Unveiling genetic variation in tomato (*Solanum lycopersicum* Lam.) using DUS Characteristics

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Abstract

Tomatoes represent a highly diverse crop renowned for their nutritional properties and extensive cultivation. Their abundant reserves of vitamins, minerals, dietary fibres, essential amino acids, and organic acids make them crucial nutrient sources across various contexts. Particularly noteworthy are their substantial levels of vitamin A and C, essential minerals, and pigments such as beta-carotene and lycopene, highlighting their biotechnological importance. The exploration of tomatoes' genetic potential is essential for harnessing their inherent variability in breeding endeavours. In this present investigation, 27 distinct tomato parents and hybrids underwent principal component analysis (PCA) based on twenty diverse traits related to both yield and quality. The analysis identified eight principal components, collectively accounting for 80.4% of the variance. Constructing a bi-plot using the initial two principal components revealed that parents and hybrids CBESL160, CBESL164, CBESL168, H4xH5, H1xH5, and H5xH7 exhibited significant genetic divergence, dispersing across all quadrants. These genetic variations, categorized within PC1 and PC2, are potentially valuable additions to initiatives aimed at boosting crop yields through breeding efforts. Several traits, including growth habit, leaflet dimensions, fruit characteristics, and soluble solids content, demonstrated notable and statistically significant associations with yield per plant. Engaging Agglomerative Hierarchical Clustering and PCA, parents and hybrids CBESL160, CBESL164, CBESL168, H4xH5, H1xH5, and H5xH7 emerged as the most diverse parents and hybrids, offering potential avenues for further advancements in breeding efforts.

Key words: PCA, diversity, tomato, correlation, multivariate analysis

Introduction

Tomatoes, scientifically known as *Solanum lycopersicum* L. and belonging to the Solanaceae family, are globally cultivated and valued as a fundamental dietary staple, often referred to as a "protective food." They hold a prominent position among processed vegetables, with India ranking fourth in cultivation area (Kumar *et al.*, 2023). Tomatoes, introduced to India by the Portuguese, originated from Peru and Mexico, and have gained worldwide popularity due to their wide availability, consumption, and recognized health benefits (Iqbal *et al.*, 2014).

Wild tomatoes and landraces play a crucial role in breeding efforts, providing valuable traits for evolutionary studies and crop enhancement (Corrado *et al.*, 2014). However, their effective integration into breeding programs is challenged by insufficient data on their phenotypic characteristics, geographic distribution, and genetic connections with related landraces. A comprehensive understanding of both parents and hybrids is essential for breeders to devise successful breeding strategies (Vishwanath *et al.*, 2014). Morphological characterization is key in evaluating genetic diversity, aiding in resource preservation and conservation efforts (Osei *et al.*, 2014; Sacco *et al.*, 2015). Utilizing a diverse set of descriptors captures various variations, distinguishes different varietal groups, and sheds light on phenotypic and morphological diversity, often complemented by biochemical analyses (Mohan *et al.*, 2021).

Descriptors established by "The Protection of Plant Varieties and Farmers' Rights Authority" (PPV&FRA) for *Solanum* species

are instrumental in recognizing varieties, distinguishing newly emerging unique varieties, and evaluating varietal purity. The scoring mechanism of distinctness, uniformity, and stability (DUS) assists in identifying highly diverse tomato parents and hybrids, enabling their effective integration into breeding programs and the creation of new varieties harboring desired traits for commercial use (Tejaswini *et al.*, 2023).

Tomato landraces often exhibit morphological variation, with closely related morphotypes differing in certain attributes. Characterizing these distinctive qualities is important not only for distinguishing plant species but also for promoting their economic significance. Tomato descriptors display a wide range of variations, identifying distinct varietal groups and describing phenotypic and morphological diversity (Pereira-Dias *et al.*, 2020). Morphological characterization focuses on highly heritable characters that are easily distinguishable by the naked eye and expressed across different environments. Thus, characterizing parents and hybrids is an effective approach to identifying promising sources and utilizing them for creating improved tomato varieties (Grozeva *et al.*, 2020; Corrado *et al.*, 2014).

The objectives of this study were to characterize tomato parents and hybrids using DUS descriptors, focusing on morphological and fruit quality traits, and to identify those with high yield and enhanced fruit quality for long-distance transport. This research aims to develop improved tomato varieties with superior fruit quality and higher yield potential, supporting breeding programs and commercial production.

Materials and methods

Experimental site: This study was conducted from 2022 to 2024 in the orchard of the Department of Vegetable Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, India, located at a latitude of 11°N and longitude of 77°E.

Plant material: The study included 12 parental lines (CBE SL 129, CBE SL 133, CBE SL 142, CBE SL 143, CBE SL 146, CBE SL 154, CBE SL 159, CBE SL 160, CBE SL 162, CBE SL 164, CBESL168, CBESL169) sourced from AVRDC (Taiwan), IIHR (Bangalore), and TNAU (Coimbatore, Tamil Nadu). Eight hybrids (H1: CBE SL 142 x CBE SL 160, H2: CBE SL 146 x CBE SL 160, H3: CBE SL 154 x CBE SL 168, H4: CBE SL 142 x CBE SL 168, H5: CBE SL 133 x CBE SL 169, H6: CBE SL 143 x CBE SL 159, H7: CBE SL 146 x CBE SL 162, H8: CBE SL 129 x CBE SL 164) and seven double cross hybrids (H1 x H7, H5 x H7, H7 x H5, H1 x H5, H8 x H5, H8 x H7, H4 x H5) were also assessed.

Nursery and experimental design: Seeds of parental lines and hybrids were sown in pro-trays filled with enriched coco-peat. The trays were covered with a polyethylene sheet for three days to encourage rapid germination. Daily watering with a rose can supported healthy growth, and protective measures were taken against pests and damping off. The plants were grown in a Randomized Block Design with two replications, following the maintenance protocols recommended by TNAU, Coimbatore (CPG, 2020).

Observations recorded: All traits were documented using predetermined guidelines specific to each trait state, adhering to the standards outlined by PPV and FRA (2001) for DUS assessment. Morphological characteristics of all parents and hybrids were evaluated in the field from the seedling stage to harvest, covering various crop growth stages. Each parent and hybrid was scored from one to nine across twenty traits (Table 1).

Visual assessments (VG) were conducted on plant groups or parts to evaluate traits such as anthocyanin pigmentation of hypocotyls, abscission layer on the peduncle, flowering time, fruit shape, indentation at the peduncle end, presence and intensity of a green shoulder, fruit coloration at ripening, and hairiness on the flower style and stem.

Single observational measurements (MG) were performed on plant clusters or their components, including plant height (cm), leaf length and width (cm), leaflet length and width (cm), segmented peduncle length (cm), flower calyx size (cm), and fruit size (g). Additionally, fruit quality characteristics such as TSS (°Brix), number of locules, and pericarp thickness (cm) were measured (Table 1).

Statistical Analysis: Principal component analysis (PCA) and correlation analysis of different traits were performed using R Studio version 4.3.3. Shannon-Weaver diversity indices (H') were calculated using PAST 3 software. Cluster analysis on diverse traits was conducted using XLSTAT 2.

Result and discussion

Genetic variability in morpho-agronomic traits: The study assessed 25 morpho-agronomic traits in tomato parents and hybrids to explore genetic variability, which is crucial for crop breeding. The observed variability in these traits forms the

Table 1. Scoring system for morphological traits in tomato parents and hybrids

S. No.	Characteristics	Code	States	Notes	No of variants
1.	Plant: Growth type	GH	Determinate	1	14
			Semi determinate	2	13
2.	Seedling: Anthocyanin colouration of hypocotyl	SACH	Absent	1	21
			Present	9	6
3.	Leaf: Length (cm)	LL	Short (<25)	3	26
			Medium (25-30)	5	1
			Long (>30)	7	-
4.	Leaf: Width (cm)	LW	Narrow (<15)	3	27
			Medium (15-20)	5	-
			Broad (>20)	7	-
5.	Leaflet: Length (cm)	LLL	Short (<5)	3	10
			Medium (5-10)	5	
			Long (>10)	7	4
6.	Leaflet: Width (cm)	LLW	Narrow (<4))	3	16
			Medium (4-6)	5	9
			Broad (>6)	7	1
7.	Leaflet: Serration	LLS	Absent (potato type)	1	4
			Less serrated	3	14
			Highly serrated	7	9
8.	Leaf: Attitude of petioles of leaflets in relation to main axis	LAP	Semi-erect	3	12
			Horizontal	5	-
			Semi-drooping	7	15
9.	Peduncle: Abscission layer	PA	Absent (jointless)	1	-
			Present (jointed)	9	27
10.	Jointed peduncle: Length (From abscission layer to calyx) (cm)	JPL	Short (<1.5)	3	17
			Medium (1.5-2.0)	5	5
			Long (>2.0)	7	4
11.	Time of flowering (50% of the plants with at least one open flower from seed sowing) (days)	TOF	Early (<65)	3	23
			Medium (65-80)	5	-
			Late (>80)	7	-
12.	Flower: Calyx size (cm)	FCS	Short (<1)	3	11
			Medium (1-1.5)	5	7
			Large (>1.5)	7	8
13.	Flower: Colour	FC	Pale Yellow	1	20
			Dark Yellow	2	7
14.	Fruit: Size (average weight of 10 fruits) (g)	FS	Very small (<100)	1	-
			Small (100-200)	3	-
			Medium (201-700)	5	2
			Large (701-1000)	7	17
			Very large (>1000)	9	8
15.	Fruit: Shape in longitudinal section	FSLS	Flattened	1	6
			Slightly flattened	2	5
			Circular	3	9
			Rectangular	4	-
			Cylindrical	5	-
			Heart shaped	6	1
			Obovoid	7	3
			Ovoid	8	2
			Pear shaped	9	1
16.	Fruit: Shape at blossom end	FSBE	Indented	1	-
			Indented to flat	2	14
			Flat	3	7
			Flat to pointed	4	5
			Pointed	5	-
			Circular	6	1
17.	Fruit: Depression at peduncle end	FDPE	Absent	1	11
			Shallow	3	8
			Medium	5	7
			Deep	7	1
18.	Fruit: Thickness of the pericarp (cm)	FTP	Thin (<0.3)	3	-
			Medium (0.3 to 0.6)	5	7
			Thick (>0.6)	7	20
19.	Fruit: Number of locules	FNOL	2	1	2
			3-4	2	20
			>4	3	5

Table 1 (contd.). Scoring system for morphological traits in tomato parents and hybrids

S .No.	Characteristics	Code	States	Notes	No of variants
20.	Fruit: Green shoulder (before maturity)	FGS	Absent	1	17
			Present	9	10
21.	Fruit: Intensity of green colour (before maturity)	FIGC	Light	3	16
			Medium	5	6
			Dark	7	5
22.	Fruit: Colour at maturity	FCM	Yellow	1	-
			Orange	2	10
			Pink	3	-
			Red	4	17
23.	Fruit: Total soluble solids (0 Brix)	FTSS	Low (<3)	3	-
			Medium (3.1 -4)	5	1
			High (4.1-5.0)	7	12
			Very high (>5)	9	14
24.	Flower: Pubescence of style	FP	Absent	1	1
			Present	9	26
25.	Stem: Pubescence	SP	Partial	1	11
			Dense	9	16

foundation for selecting superior parents and hybrids. Greater variation within breeding material indicates a higher potential for improvement through selection (Chime *et al.*, 2017). Characterizing tomato parents and hybrids is vital for agronomists and genetic improvement specialists, as it documents existing genetic variability, a critical aspect of breeding programs. Morphological traits serve as essential diagnostic features for distinguishing between parents and hybrids.

Principal component analysis (PCA): Principal Component Analysis (PCA) was employed to assess the genetic variation among tomato genotypes from different regions of India, based on 20 quantitative and qualitative traits. The analysis identified key components that accounted for the maximum variability within the dataset, providing insights into the traits responsible for clustering within the population. The PCA revealed distinct groupings of genotypes and ranked the parents and hybrids according to their principal component (PC) scores. Traits contributing the most to genetic variation were prioritized in the analysis. The analysis was performed using the PAST software (Hammer and Harper, 2001), facilitating the interpretation of complex trait relationships and variability across the studied genotypes. The methodology applied followed the framework established by Purushothaman et al. (2020).

Trait analysis and component scores: Analysis of 20 traits using DUS descriptors yielded eight principal components with eigenvalues exceeding one, collectively explaining 80.46% of the total variability (Table 2). Among these, four components
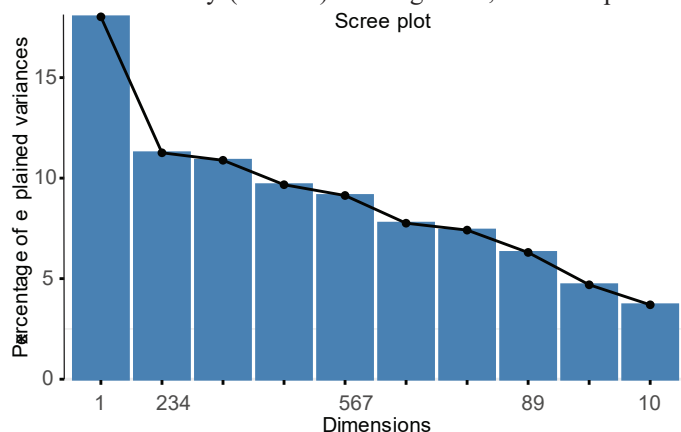

Fig. 1. Scree plot showing contribution of various principal components towards divergence

Table 2. Eigen values, variance and cumulative variability towards principal components

Principal component	Eigen value	Percent variance	Cumulative variance
1	3.605	18.025	18.025
2	2.252	11.261	29.286
3	2.178	10.889	40.176
4	1.935	9.675	49.851
5	1.828	9.137	58.989
6	1.551	7.759	66.748
7	1.483	7.415	74.164
8	1.261	6.305	80.469

accounted for 49.85% of the variability. A scree plot depicted the percentage of variance attributed to each component (Fig. 1). PC1, PC2, PC3, PC4, PC5, PC6, PC7, and PC8 had eigenvalues of 3.605, 2.252, 2.178, 1.935, 1.828, 1.551, 1.483, and 1.261, respectively (Table 2).

The PCA evaluation of traits through their principal component scores (PC scores) provided accurate selection criteria. A genotype with the highest PC score within a component indicates maximum values for the associated variables. Selection of parents and hybrids was guided by PC scores, with positive values exceeding >1.0 in each PC (Table 4).

The rotated component matrix indicated that PC1 correlated with growth habit, fruit shape (longitudinally and at the blossom end), depression at the peduncle end, and total soluble solids content (Table 3). Fruit shape and size significantly influence consumer acceptability, packaging efficiency, and space optimization. The variability in morphometric traits suggests that tomato producers prefer specific fruit types, laying the groundwork for developing varieties with desirable characteristics (Nankar *et al.*, 2020). Fruit shape, easily recognizable by the naked eye, aids in identifying tomato cultivars during field inspections. PC2 linked with traits like leaflet length, leaflet width, and fruit green shoulder, while PC3 accounted for stem pubescence. PC4 was associated with flower color and the intensity of green color in the fruit. Vibrantly colored flowers attract more pollinators, crucial for plant reproduction and subsequent fruit formation (Chime *et al.*, 2017)

Table 3. Rotated matrix results of agro-morphological traits of tomato

PC1	PC2	PC3	PC4	PC5
Growth habit	Leaflet length	Stem pubescence	Flower colour	Seedling: Anthocyanin colouration of hypocotyl
Fruit Shape in longitudinal section	Leaflet width			Fruit: Thickness of the pericarp (cm)
Fruit: Shape at blossom end	Fruit green shoulder			
Fruit: Depression at peduncle end				
Fruit: Total soluble solids				

PC5 was associated with seedling traits such as anthocyanin coloration of the hypocotyl and fruit traits like pericarp thickness. While all cultivars displayed pubescence on their hypocotyls, only hypocotyl color and its intensity were useful for distinguishing tomato cultivars during the seedling stage.

Seedling morphological traits broadly classified parents and hybrids but were not effective for identifying individual cultivars (Salim *et al.*, 2020).

PCA Bi-Plot analysis: A bi-plot graph integrating parents and hybrids and variables was generated using the two main principal components (PC1 and PC2). The PCA bi-plot illustrated that the most distinguishing variables included growth habit, fruit shape (longitudinally and at the blossom end), depression at the peduncle end, total soluble solids content, leaflet length, leaflet width, and presence of a green shoulder on the fruit, explaining 29.2% of the total variability (Fig. 3). Three parents (CBESL160, CBESL164, CBESL168) and three double hybrids (H4XH5, H1XH5, and H5XH7) were positioned farthest from the bi-plot origin, indicating higher variability for traits corresponding to their principal components compared to other parents and hybrids (Rai *et al.*, 2017).

Selection based on PCA scores: Parents and hybrids were selected based on their scores across multiple principal components (PCs), as shown in Table 3. For PC1, positive scores ranged from 0.71 (H7XH5) to 3.67 (CBESL168), while for PC2, positive values ranged from 0.73 (H8) to 3.77 (H4XH5). PCA revealed that PC1 and PC2 exhibited the highest variability in traits such as growth habit, leaflet length, leaflet width, presence of a green shoulder on the fruit, fruit shape (longitudinally and at the blossom end), depression at the peduncle end, and total soluble solids content, consistent with findings by Purushothaman *et al.* (2021) (Fig. 2). The top-performing parents and hybrids based on these components were CBESL160, CBESL164, CBESL168, H4xH5, H1xH5, and H5xH7. Therefore,
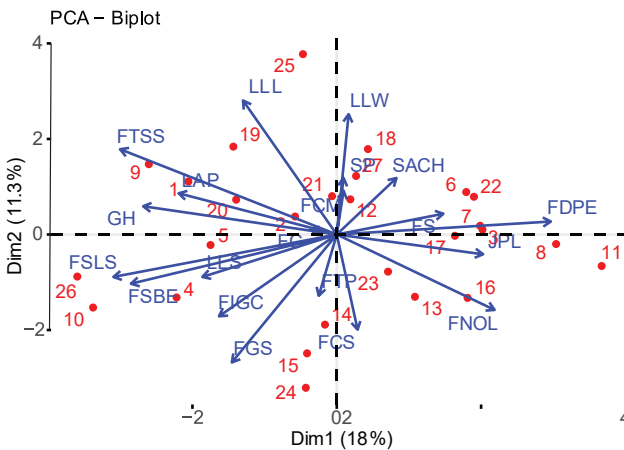

Fig 2. A Biplot depicting the correlation between PC1 and PC2 in parents and hybrids.

(1.CBE SL 129, 2.CBE SL 133, 3.CBE SL 142, 4.CBE SL 143, 5.CBE SL 146, 6.CBE SL 154, 7.CBE SL 159, 8.CBE SL 160, 9.CBE SL 162, 10.CBE SL 164, 11. CBESL168, 12. CBESL169, 13.H1, 14.H2, 15.H3, 16.H4, 17.H5, 18.H6, 19.H7, 20.H8, 21.H1xH7, 22.H5xH7, 23.H7xH5, 24.H1xH5, 25.H4xH5, 26.

parents and hybrids within the PC1 and PC2 range hold significant potential for improving yield and associated traits in future breeding efforts.

Correlation analysis: Understanding correlations between morphological traits is crucial for successful hybridization programs (Popoola *et al.*, 2016). Positive correlations among morphological traits, especially those contributing to
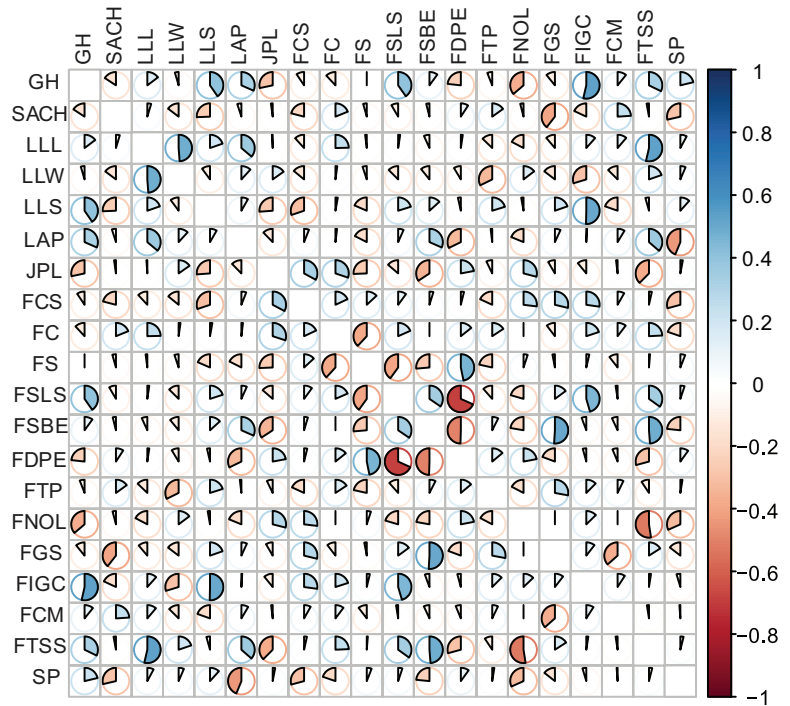

Fig 3. Correlation analysis of morphological traits (GH-Growth habit , SACH-Seedling: Anthocyanin colouration of hypocotyl, LLL- Leaflet: Length, LLW-Leaflet: Width, LLS- Leaflet: Serration, LAP- Leaf: Attitude of petioles of leaflets in relation to main axis, JPL- Jointed peduncle: Length , FCS- Flower: Calyx size, FC- Flower: Colour, FS- Fruit: Size, FSLS- Fruit: Shape in longitudinal Section, FSBE- Fruit: Shape at blossom end, FDPE- Fruit: Depression at peduncle end, FTP- Fruit: Thickness of the pericarp, FNOL- Fruit: Number of locules, FGS- Fruit: Green shoulder, FIGC- Fruit: Intensity of green colour, FCM- Fruit: Colour at maturity, FTSS- Fruit: Total soluble solids, SP- Stem: Pubescence)

Table 4. Selection of parents and hybrids based on PC score in each component having positive values and more than > 1.0 in each PCs

PC1	PC2	PC3	PC4	PC5
CBESL142 (2.019)	CBESL129 (1.11)	CBESL129 (2.33)	CBESL143 (1.89)	CBESL142 (1.36)
CBESL146 (1.79)	CBESL162 (1.47)	CBESL133 (2.19)	CBESL159 (1.26)	CBESL143 (1.75)
CBESL154 (1.98)	CBESL169 (0.74)	CBESL142 (2.77)	CBESL160 (1.77)	CBESL159 (1.63)
CBESL160 (3.04)	H6 (1.78)	CBESL143 (1.66)	CBESL162 (1.31)	CBESL164 (0.97)
CBESL168 (3.67)	H7 (1.84)	CBESL146 (2.18)	H1 (2.54)	H1 (1.08)
H1 (1.08)	H8 (0.73)	CBESL154 (2.17)	H2 (1.62)	H2 (0.73)
H4 (1.81)	H1xH7 (0.80)	H2 (1.17)	H6 (0.69)	H5 (2.32)
H5 (1.64)	H5xH7 (0.79)		H8 (1.73)	H7 (2.08)
H5xH7(1.90)	H8xH5 (1.22)		H1xH7 (0.95)	H5xH7 (1.59)
H7xH5 (0.71)	H4xH5 (3.77)			

quality characteristics, are often sought. In this study, fruit size positively correlated with fruit depression at the peduncle end (r = 0.46). Leaflet serration (r = 0.45), fruit shape in longitudinal section (r = 0.403), intensity of green color in fruit (r = 0.54), and leaflet attitude in relation to the main axis (r = 0.3) demonstrated positive correlations with growth habit. The presence of a green shoulder in fruit (r = 0.3) positively correlated with pericarp thickness. A negative correlation was observed between the number of locules and total soluble solids. Fruit shape exhibited a negative correlation with the flower color and fruit shape in the longitudinal section and fruit depression at the peduncle end.

Based on these findings, parents and hybrids characterized by a thick pericarp, the presence of a green shoulder, and depression at the peduncle end are advisable for long-distance transport, as these traits significantly

Table 4. Shannon-Weaver diversity indices (H')

DUS descriptors	(H')
Growth habit	3.21
Seedling: Anthocyanin colouration of hypocotyl	3.06
Leaf: Length	3.22
Leaf: Width	3.22
Leaflet: Length	3.21
Leaflet: Width	3.21
Leaflet: Serration	3.16
Leaf: Attitude of petioles of leaflets in relation to main axis	3.20
Peduncle: Abscission layer	3.30
Jointed peduncle: Length (From abscission layer to calyx)	3.20
Time of flowering (50% of the plants with at least one open flower from seed sowing) (days)	3.22
Flower: Calyx size	3.21
Flower colour	3.14
Fruit: Size (average weight of 10 fruits) g	3.44
Fruit: Shape in longitudinal section	3.13
Fruit: Shape at blossom end	3.19
Fruit: Depression at peduncle end	3.15
Fruit: Thickness of the pericarp(cm)	3.43
Fruit: Number of locules	3.21
Fruit: Green shoulder (before maturity)	2.99
Fruit: Intensity of green colour (before maturity)	3.20
Fruit: Colour at maturity	3.21
Fruit: Total soluble solids (°Brix)	3.43
Flower pubescence	3.23
Stem pubescence	3.08

influence fruit shape.

Shannon-Weaver Diversity Index: The Shannon-Weaver diversity index (H') is widely used to assess diversity across habitats (Clarke and Warwick, 2001). This index assumes that individuals are randomly sampled from an independent population and that all species are adequately characterized (Shannon and Weaver, 1949). Typically, values range from 1.5 to 3.5. In this study, the Shannon-Weaver diversity indices (H') indicated that fruit size (3.44), total soluble solids content, and pericarp thickness (3.43), along with the presence of the peduncle abscission layer (3.3), contributed the most variation among parents and hybrids (Table 4).

Cluster analysis: Cluster analysis of parents and hybrids based on 25 qualitative characters was performed using XLXSTAT 2024 software (Fig. 4). The analysis identified two distinct clusters (Cluster 1 and Cluster 2) based on morphological traits (Shoba *et al.*, 2019). Cluster 1 exhibited higher values across most variables than Cluster 2, indicating distinct morphological traits. The within-cluster variance was slightly higher in Cluster 2, suggesting more diverse parents and hybrids regarding morphological characteristics. Cluster 1 contained 9 parents and hybrids, while Cluster 2 contained 18. Cluster 1 (G14) and Cluster 2 (G17) represent two distinct groups with differences in centroid values and within-cluster variance. These results indicate clear differentiation between clusters, highlighting the potential for grouping parents and hybrids based on morphological characteristics. This clustering has important implications for breeding programs and crop improvement strategies, as it helps streamline breeding efforts by focusing on parents and hybrids that possess desirable traits for yield, quality, or resilience to environmental stressors (Feng-Mei *et al.*, 2006; Williams *et al.*, 2023).
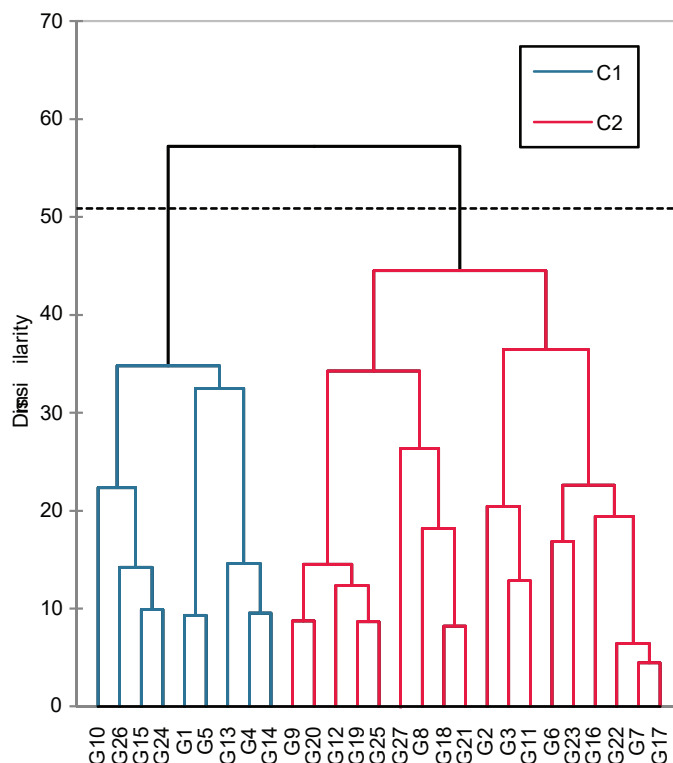

Fig 4. Cluster Dendrogram of tomato parents and hybrids

The exploration of genetic diversity in tomato (*Solanum lycopersicum* Lam.) through DUS descriptors and PCA identified key traits influencing yield and quality. Significant genetic variation was found in three parents (CBESL160, CBESL164, CBESL168) and three double hybrids (H4xH5, H1xH5, H5xH7), making them promising candidates for breeding programs. Growth habits, fruit shape, and leaflet dimensions showed strong correlations with yield. Clustering analysis effectively distinguished distinct groups based on morphological profiles. These findings underscore the importance of genetic diversity in tomato breeding and provide valuable insights for developing improved commercial varieties.

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