

Research Article

Qualitative characterization and clustering in tomato (*Solanum lycopersicum* L.) germplasm accessions

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Abstract

Most of the tomato genotypes are inbred in nature, indicating the need to assess and characterize germplasm accessions as they are the reserve for genotypes with desired traits. Documentation on morphological traits is quite informative in tomato breeding programs since higher levels of diversity on morphological traits are associated with genotypes with lower genetic diversity when assessed using molecular markers. The present investigation aimed to evaluate morphological diversity in tomato germplasm accessions. Morphological characterization was performed in 104 genotypes acquired from various sources. Thirty-three morphological traits, such as seedling, plant, inflorescence and fruit, were scored based on the tomato descriptors. Out of 33 characters studied, 26 traits exhibited diverse modalities, 4 traits exhibited varied classes in genotypes and 3 traits did not show any variants. Qualitative characterization highlighted greater variability among genotypes, as witnessed by their diverse modalities for each trait. The collected data was subjected to Agglomerative Hierarchical Clustering following Ward's method. Cluster analysis and dendrogram construction displayed genetic diversity's richness in the germplasm accessions. Cluster analysis placed these 104 genotypes in six clusters. The largest cluster comprised 55 genotypes, whereas the smallest cluster had three. Agglomerative Hierarchical Clustering helped to find similarities between genotypes. This efficiently assigned genotypes into groups and thus provided guidelines for parental selection in tomato hybridization and breeding programmes.

Keywords: Clustering, Germplasm, Morphological characterization, Parental selection, Variability

INTRODUCTION

Solanum lycopersicum L. (Tomato) with diploid chromosome number (2n) is believed to be native to Andean region. In the early sixteenth century, they were considered ornamental plants (Bauchet and Causse, 2012), but within 200 years, they became a precious crop with greater social and economic value. Globally, tomatoes were one of the most highly appraised, economically important versatile vegetable crops. It is extensively cultivated for fresh-fruit consumption or processed (concentrate, juice, paste, powder, soup or sauce) products (Bhattarai *et al.*, 2016). They are the main dietary source for various nutrients such as vitamins, minerals, antioxidants like lycopene, β -carotene, flavonoids (Eg: quercetin), lutein compounds and quenchers of ROS (Jomova and Valko, 2013). Especially, lycopene

an antioxidant is linked to numerous health benefits, including low risk for cancer and heart disease. It has been scientifically proven that the consumption of tomatoes has a strong correlation with reduced risk of various cancers and cardiovascular problems (Kotsanopoulos and Uddin, 2022). Tomato constitutes about 72% of the total economic value in fresh vegetables produced worldwide (Hanssen *et al.*, 2010), indicating its significance across countries. For the past two decades, tomato production has doubled (Bergougnoux, 2014) and its annual global production is now nearly 182.3 million tons (Vats *et al.*, 2022). Globally tomato is the second most important crop (Kulus, 2022). Correspondingly, its taxonomic and ecological diversity made it a model species for evolutionary studies, metabolite accumulation and fruit development.

Most of their genotypes (till 19th century) were open-pollinated. The mating system in diverse tomato genotypes ranges from allogamous self-incompatible to facultative allogamous and autogamous self-compatible (Bauchet and Causse, 2012). Farmers and breeders have played a key role in shaping crop diversity over the decades (Agrawal *et al.*, 2021). In general, it is believed that domestication and breeding lead to genetic erosion, including loss of vital traits like resistance to pests, diseases and nutritional value (Bauchet and Causse, 2012). As a result of rigorous selection for production traits, modern commercial varieties have significantly lost several essential flavor chemicals compared to older varieties (Tieman *et al.*, 2017). Introgression from promising wild relatives leads to quick progress and is a novel source of diversity. Thus, unravelling the genetic potential of diverse wild relatives for breeding purposes emerged. The present investigation aimed to evaluate morphological diversity in tomato, *S. lycopersicum* L. germplasm accessions.

MATERIALS AND METHODS

Plant material, nursery and field layout

One hundred four tomato accessions (including germplasm accessions collected from Gene Bank, National Bureau of Plant Genetic Resources, local landraces and a few varieties) served as a base material for this investigation. The acquired seeds were sown in pro trays filled with an admixture of organically enriched compost and topsoil. Nursery management practices were carried out, which aided in the production of vibrant seedlings. Seedlings were transplanted on the 30th day after sowing. An augmented design with fifteen blocks and three controls was formed for morphological assessment. Seedlings were planted with a spacing of 60×45 at the Plant breeding farm, Department of Plant Breeding and Genetics, Annamalai University, Chidambaram, from January to May 2022.

Morphological characterization

All standard horticultural practices for tomato production were taken up to raise the crop. Thirty-three morphological traits, *viz.*, seedling, plant, inflorescence and fruit characters, were scored based on the tomato descriptors IPGRI (1996).

Seedling traits

Seedling traits like Hypocotyl colour, hypocotyl colour intensity and hypocotyl pubescence were recorded when the primary leaves were fully open and the terminal bud was 5 mm in size.

Plant traits

Plant traits like plant size, stem pubescence density, stem internode length, foliage density, number of

leaves under first inflorescence and leaf attitude were recorded when fruits of the 2nd and 3rd truss were ripened.

Inflorescence traits

Inflorescence traits like Inflorescence type, corolla colour, corolla blossom type and style position were recorded on the 2nd and 3rd truss of 10 plants.

Fruit traits

Fruit traits like Exterior colour of immature fruit, presence of green trips on the fruit, intensity of greenback, fruit pubescence, predominant fruit shape, fruit size, fruit size homogeneity, exterior colour of mature fruit, intensity of exterior colour, ribbing at calyx end, fruit shoulder shape, width of pedicel scar, size of corky area around pedicel scar, flesh colour of the pericarp, flesh colour intensity, colour intensity of core, fruit cross-sectional shape, shape of pistil scar, fruit blossom end shape, fruit firmness were recorded on the 3rd fruit of 2nd or 3rd truss when the fruits were fully matured.

Statistical analysis

The collected data was analysed using R statistical software (version 3.2.0), for Agglomerative Hierarchical Clustering following Ward's method. Euclidean distances between the genotypes were calculated from the unweighted pair group method using arithmetic averages (UPGMA).

RESULTS AND DISCUSSION

Morphological characterization

Documentation on morphological traits is quite informative in tomato breeding programs since higher levels of diversity on morphological traits are associated with genotypes with lower levels of genetic diversity when assessed using molecular markers (Cebolla-Cornejo *et al.*, 2013). Morphological characterization is the classical method of varietal identification and purity testing (Vishwanath *et al.*, 2014b). Morphological characterization done in 104 genotypes and phenotypic variants for 33 qualitative traits are tabulated in Table 1. In this study, among 104 accessions, one pimpinellifolium genotype and 8 cherry tomato types were found. Cherry-type tomatoes were the genetic admixture of cultivated accessions and *S. pimpinellifolium* (Peralta and Spooner, 2007). The characterization highlighted greater variability among genotypes, as evidenced by diverse modalities for each trait. Traits like corolla color, corolla blossom type and fruit pubescence did not show any phenotypic variants in the 104 genotypes. All other 30 traits showed varied phenotypic classes.

Seedling morphological characters are an important tool in characterising and differentiating tomato cultivars, even at the seedling stage (Salim *et al.*, 2020).

The present study showed four types of hypocotyl color variants and two different colors. Most of the genotypes showed $\frac{1}{4}$ purple from the base type variant and the major colour observed was purple. No other color combinations were observed. This was in accordance with the results of (Salim *et al.*, 2020) in twenty-two tomato inbred lines.

Variations in flower complexity and style exertion could be other interesting traits related to domestication and selection processes. Style insertion is related to increasing autogamy levels. Flower stigma exertion and gametophytic incompatibility system contribute more to natural outcrossing and genetic diversity (Bauchet and Causse, 2012). Mata-Nicolás *et al.* (2020) reported that style exertion is related to fasciation and big-sized fruits. But in contrast, the genotypes with highly exerted style bore small-sized fruits in this investigation. Also, they had delayed fruiting compared to other genotypes with inserted style, the same level as stamen and slightly exerted style. These striking changes in style length were due to gene mutation controlling stigma length (Chen *et al.*, 2007). In the present study, inflorescence in tomato lines was mostly multiparous (66%) followed by both uniparous and multiparous (32%) and uniparous (2%). Plants with multiparous inflorescence having more than ten fruits per inflorescence were desirable as it is a yield-attributing trait. But most of the multiparous inflorescence bore smaller fruits.

Carotenoids impart coloration in fruits and fruit color can discriminate wild relatives of tomato. Most of their wild relatives produce green-coloured fruits. Only two species from Galapagos island have yellow and orange-coloured fruits, whereas *Solanum pimpinellifolium* is the only relative with red coloured fruits (Paran and Van Der Knaap, 2007). In the present study with 104 genotypes, five fruit colours such as red, yellow, orange, pink and greenish-black were noticed. No green-coloured fruits have been observed. A total of 58 genotypes produced red fruits (55.77%) indicating the presence of lycopene, 33 genotypes produced orange-coloured fruits (32%). Orange colour indicates the richness of β carotene in fruits (Saini *et al.*, 2015). In the present study, pink and yellow coloured fruits were in low frequency (3.85%). The predominant nature of red-coloured mature tomato fruit was supported by various authors (Kenneth, 2016; TRAORE *et al.*, 2019; Salim *et al.*, 2020).

Genus *Lycopersicon* comprises nine species, of which only *L. esculentum* was domesticated and is the only cultivated species that finds significant phenotypic variation in fruit shape and size (Tanksley, 2004). Tomato accessions in the present study contained all variants for fruit shape, whereas rounded fruit shapes appeared to be predominant (31.73%). Round-shaped fruits have consumer preferences and can be used for table purposes, whereas the other shaped fruits can be used in

the processing industry. A strong association does exist between the total carotenoid content of ripe tomatoes and blossom colour (Saltveit, 2005). Hence, genotypes with darker blossom colour have higher total carotenoid content. All the 104 genotypes used in this study exhibited yellow-coloured blossom, but differences have been observed in their intensity. The result is consonant with Vishwanath *et al.* (2014a), who reported that differences were observed in the intensity of tomato blossom colour. Thirt two lines produced dark yellow blossom, whereas 72 genotypes produced light yellow colour blossom. Darker blossom in tomatoes acts as an aid in the selection of genotypes with higher carotenoid content. In the present study, with 104 genotypes, 28 genotypes produced dark-coloured fruits, 70 genotypes showed intermediate-coloured fruits, whereas 6 genotypes bore light-coloured fruits. Lycopene is generally accumulated in tomato skin and it imparts dark colour in tomato fruits. Genotypes with dark-coloured fruits have a greater chance of having higher lycopene content.

Foliage density has a greater concern in tomato breeding. Genotypes with dense foliage exhibit more tolerance/resistance to biotic and abiotic stresses. It also protects fruits from birds and direct sunlight (Kalloo, 2012). In the present study, 23% of genotypes showed dense foliage and 76% showed intermediate foliage density. Due to dense and intermediate foliage in genotypes, sun scalding was not a problem in these 104 genotypes, even if it is unconditioned.

Ideotypes for tomatoes include semi-erect upper leaves and spreading lower leaves (Hui *et al.*, 2008). Photoreception posture and optimum leaf area index contribute to higher photosynthetic rates (Hui *et al.*, 2008). The photosynthetic rate in cultivars mainly relies on their leaf orientation and leaf area exposed to sunlight (Zhang *et al.*, 2022). In the present study, semi-erect type of leaf orientation was found in 22% of genotypes, followed by 23% of genotypes with horizontal leaves and 55% with drooping leaves.

Improving the texture quality of fruit encourages a healthier diet, simplifies food chain logistics, and reduces postharvest waste (Barrett *et al.*, 2010). Enhanced fruit firmness in tomato leads to extended shelf-life of fruits and reduced postharvest waste. In the present study, 68% of genotypes produced fruits with soft texture whereas 21% of genotypes produced fruits with intermediate texture and 11% produced fruits with firm texture. Hence, these 11 genotypes with firm texture can be further used in breeding programmes to enhance shelf life.

Agglomerative clustering

Cluster analysis is an efficient tool for defining clustering patterns and helps establish relationships between genetic divergence (Shoba *et al.*, 2019). Agglomerative

Table 1. Phenotypic variants for 33 qualitative traits in 104 genotypes

Sl. No.	Character	Score	Phenotype	Number of variants	Percentage of variants
	Hypocotyl colour	1	Green	25	24.04
		2	¼ purple from the base	32	30.77
		3	½ purple from the base	18	17.31
		4	Purple	29	27.88
	Hypocotyl colour intensity	3	Low	13	12.50
		5	Intermediate	64	61.54
		7	High	27	25.96
	Hypocotyl pubescence	0	Absent	58	55.77
		1	Present	46	44.23
	Plant size	3	Small	1	0.96
		5	Intermediate	98	94.23
		7	Large	5	4.81
	Stem pubescence density	3	Sparse	62	59.62
		5	Intermediate	32	30.77
		7	Dense	10	9.62
	Stem internode length	3	Short	32	30.77
		5	Intermediate	68	65.38
		7	Long	4	3.85
	Foliage density	3	Sparse	1	0.96
		5	Intermediate	79	75.96
		7	Dense	24	23.08
	Number of leaves under first inflorescence	3	Few	6	5.77
		7	Many	98	94.23
	Leaf attitude	3	Semi-erect	23	22.12
		5	Horizontal	24	23.08
		7	Drooping	57	54.81
	Inflorescence type	1	Generally uniparous	2	1.92
		2	Both	33	31.73
		3	Generally multiparous	69	66.35
	Corolla colour	1	White	0	0
		2	Yellow	104	100
		3	Orange	0	0
		4	Other	0	0
	Corolla blossom type	1	Open	104	100
		2	Closed	0	0
	Style position	1	Inserted	72	69.23
		2	Same level as stamen	25	24.04
		3	Slightly exerted	6	5.77
		4	Highly exerted	1	0.96
	Exterior colour of immature fruit	1	Greenish white	4	3.85
		3	Light green	33	31.73
		5	Green	4	3.85
		7	Dark green	58	55.77
		9	Very dark green	5	4.81
	Presence of green trips on the fruit	0	Absent	79	75.96
		1	Present	25	24.04
	Intensity of green back	0	Absent	79	75.96
		3	Slight	13	12.50
		5	Intermediate	6	5.77
		7	Strong	6	5.77
	Fruit pubescence	3	Sparse	104	100
		5	Intermediate	0	0
		7	Dense	0	0
	Predominant fruit shape	1	Flattened	5	4.81
		2	Slightly flattened	16	15.38
		3	Rounded	33	31.73
		4	Highly rounded	23	22.12
		5	Heart shaped	5	4.81
		6	Cylindrical	5	4.81
		7	Pyriform	6	5.77
		8	Ellipsoid	11	10.58
		9	Other	0	0

Contd.....

Table 1. Contd.....

Fruit size	1	Very small	12	11.54
	2	Small	30	28.85
	3	Intermediate	61	58.65
	4	Large	1	0.96
	5	Very large	0	0
Fruit size homogeneity,	3	Low	10	9.62
	5	Intermediate	46	44.23
	7	High	48	46.15
Exterior colour of mature fruit	1	Green	0	0
	2	Yellow	4	3.85
	3	Orange	33	31.73
	4	Pink	4	3.85
	5	Red	58	55.77
	6	Other	5	4.81
Intensity of exterior colour	3	Light	6	5.77
	5	Intermediate	70	67.31
	7	Dark	28	26.92
Ribbing at calyx end	1	Very weak	18	17.31
	3	Weak	60	57.69
	5	Intermediate	19	18.27
	7	Strong	7	6.73
Fruit shoulder shape	1	Flat	17	16.35
	3	Slightly depressed	55	52.88
	5	Moderately depressed	25	24.04
	7	Strongly depressed	7	6.73
Width of pedicel scar	3	Narrow	90	86.54
	5	Medium	14	13.46
	7	Wide	0	0
Size of corky area around pedicel scar	3	Small	80	76.92
	5	Intermediate	24	23.08
	7	Large	0	0
Flesh colour of pericarp	1	Green	2	1.92
	2	Yellow	8	7.69
	3	Orange	10	9.62
	4	Pink	25	24.04
	5	Red	57	54.81
	6	Other	2	1.92
Flesh colour intensity	3	Light	23	22.12
	5	Intermediate	68	65.38
	7	Dark	13	12.50
Colour intensity of core.	1	Green	10	9.62
	2	White	51	49.04
	3	Light	0	0
	5	Intermediate	40	38.46
	7	Dark	3	2.88
Fruit cross sectional shape	1	Round	78	75
	2	Angular	11	10.58
	3	Irregular	15	14.42
Shape of pistil scar	1	Dot	70	67.31
	2	Stellate	20	19.23
	3	Linear	2	1.92
	4	Irregular	12	11.54
Fruit blossom end shape	1	Indented	15	14.42
	2	Flat	81	77.88
	3	Pointed	8	7.69
Fruit firmness	3	Soft	71	68.27
	5	Intermediate	22	21.15
	7	Firm	11	10.58

clustering helps to find the similarities between genotypes. Cluster analysis placed these 104 genotypes in six clusters (Fig. 1).

The smallest cluster consisted of three genotypes with hypocotyl pubescence, intermediate plant size, intermediate stem internode length, and drooping leaves

and these genotypes produced fruits with indented blossom ends, irregular cross-sectional shape and small corky area with the absence of green trips. The genotypes in this cluster have lesser consumer preference because of their irregular fruit shape.

The largest cluster contained 55 genotypes with inter-

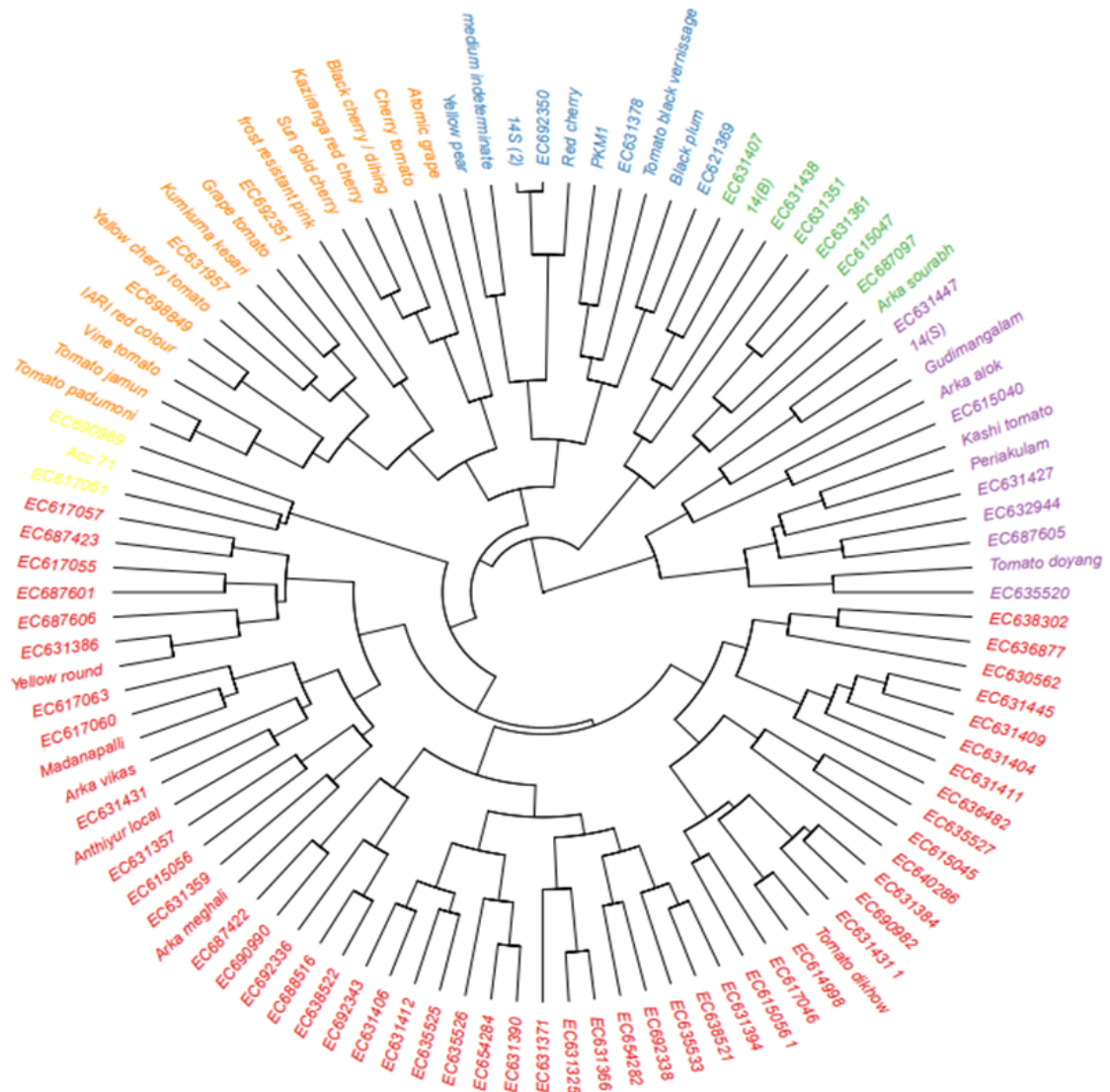


Fig. 1. Agglomerative Clustering of 104 genotypes showing six clusters of tomato genotypes

mediate foliage density, more leaves under first inflorescence, horizontal and drooping leaf type having both uniparous and multiparous inflorescence and these genotypes produced orange to red colour flattened fruits with weak ribbing at calyx end, flat blossom end shape, dot-shaped pistil scar and round cross-sectional shape. The genotypes with weak ribbing produced fruits with round cross-sectional shapes. The members of this cluster were domesticated lines which are clearly evident from the fruit colour and shape.

The third cluster consists of 16 genotypes under two subgroups. The first subgroup comprises six small cherry tomato types and one *pimpinellifolium* genotype. They were grouped under a single cluster due to smaller fruit size (Rick and Holle, 1990; Peralta and Spooner, 2007) reported that these cherry tomato accessions were a possible genetic admixture of cultivated and wild germplasm. The second subgroup consisted of

nine genotypes ranging from small to intermediate-sized highly homozygous fruits.

The fourth cluster consists of 12 genotypes with intermediate stem pubescence and produced pink to red, fleshed fruits with depressed fruit shoulder and irregular shape of pistil scar. The fifth cluster consists of 8 genotypes with semi-erect leaves, which produced fruits with slightly depressed fruit shoulders and medium-sized corky area around the pedicel. The members of this cluster have genotypes with semi-erect leaves, an ideotype trait in tomato breeding for higher photoreception. The sixth cluster has 10 genotypes that produced round, cross-sectional-shaped fruits, stellate pistil scar, and flat fruit blossom end.

It is evident from this study that clustering is one of the efficient tools that aided in categorizing germplasm and serves as a reliable basis for choosing base material in future breeding programmes, as reported earlier (Sušić

et al., 1999; Jin *et al.*, 2006; Sinha *et al.*, 2021) in tomato.

Conclusion

Across the world, diversity in tomato genotypes has been narrowed down to cultivated types. In the present study, 104 genotypes and 11 accessions produced firm fruits, which can be used in further breeding programs to enhance their shelf life. The accessions used in the present study could be valuable germplasm for genetic improvement programs in tomatoes. Hence, a diverse tomato germplasm pool can be developed by crossing desirable wild genotypes with cultivated ones that can enhance future tomato breeding programs, production and quality.

Conflict of interest

The authors declare that they have no conflict of interest.

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