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Genetic Divergence for Seedling Traits in Tomato (*Solanum lycopersicum*)

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Abstract

Three hundred and eighty genotypes of tomato were investigated for genetic diversity for nine seedling traits and considerable genetic variation was observed for all the traits except pubescence. Only two genotypes (19901 and 6836-9) were glabrous, whereas all others had hair on the hypocotyl. Eight clusters were observed based on *k-means* clustering with average distance ranging from 0.47 (cluster 6) to 0.79 (cluster 8). Some of the discrete traits did not exhibit variation within individual cluster that could be one of the bases for clustering pattern. The scatter diagram partially indicated the clustering pattern and the clusters 3, 4 and 5 intermixed. Sub clustering of individual clusters revealed 5 sub clusters of cluster 1, three of cluster 2 and 5 in each case, six in cluster 3, 4 and 7 and cluster 6 and 8 had 4 each. The cluster 2, 3, 4 and 6 joined at higher genetic linkage with induction of single genotype in the cluster 2, 4 and 6, whereas in cluster 3, two main groups joined at higher distance including one group comprising of the 41 genotypes and the second comprising 19 genotypes, which joined at 80% linkage distance. The data on seedling traits along with other data will be utilized for establishment of core set and the genotypes with maximum genetic distance from individual clusters along with representative samples from low distance groups are likely to be chosen for core set.

Keywords: K-means clustering; Seedling vigor; Hairy hypcotyl; Wild germplasm; Tomato

Introduction

Tomato, a member of *Solanaceae* family is short-lived perennial usually grown as annual plant almost throughout the world. It is highly self-pollinated plant and may be determinate or indeterminate in growth habit. The cultivated Tomato (*Solanum lycopersicum* L.) is relatively a new addition to world food crops, but has gained popularity very rapidly and has attained the status of most widely consumed vegetables in world (Ojo *et al*., 2009). It is believed that tomato is native to South America, the Incas and Aztecs began cultivating tomato plants as early as 700AD (Tam *et al*., 2007). Genetic evidence shows that the progenitors of tomatoes were herbaceous green plants with small green fruit and a center of diversity in the highlands of Peru (Jenkins, 1948). In Indo-Pak region, tomato is thought to be introduced by British colonists in the beginning of 19th century (Khan, 2009). The large, lumpy tomato, a mutation from a smoother, smaller fruit, originated in Mesoamerica and may be the direct ancestor of some modern cultivated tomatoes (Abdullahi and Choji, 2009). Thousands of tomato cultivars are being grown worldwide for its edible fruit with a world production of 130 million tons (MT), China being the largest producer (34 MT), followed by USA (12.7 MT) and Pakistan is at the 34th position in fresh tomato production with production of 0.47 MT (http://faostat.fao.org).

Tomatoes are rich source of nutrition and contain lycopene, one of the most powerful natural antioxidants (Cohen *et al*., 2000; Marković, 2010). Assessment of the intra-population genetic variability in tomato has been reported important by Mazzucato *et al*. (2010). These authors analyzed morphological and molecular descriptors in Italian landraces of tomato. Seedling traits give an indication for plant growth and could also be used as plant descriptors as well as markers. Monogenic traits were the first to be employed for varietal identification and for markers which are still important for most of the crops (Gul *et al*., 2010; Xu *et al*., 2010). The seedling qualitative descriptors have been utilized for identification of various crop varieties and as genetic markers for investigation of quantitative traits loci (Basunanda *et al*., 2010; Ashfaq *et al*., 2012). The present study was initiated to characterize tomato germplasm for seedling traits, both qualitative and quantitative to investigate the magnitude of diversity among genotypes and clusters. This data will serve the purpose for genotypic identification and later will be utilized for establishing core collection to have the maximum diversity in less number of genotypes.

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Materials and Methods

Three hundred and eighty genotypes of tomato including five commercial cultivars (Tom Round, Roma King, Money Maker, Saryab Long, BARI-5), and four wild species *Solanum pimpinellifolium* (eight accessions), *S. habrochaites* (2 accessions), *S. pennellii* and *S. chilense* (one accession of each) were investigated for genetic diversity on nine seedling traits *i.e*., hypocotyl color, hypocotyl color intensity, hypocotyl pubescence, overall leaf color, midrib color, seedling vigor, primary leaf length (mm), primary leaf width (mm) and hypocotyl length (mm) according to the descriptors by IPGRI (http://www.bioversityinternational.org/publications/pubfile.asp?ID_PUB=286). All of these genotypes have been maintained in the genebank at three storage conditions for active/short-term, medium-term and long-term/base collection for conservation and future utilization for crop improvement program. The seed was placed in the paper towel till germination at 25ºC in the growth chamber and then each genotype was transferred in plastic pots accommodating twenty seedlings with equal plant spacing. The pots were kept under greenhouse conditions with appropriate temperature and irrigated alternate days with the help of sprinkler. Data were recorded when seedlings were three weeks old after transferring in the plastic pots. The data for hypocotyl color intensity, hypocotyl pubescence, overall leaf color, midrib color and seedling vigor were recorded on genotype basis as discrete traits, and thus can be used as plant descriptors representing single value for each genotype, whereas primary leaf length and width (mm) and hypocotyl length (mm) were measured on 10 seedlings sampled at random within each genotype and then averaged for analysis and presentation.

Measureable data for seedling traits (primary leaf length and width, and hypocotyl length) were analyzed for descriptive statistics including means and variance. For easy comparison, the variance was expressed as percent of mean for measureable traits. Other discrete data were classified into groups, whereas all the nine seedling traits were analyzed for genetic diversity according to Nei and Li (1979). The data were analyzed for *k-means* clustering and then each cluster was presented as dendrogram using computer software STATISTICA Version 6.01 for Windows (http://www.statsoft.com/). The data were standardized prior to cluster analysis due to variation in measuring scales. Based on two Principal Components, the genotypes were plotted on the basis of clusters with the help of SPSS, Version 11.0 for Windows (http://www.spss.com/).

Results

Table 1 presents the description of all the nine seedling traits and their classification in various categories. One hundred seventy seven genotypes produced full purple hypocotyl, whereas eighty three were green. Others exhibited intermediate level either having one fourth purple color (36 genotypes) or half purple color from the base (84 genotypes). Two genotypes (19901 and 6836-9) were glabrous, whereas all others had hairs on the hypocotyl. In the germplasm under study, 284 genotypes produced good seedling vigor and among these, high yielding cultivars could be selected. The measurable seedling traits showed a normal distribution that could facilitate selection to genetic gain for these traits. The range for three measured traits indicated a considerable variation among the genotypes investigated in the present study (Table 2). Variation expressed as percentage of means indicated that hypocotyls length had the maximum variation that was followed by primary leaf length and primary leaf width.

Eight clusters were observed based on *k-means* clustering. Data indicated the members of each cluster and average distance (Table 3). Fifty one genotypes were grouped in cluster 1, 48 in cluster 2, 60 in cluster 3, cluster 4 and 6 got 40 each, 52 in cluster 5, 63 in cluster 7 and 26 genotypes were grouped in cluster 8. The average distance for individual clusters ranged from 0.47 (cluster 6) to 0.79 (cluster 8). The cluster with higher genetic distance grouped the genotypes with distinct characteristics. Among discrete traits, hypocotyl pubescence (HP), midrib color (MRC) and seedling vigor (SLV) did not exhibit variation in the cluster 1 and 5, whereas the cluster 2, 3 and 8 were monomorphic for HP. The genotypes in cluster 6 did not reveal variation for HP and MRC, while the members of cluster 7 did not show variation for HP and SLV. Among cultivated varieties, Saryab Long was in cluster 2, Tom Round and BARI 5 were grouped in cluster 3, Roma King was in cluster 6 and Money maker was in the cluster 7, while in wild genotypes, three accessions of *S. pimpenellifolium* (19888, 19895, 19896) were placed in cluster 2, two (19897, 19903) in cluster 7, whereas cluster 1, 4 and 5 had the accessions 19898, 19889 and 19899, respectively. Two accessions of *S. habrochaites* were grouped in two clusters, *i.e*., 19902 in cluster 2 and 19901 in cluster 4. *S. chilense* (19906) was in the cluster 2 and *S. pennelli* (19905) in the cluster 4. The average distance for grouping of commercial varieties in different clusters indicated the distinctness of tomato varieties for seedling traits.

Maximum genetic distance (1.61) was observed between cluster 6 and 8, whereas a minimum distance (0.79) was observed in two cases *i.e*., between cluster 2 and 4, and cluster 3 and 5 (Table 4). The clusters thus observed through *k-means* clustering were plotted for the first two principal components contributing 44% of variation against *x-y* coordinated (Fig. 1). More than half of the genotypes in cluster 6 and 7 were at the peripheral boundaries of the graph and only cluster 8 was clearly grouped in the upper right box indicating both the factors with positive axes. The genotypes observed on the graph were categorized and the clustering pattern was presented in the Fig. 1 for understanding and easy interpretation, because due to large

Table 1: Classification of seedling traits in 380 genotypes of tomato

Traits	Notation	Classes	Frequency	Percentage
Discrete Traits				
Hypocotyl color	*HC*			
		Green	83	21.8
		One fourth purple from base	36	9.5
		Half purple from base	84	22.1
		Purple	177	46.6
Hypocotyl color intensity	*HCI*	Low	122	32.1
		Intermediate	176	46.3
		High	82	21.6
Hypocotyl pubescence	*HP*			
		Present	378	99.5
		Absent	2	0.5
Overall leaf color	*OLC*			
		Low	28	7.4
		Intermediate	207	54.5
		Dark	145	38.2
Midrib color	*MRC*			
		Green	244	64.2
		Purple	136	35.8
Seedling vigor	*SLV*			
		good	284	74.7
		weak	96	25.3
Measureable Traits				
Primary leaf length (mm)	*PLL*			
		Up to 18.0	24	6.3
		18.1 – 24.0	153	40.3
		24.1 – 30.0	142	37.4
		30.1 – 36.0	57	15.0
		36.1 – 42.0	4	1.1
Primary leaf width(mm)	*PLW*			
		≤ 4.0	6	1.6
		4.1 – 6.0	112	29.5
		6.1 – 8.0	232	61.1
		8.1 – 10.0	28	7.4
		10.1 – 12.0	2	0.5
Hypocotyl length (mm)	*HL*			
		≤ 20.0	36	9.5
		20.1 – 40.0	292	76.8
		40.1 – 60.0	47	12.4
		60.1 – 80.0	3	0.8
		80.1 – 100.0	2	0.5

HC: Hypocotyl color, *HCI*: Hypocotyl color intensity, *HP*: Hypocotyl pubescence, *OLC*: Overall leaf color, *MRC*: Midrib color, *SLV*: Seedling vigor, *PLL*: Primary leaf length (mm), *PLW*: Primary leaf width (mm), *HL*: Hypocotyl length (mm)

Table 2: Basic statistics for seedling traits in 380 genotypes of tomato

Growth parameters	Mean±SE	Standard Deviation	Variance % of means	Range
Primary leaf length (mm)	25.0±0.2	4.8	90.9	12.0-42.4
Primary leaf width (mm)	6.6±0.1	1.1	18.6	3.0-12.0
Hypocotyl length (mm)	30.3±0.5	10.4	357.4	1.2-96.0

number of genotypes (380 in this case), the graph was intermingled. With the intermixing of clusters 3, 4 and 5, all the eight clusters were further analyzed individually keeping same axis values for distance for easy comparison to present in the dendrogram (Fig. 2). The dendrograms grouped varying numbers of genotypes as presented in Table 3. Sub clustering was observed at 50% inter-cluster dissimilarity and cluster 1 was divided into 5 sub clusters, cluster 2 and 5 into three sub-clusters each, six sub clusters were observed in each of the cluster 3, 4 and 7 and cluster 6 and 8 were divided into four sub clusters in each case. Among all the clusters, cluster 2, 3, 4 and 6 joined at higher genetic linkage. Single genotype 19896 (*S. pimpinellifolium*) was joined at higher distance in the cluster 2, 1803083 in cluster 4 and LO5860 in cluster 6, whereas in cluster 3, two main groups joined at higher distance. One group comprising of the genotypes, viz., 10576, Tom Round, Avinash, 17889, 17870, LO5596, 6836-2, 6863-10, 6836-8, 6836-4, 19294, LO5633, 10573, 10587, LO5632, LO5840, Nozami, 17885 and 6234 joined with other group of genotypes, viz., LO6029, LO5839, LO6021, LO2401, NGB2407, CN1516, CLN2768A, PL12583157AL, 17882, 19291, CN112,

Table 3: Clusters for seedling traits in 380 genotypes of tomato

Cluster	Frequency	Genotypes	Average distance	Traits with no variation
1	51	17862, 17873, 17875, 17883, 17887, 17888, 17890, 17902, 17903, 17904, 17906, 17909, 19898, 19908, 23666A, 2366C, 2777B, 99S-C-39-20-11-240, CH154, CL5615-93014-1-0-3, CL5915-206, CLN 2413J, CLN 5615-93D4-1, CLN 3022F-10-32-2, CLN1462A, CLN1466P, CLN1555A, CLN2026D, CLN2070A, CLN22641, CLN2264J, CLN2366C, CLN2400B, CLN2413D, CLN2413J, CLN2413R, CLN2418A, CLN2498, CLN2498D, CLN2762A, CLN2764A, CLN2777C, CLN2777F, CLN2777G, CLN2777H, CLN3022-F2-10-16, CLN5915-204D4, LRB9, PT4664B, PT4719A, Walter	0.60	HP, MRC, SLV
2	48	6232, 10580, 10585, 10588, 17859, 17860, 17865, 17872, 17874, 17878, 17880, 17881, 19288, 19295, 19296, 19887, 19888, 19895, 19896, 19900, 19902, 19904, 19906, 19907, 19913, CH-151, CN1498, CN302, DR 4, FLA456-4, FLA478-6-3-1-11, FLA496-11-6-1-0, FLA653-3-1-0, LO0746, LO0868, LO2649, LO4034, LO5936, LRB10, NGB11910-2, NGB15858-1, NGB2408-2, NGB5021-2, PL12902658AL, PL12903367AL, PL29133765AL, Punjab Chuhara, Saryab Long	0.69	HP
3	60	6234, 10573, 10576, 10579, 10583, 10587, 10589, 10592, 17856, 17858, 17870, 17882, 17885, 17889, 17900, 19291, 19294, 19891, 6836-2, 6836-4, 6836-8, 6863-10, Avinash, BARI-5, CLN2768A, CN112, CN1516, LO0760, LO0769, LO0790, LO0853, LO1891, LO1902, LO1921, LO2064, LO2401, LO2540, LO2560, LO2663, LO5596, LO5632, LO5633, LO5839, LO5840, LO5848, LO5995, LO6021, LO6022, LO6029, LRB11, LRB6, LRB7, NGB2407, Nozami, PL12166295GL, PL12583157AL, PL26299560AL, PL40695276AL, PL64744505GL, Tom Round	0.63	HP
4	40	6235, 17863, 17869, 17876, 17884, 19290, 19297, 19889, 19890, 19893, 19901, 19905, 19910, 6836-9, AARI local, CN1506, CN18862, CN7232, H 24, LO1923, LO1936, LO1941, LO1996, LO2559, LO2738, LO2739, LO5595, LO5605, NGB11900-2, NGB7769-2, PL11878384AL, PL12858695GL, PL21206269AL, PL25847885AL, PL27020663AL, PL28155566AL, PL45202678AL, PL45202777AL, PL64730599GL, PL9809766AL	0.77	-
5	52	6233, 10574, 10575, 10578, 10581, 10582, 17864, 17877, 19899, 15876095, Madona, 6836-7, CN100, CN123, CN126, CN1632, CN18078, CN1855, CN1857, CN342, CN612, CN74, CN85, CN87, CN89, FLA47-6-3-1-11, HGB15846-1, LO2017, LO5822, LO5905, LO5926, LO5992, NGB11704-2, NGB15851-1, NGB2048, NGB2050, PL11756384AL, PL12403596GL, PL12782570AL, PL12859269AL, PL12912858AL, PL15537256AB, PL19629700GL, PL25847466AL, PL2943865AL, PL34113498GL, PL39051075AL, PL64744791GL, PL64748699GL, PL64755601GL, PL64756602GL, T5020	0.56	HP, MRC, SLV
6	40	6237, 10584, 19894, 19912, 19914, Rio-China, CN1707, CN345, FLA505, LO1231, LO1715, LO1878, LO1917, LO2598, LO3873, LO5860, LO6017, NGB13643-3, NGB15845-1, NGB15847-1, NGB18109-1, PL10983484AL, PL12782059AL, PL12908468AL, PL12914258AL, PL15799368AL, PL15900970AL, PL15919885AL, PL2681072AL, PL27040861AL, PL27043096GL, Pl27270361A1, PL40695276AL, Pl50531784AL, PL60092005GL, PL97538704L, PL9978275AL, Roma King, T 4065, TY52	0.47	HP, MRC
7	63	6238, 17857, 17868, 17871, 17895, 17899, 19321, 19897, 19903, 19909, 19911, Peto-86-China, 6836-3, 6836-5, 6836-6, 99S-C-39-20-11, Arka Abha, CH 68, CLN 1314G, CLN 2001 A, CLN 2071C, CLN 2123C, CLN 2366A, CLN 2400B, CLN 2413D, CLN 2418 A, CLN1621L, CLN2001A, CLN2026M, CLN2123C, CLN2123D, CLN2123E, CLN2277C, CLN2366 B, CLN2400A, CLN2585A, CLN2585D, CLN2777A, CLN2777B, CLN2777E, CN1502, DR 2, DR 2-1, DR 3, LO0244, LO0818, LO1788, LO4020, LO5833, LO5861, LO5877, LO5913, LRB16, LRB17, Money Maker, Nagina, NGB12213-1, Pant Bahar, Pusa Ruby, Rio-Early, T-4, TLB 111, TMV F1	0.68	HP, SLV
8	26	6231, 17867, 17879, 19289, 19292, 19293, 19892, 6836-1, 6836-11, Bio Blitz, CLN3022F-183-11, CLN2460E, CLN3022F2-10-55, CLN3022F2-11-16, CN117, CN634, FLA 496-11-6-1-0, LO0854, LO0981, LO1733, LO1967, LO2299, LO5571, LO5835, LO5947, LO6003	0.79	HP

17858, PL26299560AL, PL12166295GL, LO2540, LO0853, LO0769, LRB7, LO1921, LO1891, LO5995, LO0790, LO0760, LO5848, 19891, LO6022, LO2064, 10592, BARI-5, 10589, 17856, 10583, LO1902, PL64744505GL, 17900, LO2663, LO2560, PL40695276AL, LRB11, LRB6, 1057 at 80% linkage distance. The genotypes with maximum genetic distance from individual clusters along with representative samples from low distance groups are likely to be chosen for the establishment of core collection.

Discussion

The loss of genetic diversity is a major threat for the maintenance and adaptive potential in genetic improvement of crop species (Olivera and Steffenson, 2009). Tomato is also enlisted among the autogamous crops, which have undergone loss of genetic diversity due to intense natural and artificial selection during domestication (Saavedra *et al*., 2001; Akhtar *et al.*, 2011). Huge genetic resources of tomato are available in various genebanks and are accessible worldwide for research purposes. Significant improvement in tomato along with basic information has been reported **(**Kamenetzky *et al*., 2010). The magnitude of diversity and availability of information on plant descriptors and agronomic data are crucial for crop improvement (Cruz *et al*., 2010). The first step to deal with any crop germplasm is to evaluate and characterize. We initiated characterization of seedling traits in tomato as a first step in the study.

For seedling traits of qualitative nature, the germplasm possessed almost all the categories reported, elsewhere representing optimum diversity for discrete

Table 4: Genetic distances between clusters based on *k-means* analyses for seedling traits in 380 genotypes of tomato

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
Cluster 2	1.09						
Cluster 3	0.85	1.12					
Cluster 4	1.18	0.74	0.90				
Cluster 5	0.79	0.85	0.74	0.83			
Cluster 6	1.20	0.85	1.19	0.76	0.85		
Cluster 7	0.85	1.05	0.98	1.25	0.97	1.26	
Cluster 8	1.27	1.56	0.71	1.14	1.24	1.61	1.36

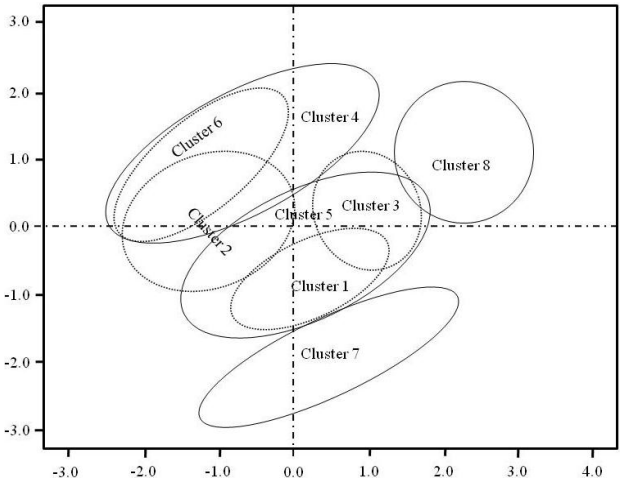

Fig. 1: Scatter diagram of tomato germpalsm based on nine seedling traits. Eight clusters were constructed on the basis of *k-means* clustering and the graph was plotted depicting individual genotypic position on *x-y* coordinates presenting the cluster number

traits in the tomato germplasm conserved in the genebank. Among measureable traits, hypocotyl length exhibited maximum variation. The hypocotyl length determines the plant vigor at later stage and a medium seedling height with stuff growth is likely to produce healthy plants. According to Ellis (1992) seed germination, vigor and size influence crop yield through both indirect and direct effects. Rasmussen and Rasmussen (2000) suggested potential possibilities for successful integrated weed control with the use of high quality seed to a weed harrowing strategy. Similar model can be adopted in tomato, where weeds are serious problem and to avoid chemical weedicides, the genotypes with better seedling vigor and quick growth habit could be selected to cope with weed control strategy.

A considerable variation was observed for most of the seedling traits. Among nine seedling traits, six were of discrete nature and can be incorporated as genotype identification singly or in combination (Smýkal *et al*., 2008). In the present study, tomato germplasm contained genotypes from various sources and the clustering pattern exhibited the formation of well characterized and coherent groups that indicated the practical value of data sets and analysis (Rodiño *et al*., 2010). In our study, two genotypes19901 (*S. habrochaites*) and 6836-9 (*S. lycopersicum*) were glabrous and there might be a link between the morphology and chemical composition of glandular trichomes in these two genotypes. Kang *et al.* (2010) has already reported that *hl*-mediated changes in tomato leaf surface traits correlate with decreased resistance to insect herbivory. In current study tomato seedlings fell in two categories on basis of purple color distribution in hpocotyl; one with purple colored hypocotyls and one category with green hypocotyls. Hypocotyls with purple color exhibited not only different levels but variation in color intensity was also obvious. Purple color in tomato vegetative tissues is a routine attributed to anthocyanins. Petunidin 3-(*p-coumaryl rutinoside*)-5-glucoside (Von Wettstein-Knowles, 1968) were extracted from tomato seedlings. These are group of purple, red and blue pigments (Mazza and Miniati, 1993), strong antioxidants and phytonutrients (Ames, 1983; Ames *et al*., 1993). Different levels of anthocyanins are attributed to various genes. Recessive anthocyanin free gene (*af*) have also been reported due to which anthocyanins are lacking in vegetative tissues (Burdick, 1958).

In the data analyzed genotypes of wild species were distributed in different clusters along *S. lycopersicum*. In the cluster 2 a genotype 19896 (*S. pimpenellifolium*) was seen to join at higher distance. Rick (1976) considered *S. pimpenellifolium* as either a direct ancestor of cultivated tomato or parallel evolution of both from a green fruited ancestor. Having close relationship and ease of backcrossing with *S. lycopersicum* it is considered a valuable source of germplasm. Tomato wild germplasm is known to contain various important genes like resistance to insects (Thurau *et al.,* 2010), diseases (Cano *et al.,* 2010) and introgressed successfully into cultivated tomato for improvement in breeding programs (Lin *et al.*, 2010). However, we intend to evaluate further these wild accessions in our studies. Estimation of genetic diversity and relationships between germplasm collections are important for facilitating efficient germplasm collection, evaluation and utilization **(**Rafalski, 2009). Terzopoulos and Bebeli (2010) reported wide intra-population diversity in tomato landraces and suggested modified approach to population characterization than that used for the homogeneous varieties.

In conclusion either used *per se* or as a very interesting genetic resource in breeding programs, investigations on the genetic structure of tomato landraces is of high importance. If the germplasm is characterized/evaluated for various traits, the spectrum of utilization will be enhanced. Further

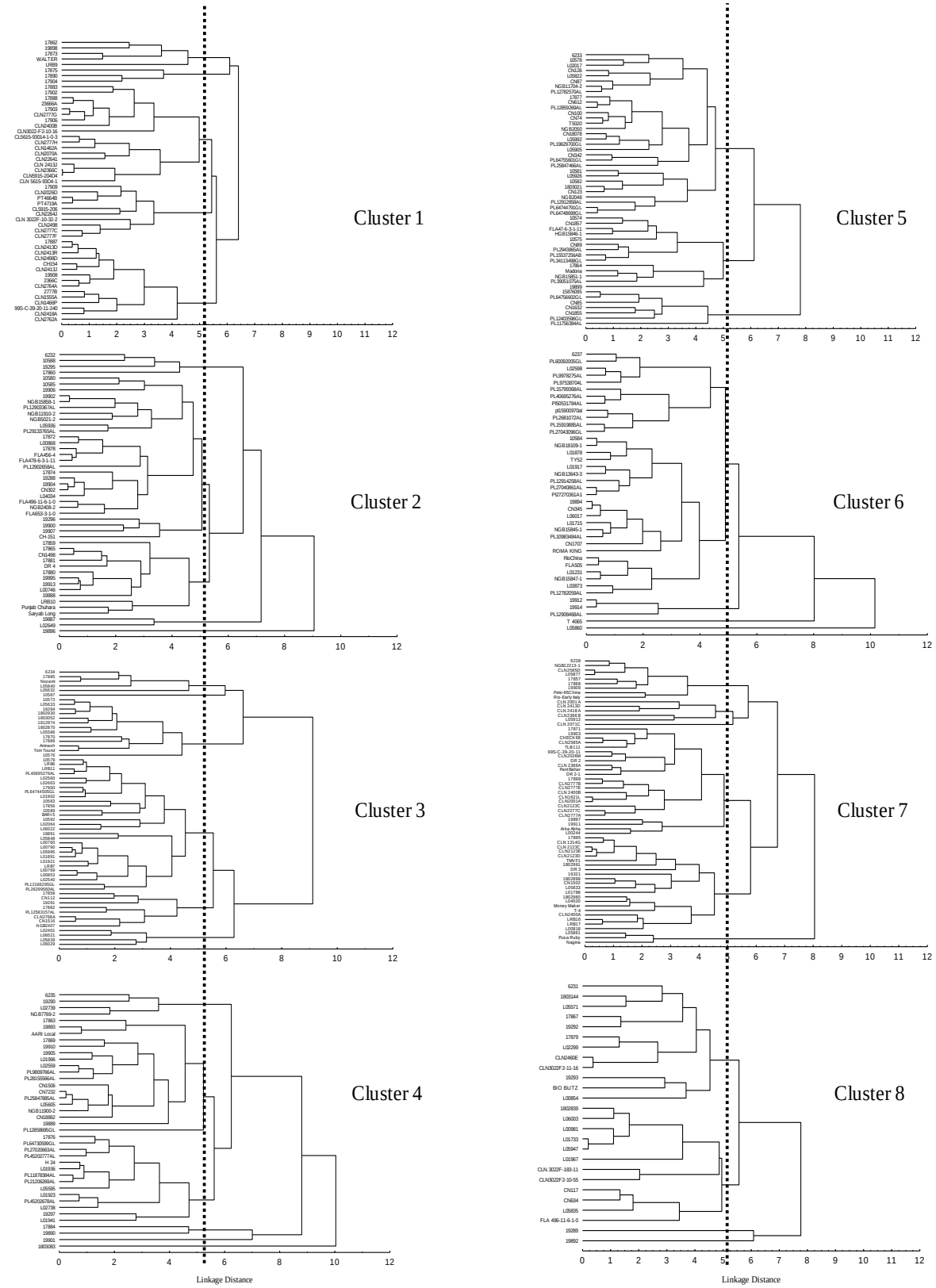

Fig. 2: Dendrograms of individual cluster of tomato germplasm grouped in to eight clusters on the basis of *k-means* clustering and the graph was plotted keeping linkage distance constant and the dotted line has been drawn at 50% dissimilarity. The names of genotypes are also mentioned in the Table 3 for individual clusters

characterization of tomato germplasm is suggested for broadening the use of core collections so as to use crop germplasm more efficiently in minimum time.

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