato accessions using morphometric data

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Abstract

Genetic diversity within germplasm collections is a cornerstone for effective plant breeding, providing a reservoir of traits for crop improvement, particularly in the face of escalating climate change challenges. This study aimed to characterize the morphometric diversity of 57 tomato accessions to identify key traits contributing to variation and to group genotypes with similar profiles for targeted breeding. Six morphometric traits (5 vegetative and 1 reproductive) were evaluated and found to be significantly different. Principal component analysis (PCA) and hierarchical clustering were performed. PCA revealed that the first 3 principal components, with eigenvalues of 2.4, 1.5 and 1.1, collectively accounted for the majority of the total variation. Hierarchical clustering categorized the accessions into 6 distinct morphotype-based clusters. The findings highlight the existence of multidimensional variation within the germplasm, enabling the identification of genetically divergent accessions for future breeding activities. These divergent genotypes can be leveraged as valuable parental lines to develop new cultivars with improved attributes such as high yield and compact growth habit, which are crucial for enhancing tomato breeding efforts and fostering climate-resilient agriculture.

Keywords: clustering; diversity; morphometric traits; PCA; tomato

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops worldwide, cultivated for both fresh consumption and processing industries. According to Food and Agriculture Organization (FAO) statistics, global tomato production reached approximately 186.1 million tonnes in 2022, with China, India and Turkey being the leading producers. Besides economic significance, the tomato also serves as a model plant system for studies on fruit development, ripening, stress physiology and molecular genetics (1, 2).

Nutritionally, tomatoes are a rich source of vitamins (C, A, K, folate), minerals, dietary fiber and phytochemicals, making it vital for human health. Among its bioactive compounds, lycopene has received particular attention due to its strong antioxidant properties, which are also correlated with reduced risk of cardiovascular diseases and certain cancers (3, 4). Other components such as β -carotene, flavonoids and phenolic acids contribute further to its nutraceutical value (5).

Despite its importance, tomato production is constrained by biotic and abiotic stresses. Major biotic stresses include

tomato yellow leaf curl virus (TYLCV) (6), bacterial wilt (7) and fusarium wilt (8). Abiotic stresses such as heat, drought and salinity impair photosynthesis, reduce pollen viability, pollen tube growth and lower fruit set and yield. These challenges are expected to intensify under the conditions of climate change, where global warming of 1.5 °C is anticipated between 2030 and 2052 (9), making stress tolerance a critical breeding objective. Tomato domestication and modern breeding have led to significant improvements in yield, fruit quality and adaptability. However, domestication has also resulted in a narrow genetic base compared to wild relatives (1). Recent advances in molecular breeding and genome editing technologies, such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein (Cas) systems, have opened new avenues to accelerate the development of stress-resilient and nutritionally enhanced varieties (2, 10). In this study, we assembled a set of 57 diverse tomato genotypes to identify key traits underlying variation, with the aim of selecting potential parental lines for future breeding. Such characterization is essential for broadening the genetic base of cultivated tomato and addressing the dual challenges of yield stability and stress tolerance in the face of a changing climate (11).

Materials and Methods

Tomato collections

A collection of 57 tomato genotypes was used in this study. The seeds were procured from a farmer, a native of the Vellore district, Tamil Nadu, India. Since these collections were obtained directly from the farmer, no official passport data (collection site and accession details) were available apart from the local names of the genotypes, which were used for identification and further evaluation. The names of the varieties used in the study, along with their growth habits, are given in Table 1.

Experimental design

A total of 57 entries (test genotypes), along with one standard check variety (Naattu / country tomato), were evaluated under an augmented design. Although augmented designs are commonly implemented with multiple blocks and replicated checks, a single-block layout was adopted in this study due to space and resource limitations. Similar single-block augmented designs have been successfully employed in crop diversity trials, where large numbers of unreplicated test entries are evaluated with replicated checks to provide error estimation (12-14).

Tomato culture conditions

Seeds of all the genotypes were germinated in sterilized Petri-dishes containing moistened filter paper. Three to 5 days after germination, the seeds were transferred to 96-well plates containing sterilized cocopeat and then were placed in an environmentally controlled growth chamber (Perceival plant growth chamber E75L1) with the following parameters: temperature of $25 \pm 2^\circ\text{C}$, 70 % relative humidity and 16/8 hr light/dark photoperiod. A total of 5 to 6 healthy seedlings (10 - 12 cm in height) were transplanted in the main field, 25 days after sowing,

for field evaluation. Morphological evaluation of 57 tomato genotypes was carried out under field conditions at the Agriculture College and Research Institute, Madurai, where the experimental plot had a pH of ~ 7.95 , electrical conductivity of $\sim 0.37 \text{ dS m}^{-1}$, organic carbon content of $\sim 0.24 \%$, available nitrogen content of $\sim 293.1 \text{ kg ha}^{-1}$, available phosphorus content of 11.13 kg ha^{-1} and available potassium of $\sim 225 \text{ kg ha}^{-1}$. The collections were raised in individual rows containing a minimum of 5 plants per genotype. Plants were spaced $60 \times 45 \text{ cm}$ to ensure proper growth and development. All the genotypes were grown under uniform agronomic practices and all morphological data were recorded from three individual plants treated as biological replicates. Standard cultivation practices included irrigation at 3 days intervals (using furrow irrigation to maintain optimal soil moisture) and a basal application of farmyard manure and inorganic fertilizers ($100:50:50 \text{ kg ha}^{-1}$ of nitrogen (N), phosphorus pentoxide (P_2O_5) and potassium oxide (K_2O), respectively). Pest and disease management involved regular monitoring and need-based foliar sprays of emamectin benzoate and imidacloprid during early vegetative stages for fruit borer and whitefly control, supplemented with neem oil (3 %). Fungicides were also applied when necessary to manage foliar diseases. Plants were supported with stakes 30 days after transplanting to provide structural support and ensure uniform growth.

Morphological diversity

Morphological traits were recorded following International Plant Genetic Resources Institute (IPGRI) tomato descriptors (15) and International Union for the Protection of New Varieties of Plants (UPOV) guidelines (16). Three plants per genotype were tagged and used for trait measurements. The traits that were measured and methodology adopted are described below:

Table 1. List of tomato accessions used in the study and their growth habit

S. No.	Varieties	Growth habit	S. No.	Varieties	Growth habit
1	Beef steak	Indeterminate	30	New moni ribbed	Indeterminate
2	Big spoon	Indeterminate	31	Orange stuffer	Determinate
3	Black cherry	Indeterminate	32	Pink berry	Indeterminate
4	Black shade	Determinate	33	Pink grapes	Indeterminate
5	Black vernissage	Indeterminate	34	Pink oyster	Indeterminate
6	Blue green	Indeterminate	35	Pink pear	Indeterminate
7	Blueberry tomato	Determinate	36	Pink ribbed	Indeterminate
8	Brown berry	Indeterminate	37	Pink shade	Indeterminate
9	Canary yellow	Indeterminate	38	Pink stuffer	Indeterminate
10	Cherokee purple	Determinate	39	Pink vine	Indeterminate
11	Chocolate cherry	Indeterminate	40	Red apple	Determinate
12	Costoluto florentine	Indeterminate	41	Red ball	Indeterminate
13	Naattu / country tomato	Indeterminate	42	Red berry	Indeterminate
14	Cream yellow	Indeterminate	43	Red cherry	Indeterminate
15	Genovese tomato	Indeterminate	44	Red grape cluster	Indeterminate
16	Giant apple	Determinate	45	Red oyster	Indeterminate
17	Green zebra	Indeterminate	46	Red plum	Determinate
18	Ildi tomato	Indeterminate	47	Red ruffled	Indeterminate
19	Indigo berry	Indeterminate	48	Red stuffer	Indeterminate
20	Indigo rose	Indeterminate	49	Red vine	Indeterminate
21	Jamun tomato	Indeterminate	50	Ribbed grapes tomato	Indeterminate
22	Kumkum kesari tomato	Determinate	51	Roma orange tomato	Determinate
23	Large barred boar	Indeterminate	52	Roma tomato	Determinate
24	Large cherry	Indeterminate	53	Spoon tomato	Indeterminate
25	Liberian tomato	Indeterminate	54	Sungold cherry	Indeterminate
26	Little yellow pear	Indeterminate	55	Yellow cherry	Determinate
27	Madanapalle	Indeterminate	56	Yellow pear	Determinate
28	Madippu tomato	Indeterminate	57	Yellow clustered/ doyong	Indeterminate
29	Muppattai tomato	Determinate			

Plant height

Plant height was measured twice (45 days after transplantation and when the first visible fruit set was observed) in centimeters from the soil surface to the apex of the main shoot using a measuring scale.

Stem diameter

Stem diameter was measured 5 cm above the soil surface (thickest portion of the stem) using a calibrated thread manually for each variety.

Internodal distance

The average length of 3 consecutive internodes from the middle portion of the main stem was measured using a measuring scale in cm.

Leaf length

The length of the terminal leaflet (20 days after transplantation) from the petiole to the tip was measured in cm using a measuring scale.

Leaf breadth

Leaf breadth was also recorded in the same leaf at the widest portion of the terminal leaflet using a measuring scale in cm.

Number of flowers per inflorescence

A count of the number of flowers per inflorescence was made to identify if genotypes under study differed significantly.

Statistical analysis

Statistical analyses of the biometric data were performed using the software JMP Pro 16.2.0 (17). For studying the prevailing diversity among the 57 genotypes, mean values for the traits viz., plant height (cm), stem diameter (cm), internodal distance (cm), leaf length (cm), leaf breadth (cm) and number of flowers per inflorescence (count) were subjected to principal components analysis (PCA) and hierarchical clustering (Ward's method). A loading plot, a scoring plot and a dendrogram were generated to reveal contributions of different morphometric traits to the morphological diversity as well as grouping of individuals, respectively.

Results

Morphological observations

The mean values of the morphometric observations, including plant height, stem diameter, internodal distance, leaf length, leaf breadth and number of flowers per inflorescence, are presented in Table 2.

Principal components analysis

PCA is a dimensionality reduction technique that enables reducing the number of complex related traits into fewer unrelated ones that contribute to the major variance in the existing dataset. PCA was conducted on the morphometric traits to identify the primary sources of variation and to visualize the relationships among the tomato accessions and the measured variables. PCA of 6 quantitative traits (5 vegetative and 1 reproductive) generated 6 components, of which the first 3 had eigenvalues greater than 1 (2.4, 1.5 and 1.1) (eigen value table not shown), together accounting for most of the variation. The loading matrix (Table 3) and the scree plot (Fig. 1) confirmed that 3 components optimally represent the variation in the data structure. PC1 was strongly associated with

leaf length (0.7365), leaf breadth (0.6956), plant height (0.7064) and stem diameter (0.6964), reflecting overall plant size and vegetative vigor. PC2 showed contrasting loadings, differentiating genotypes with taller stems and longer internodes but smaller leaves (high PC2) from those with broader, longer leaves and shorter stems (low PC2). PC3 was dominated by flowers per inflorescence (0.9342), representing floral traits largely independent of vegetative growth.

The PCA biplot (Fig. 2) confirmed these patterns, with leaf traits clustering along PC1, stem and height traits influencing both PC1 and PC2 and flower traits contributing mainly to PC3. These results indicate that vegetative traits are the primary drivers of diversity among tomato genotypes, while floral traits provide an additional, independent axis of variation. Such multidimensional variation can inform selection and breeding strategies aimed at capturing both vegetative and reproductive diversity.

These findings suggest that vegetative traits, particularly plant height, stem diameter and leaf dimensions, are major contributors to variation among genotypes, making them useful for differentiating tomato germplasm. Floral traits, represented by PC3, provide an additional axis of diversity that is largely independent of vegetative growth. Together, these components capture the multidimensional variation necessary for the selection and breeding of diverse genotypes.

Cluster analysis

Ward's method of clustering uses 'minimization of variance' within clusters of individuals so that a homogenous group is obtained. In the dendrogram obtained (Fig. 3), the vertical lines represent the degree of dissimilarity between the clusters or individual entities. Based on the table of mean values (Table 4) for each cluster, we can precisely characterize the unique morphometric profile of each group of tomato accessions. This table serves as a vital complement to the visual analysis from the PCA and dendrogram, providing quantitative evidence for the cluster distinctions. The details of clusters can be summarized as follows:

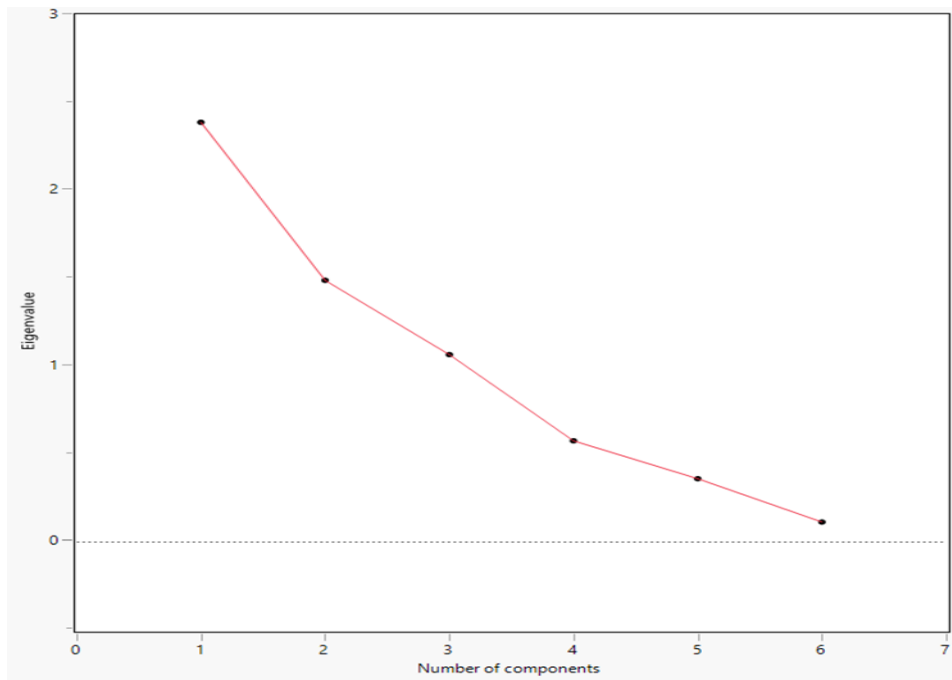
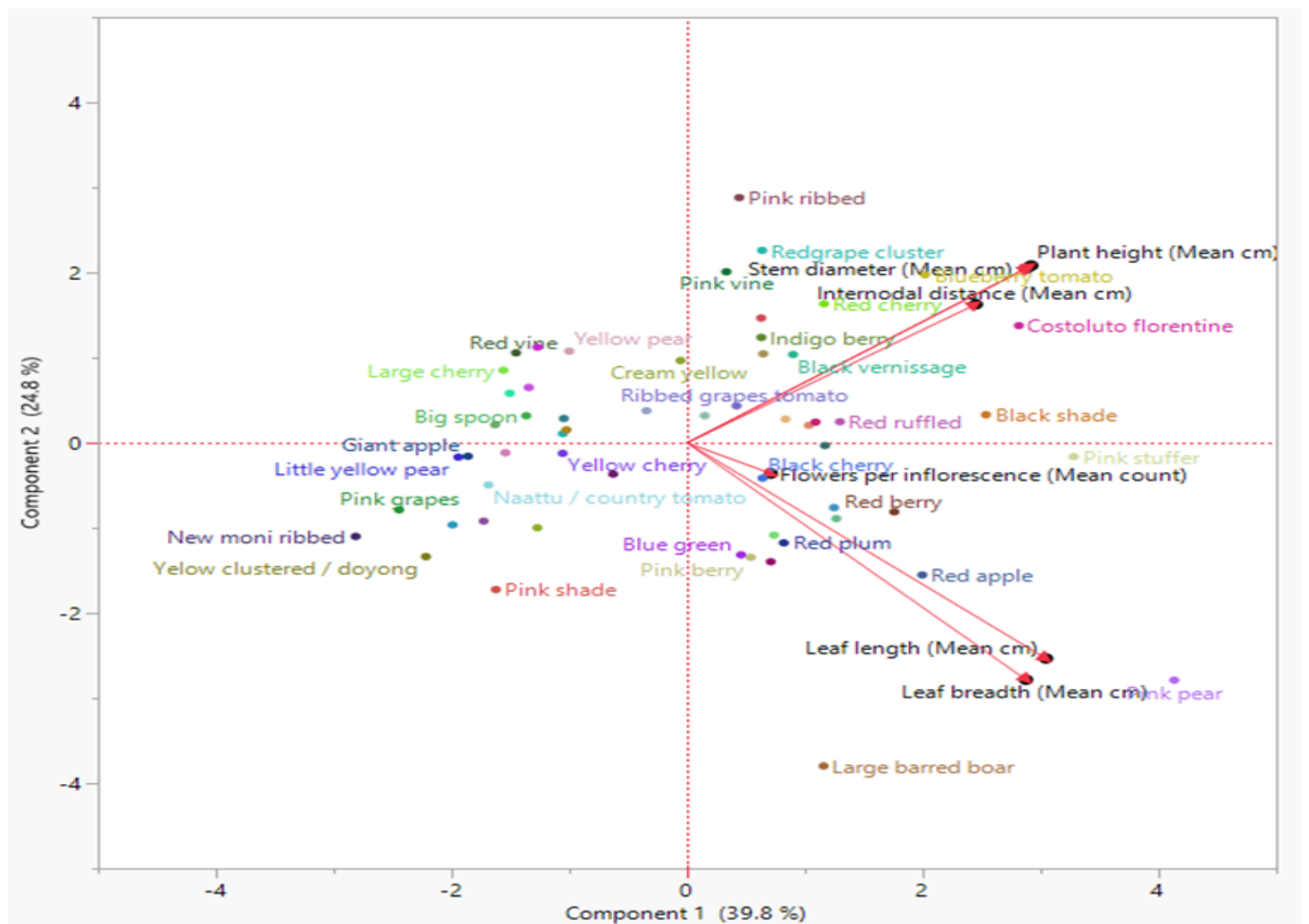
- Cluster 1 (n=15): This cluster represents the accessions with a high degree of vegetative vigor. Its members are characterized by above-average plant height (86.44 cm) and internodal distance (7.85 cm), as well as a large stem diameter (3.06 cm). The reproductive trait, flowers per inflorescence (4.68), is near the overall average. This group can be categorized as a robust, tall-growing morphotype.
- Cluster 2 (n=5): This is a small, but distinct, cluster defined by its high values for both reproductive and vegetative traits. It exhibits the highest mean plant height (94.73 cm) and has a high flowers per inflorescence count (5.53), suggesting a highly productive and vigorous growth habit. Its leaf breadth (3.25 cm) is also significantly higher than the overall average.
- Cluster 3 (n=4): This is the most unique and differentiated cluster in the dataset. Its most striking feature is the exceptionally high mean for flowers per inflorescence (12.00), which is more than double the mean of any other cluster. Despite this high reproductive capacity, the plants are relatively short (77.16 cm) and have a below-average stem diameter. This group likely contains highly productive, perhaps determinant, accessions.
- Cluster 4 (n=8): This cluster is characterized by its large leaf dimensions, showing the highest mean values for both leaf breadth (3.87 cm) and leaf length (6.80 cm). The plants,

Table 2. Biometrical data obtained from 57 tomato accessions

Variety	Mean values					
	Flowers per inflorescence (count)	Internodal distance (cm)	Leaf breadth (cm)	Leaf length (cm)	Plant height (cm)	Stem diameter (cm)
Beef steak	6.33	5.40	2.20	4.30	109.33	2.93
Big spoon	6.00	4.67	2.03	3.77	70.33	2.40
Black cherry	10.00	5.00	3.23	4.73	88.33	2.60
Black shade	6.00	7.13	3.30	6.23	100.67	3.33
Black vernissage	4.33	9.00	2.53	4.77	90.00	2.60
Blue green	6.00	5.13	3.20	6.13	76.67	2.33
Blueberry tomato	6.33	8.30	2.50	4.73	117.67	3.13
Brown berry	7.33	4.23	2.17	4.07	79.33	2.30
Canary yellow	4.67	5.90	1.67	3.53	67.33	2.70
Cherokee purple	4.67	4.60	2.33	5.23	48.33	2.40
Chocolate cherry	4.00	4.80	2.40	4.30	53.67	1.80
Costoluto florentine	3.33	9.50	2.90	6.07	87.00	3.93
Cream yellow	6.00	7.77	1.93	4.57	59.00	3.13
Genovese tomato	9.67	6.57	3.43	5.77	77.33	2.67
Giant apple	6.00	3.20	2.30	3.40	60.00	2.50
Green zebra	3.67	5.80	2.40	4.57	45.00	2.00
Ildi tomato	5.33	4.73	2.93	5.33	94.67	3.10
Indigo berry	7.00	8.33	2.17	4.53	68.00	3.30
Indigo rose	4.33	7.07	1.73	3.87	55.33	2.33
Jamun tomato	5.00	7.20	2.53	4.13	75.33	2.33
Kumkum kesari tomato	5.00	6.93	2.43	4.93	59.33	2.27
Large cherry	4.00	6.90	1.77	3.43	63.33	2.27
Large barred boar	3.67	5.00	4.93	7.60	49.33	2.30
Liberian tomato	11.00	7.03	2.73	5.53	86.33	2.67
Little yellow pear	5.00	3.80	2.20	3.57	60.33	2.30
Madanapalle	3.67	5.23	2.53	3.53	56.67	2.37
Madippu tomato	3.33	4.50	2.43	4.00	75.00	2.47
Muppattai tomato	4.33	4.33	2.13	3.60	73.33	2.20
Naattu / country tomato	4.67	4.00	2.50	3.83	56.00	2.37
New moni ribbed	3.33	4.60	2.10	4.07	44.67	1.53
Orange stuffer	4.67	10.00	3.13	5.00	71.33	2.60
Pink berry	3.33	5.10	3.13	6.63	76.67	2.40
Pink grapes	3.67	3.80	2.00	4.23	45.00	2.10
Pink oyster	4.67	7.00	3.20	6.43	71.67	2.83
Pink pear	5.00	7.60	5.53	8.63	96.67	2.67
Pink ribbed	3.67	7.57	1.77	3.17	106.33	3.20
Pink shade	6.00	2.50	2.50	5.40	44.00	2.40
Pink stuffer	3.67	6.00	3.93	6.97	104.00	3.73
Pink vine	4.33	7.20	2.00	3.73	90.33	3.20
Red apple	5.67	8.67	4.30	6.43	74.67	2.40
Red ball	5.33	4.87	1.80	3.80	69.67	2.57
Red berry	6.00	4.80	3.53	6.47	86.67	3.20
Red cherry	4.33	7.47	2.47	4.43	82.67	3.67
Red oyster	3.00	7.60	2.60	4.87	79.67	2.40
Red plum	5.33	6.10	3.67	5.67	66.33	2.73
Red ruffled	3.00	7.33	3.10	5.50	82.00	3.07
Red stuffer	4.33	8.07	2.90	5.10	80.00	2.67
Red vine	4.00	4.80	1.60	3.60	68.00	2.80
Red grape cluster	4.67	7.93	1.87	3.93	97.33	3.13
Ribbed grapes tomato	5.00	6.30	2.27	5.40	76.00	3.00
Roma orange tomato	4.33	5.80	3.07	6.90	68.33	2.53
Roma tomato	6.67	3.80	2.57	4.27	87.67	3.73
Spoon tomato	17.33	4.93	3.23	5.00	56.67	3.13
Sungold cherry	5.00	4.67	2.43	3.63	71.67	2.57
Yellow cherry	6.33	4.47	2.53	3.87	74.00	2.30
Yellow pear	5.00	7.53	2.23	2.93	73.33	2.30
Yellow clustered/ doyong	4.33	3.20	2.53	4.30	55.00	1.80

Table 3. Loading matrix of principal components

Traits	PC1	PC2	PC3	PC4	PC5	PC6
Flowers per inflorescence (mean count)	0.17174	-0.08712	0.93418	0.29475	0.04986	0.02943
Internodal distance (mean cm)	0.59249	0.39378	-0.36788	0.59857	-0.01504	-0.00609
Leaf breadth (mean cm)	0.69557	-0.67301	-0.03865	-0.01590	0.09000	-0.23108
Leaf length (mean cm)	0.73654	-0.61274	-0.12324	-0.05288	-0.10083	0.23218
Plant height (mean cm)	0.70638	0.50389	0.04963	-0.26137	0.41771	0.04314
Stem diameter (mean cm)	0.69642	0.49560	0.20122	-0.24501	-0.40644	-0.06059

**Fig. 1.** Scree plot of principal components.**Fig. 2.** PCA biplot representing principal component 1 and principal component 2.

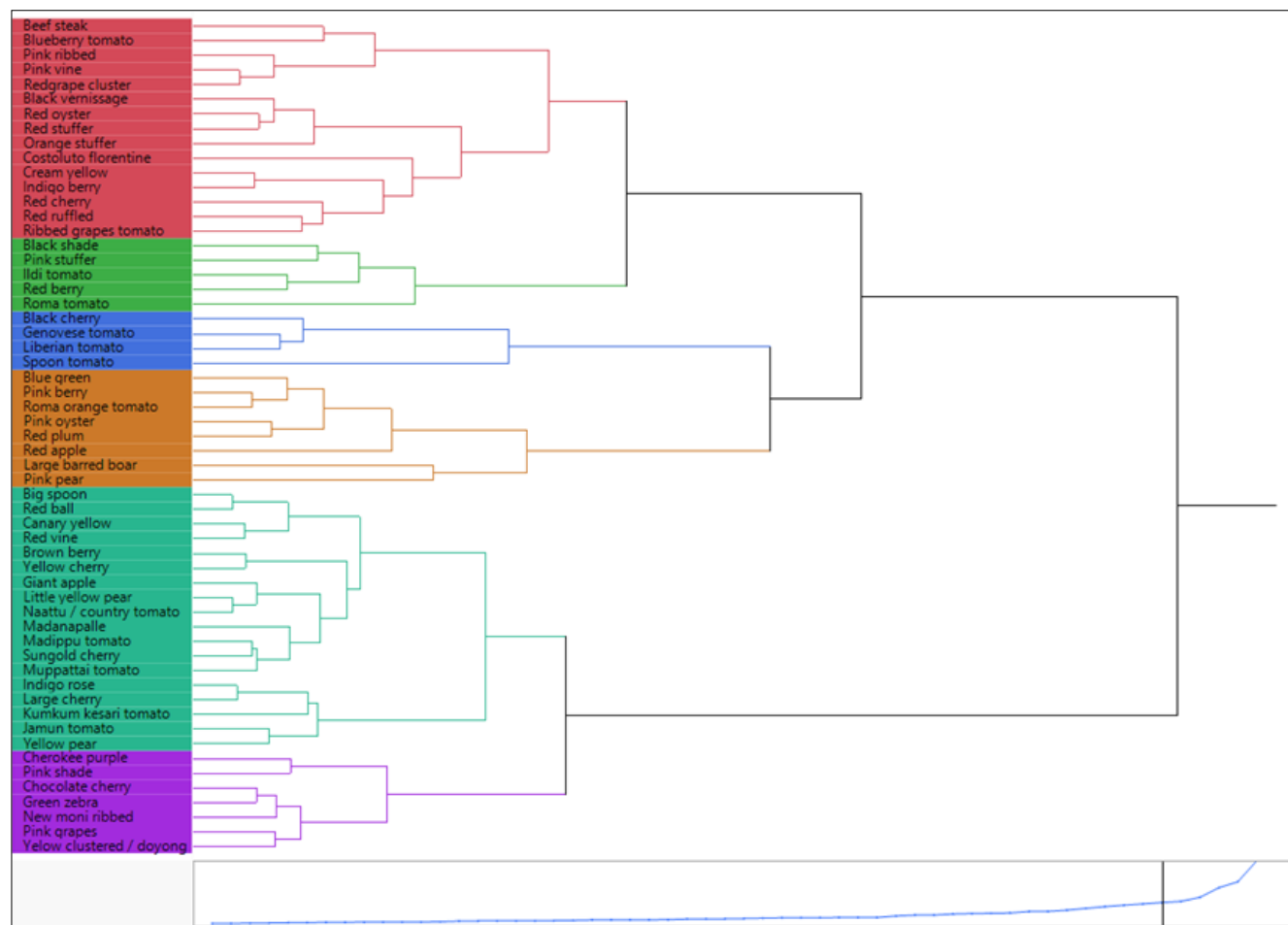


Fig. 3. Clustering of genotypes using morphometric traits based on Ward's method.

Table 4. Clustering of genotypes and their means summary

Cluster	Count	Flowers per inflorescence (mean count)	Internodal distance (mean cm)	Leaf breadth (mean cm)	Leaf length (mean cm)	Plant height (mean cm)	Stem diameter (mean cm)
1	15	4.6889	7.8511	2.4222	4.6733	86.4444	3.0644
2	5	5.5333	5.2933	3.2533	5.8533	94.7333	3.4200
3	4	12.0000	5.8833	3.1583	5.2583	77.1667	2.7667
4	8	4.7500	6.3000	3.8792	6.8042	72.5417	2.5250
5	18	4.9444	5.2389	2.1685	3.7500	67.1296	2.4074
6	7	4.2381	4.1857	2.3238	4.5857	47.9524	2.0048

however, are generally shorter (72.54 cm) and exhibit relatively lower mean stem diameter (2.52 cm) among all clusters. This pattern suggests a morphological trade-off, where investment in larger leaf area may come at the expense of stem robustness and overall plant height.

- Cluster 5 (n=18): As the second-largest group, this cluster represents a compact, small-statured morphotype. It has the lowest mean values for leaf breadth (2.16 cm) and leaf length (3.75 cm) and a low plant height (67.12 cm). This group's traits are on the opposite end of the spectrum from Cluster 4.
- Cluster 6 (n=7): This cluster is a group of very low-vigor accessions. It is characterized by the lowest mean values across all vegetative traits, including internodal distance (4.18 cm), plant height (47.95 cm) and stem diameter (2.00 cm). This group represents a distinct population of very small, low-stature plants.

Discussion

Germplasm of any crop species is a valuable resource for breeders in their plant breeding endeavors. In tomato, germplasm collections are particularly important because they harbor genetic variation for key traits such as yield, fruit quality, disease resistance and tolerance to various stressors. Knowledge of available genetic diversity, as well as important traits that contribute to the major variations in the germplasm collection, will provide informed choices in the selection of parental lines for breeding for specific objectives (18-20). In the context of climate change, it is essential to breed tomatoes that can be either tolerant or resistant to biotic (for example, fusarium wilt, bacterial wilt and yellow leaf curl viral diseases) as well as abiotic stresses (high temperature, drought, salinity, etc.). To identify key traits responsible for existing variation, which will inform the selection of parental lines for future breeding programs, a PCA was conducted on 6 different traits. Furthermore, clustering of genotypes based on 6 different morphometric traits yielded 6 homogeneous clusters.

Preliminary statistical analyses revealed that all the traits chosen were significantly contributing to the morphological diversity among the germplasm studied. To determine which of the 6 traits contributed significantly (towards morphological diversity), PCA was done (21). In the present study, among the traits evaluated, traits viz., plant height, stem diameter and internodal distance were positively correlated in the first principal component whereas leaf breadth, leaf width and number of flowers per inflorescence were positively correlated in the second principal component. It implies that taller plants tend to have thicker stems and longer internodes and, it is observed that the PC1 traits are independent of those in PC2. A similar approach has been carried out in tomato (22), rice (23), okra (24), wheat (25), soybean (26) and others. These studies point out the target traits that can be used in future breeding programs for selecting parental lines.

To identify or to group the genotypes into homogenous clusters, hierarchical clustering using Ward's method was adopted. The number of clusters was restricted to 6, which potentially can be used for selecting genotypes for future breeding activities. For example, one of the clusters contained black cherry, genovese, liberian and spoon tomato. It is a well-known fact that clustering algorithms tend to group individuals with similar traits. On careful observation, this cluster contained genotypes that have a greater number of flowers per inflorescence besides average plant height. These traits could be among the potential traits for future breeding programs targeting medium plant height with enhanced yield. In a similar study, 52 tomato accessions were evaluated for their morphological diversity and their potential use in tomato breeding. Of the 52 lines evaluated, they narrowed down to 20 % of accessions that could be used as potential parental lines for Canadian tomato breeding programs (27).

When the results of PCA are correlated with the clustering results, it can be observed that the individuals occupying different clusters are homogenous in their growth habits. Of the 6 different clusters observed, shorter plants with a higher number of flowers per inflorescence (cluster 3) tend to be useful in future breeding programs for evolving determinate and high-yielding genotypes.

Conclusion

In this study 57 tomato genotypes revealed clear morphological diversity, with 6 distinct clusters defined by key traits. Vegetative characteristics such as plant height, stem diameter and leaf size explained most of the variation, while flowers per inflorescence provided an independent axis linked to reproductive potential. Clusters like the short but highly floral group (cluster 3) and large-leaved, weak-stemmed group (cluster 4) highlight the contrasting phenotypes present within the germplasm. These findings not only help breeders identify promising parents for yield, stress tolerance and better architecture, but also provide farmers and the tomato processing industry with leads on varieties that could enhance productivity and market value. Looking ahead, combining this kind of field-based trait evaluation with molecular marker information could make breeding efforts more precise and efficient, helping speed up the development of resilient, high-yielding varieties.

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Authors' contributions

KK carried out the experimental work, data collection and preparation of the manuscript draft. PM conceived and supervised the study, helped in funding acquisition and provided continuous guidance and critical input throughout the study. MA provided the field source material and essential support in crop raising. VS, JA and GA contributed to laboratory analyses and data curation. VPS and CHB critically reviewed and provided constructive suggestions on the manuscript. SMPK and MLM assisted in experimental support and data validation. All authors read and approved final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflicts of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process:

While preparing this work, the authors used the assistance of the ChatGPT tool for guidance in using the statistical tool for statistical analysis. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the publication's content.

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