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ASSESSMENT OF GENETIC DIVERSITY IN TOMATO FOR FRUIT MORPHOLOGY, COMPOSITION AND YIELD

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Abstract

Postharvest losses are of great concern in vegetable crops, including tomato, due to their perishable nature. Damage to tomato fruit during transportation is related to its shape besides other factors. Therefore, this study was conducted to assess genetic diversity in the available tomato germplasm (35 genotypes) for fruit morphology, biochemical composition and yield. Considerable variation was observed for all studied traits except number of locules per fruit, TSS and reducing sugars, for which very low range was recorded. While, moderate level of variation existed for stem-end blockiness, blossom-end blockiness and elongated shape which suggested that fruit of most of the varieties could belong to more than one category of fruit shape. Fruit length and diameter had significantly positive correlation with heart shape of tomato, while later was also correlated with blossom-end-blockiness. Heart shape of tomato fruit was also correlated with stem-end blockiness. Yield showed significantly positive correlation with number of inflorescence per plant. Principal component analysis (PCA) revealed PC-I to PC-VI had Eigen values >1, which contributed 73.86% of total variability for different traits. The highest factor loading values for blossom-end-blockiness, stem-end-blockiness, heart shape and elongated shape was recorded in PC-6, PC-4, PC-6 and PC-2, respectively. Thirty five genotypes were grouped into three clusters. Higher yield and stem-end-blockiness was observed in genotypes from cluster II, while higher values for blossom-end-blockiness, heart shape and elongated shape were noticed in cluster I followed by cluster III. So, it can be assumed that genotypes in cluster II showed higher yield and also possessed blocky fruit, a desirable character for transportation and processing purpose. Moreover, it is suggested that genotypes of cluster I and cluster II can be crossed to find heterosis for yield and fruit shape related traits.

Key words: *Solanum lycopersicum*, Fruit shape, Blockiness, Processing, Correlation.

Introduction

Tomato (*Solanum lycopersicum* L.) is an important vegetable grown for fresh consumption (in the form of salad), cooking and processing (puree, paste, ketchup, sauce). It is grown mainly as Rabi crop, besides a minor Kharif crop, in Pakistan on 62.93 thousand hectares with production of 599.59 thousand tonnes. There is decline in total tomato production and per hectare yield (Anonymous, 2013) due to lack of locally bred material. Any breeding program cannot proceed successfully without availability of diverse germplasm as well as characterization of the available germplasm. Breeder should not only focus on yield and adaptability but should also seek the consumer preference. Consumers and farming community are more interested in shape and size of the tomato fruit and this preference vary in different geographical areas of the world; oblong shape is the most preferred one in Pakistan, while round shape is preferred in other countries. Several researchers have studied the genetics of fruit shape and size in tomato (Brewer *et al*., 2007; Gonzalo & van der Knaap, 2008; Xiao *et al*., 2008; Mazzucato *et al*., 2010; Osorio *et al*., 2011). The cultivated tomato (*Solanum lycopersicum*) exhibits great diversity in morphology of fruit *viz*., round, pear shaped, heart shaped, tapering, and pointed. Some varieties have fruit resembling the bell pepper fruit. International Union for the Protection of New Varieties of Plants (UPOV) and the International Plant Genetic Resources Institute (IPGRI; IPGRI, 1996; UPOV, 2001) has categorized the tomato varieties on the basis of fruit morphology (shape) into various classes.

Fruit shape is a quantitatively inherited trait that involves 4 to 17 loci (Gonzalo & van der Knaap, 2008). However, elongated fruit shape is controlled by only one major locus that also induces blockiness in tomato fruit (Grandillo *et al*., 1996). It is reported that blocky fruit with more length than width are preferred for processing (van der Knaap & Tanksley, 2003), because this character of tomato fruit prevents the fruit to rolling from conveyer belts (Visa *et al*., 2014). Blockiness can be at stem-end or blossom-end. Blossom-end blockiness was strongly correlated with fruit size (van der Knaap & Tanksley, 2003). While, stem-end blockiness was reported to be controlled by the same loci that controlled heart shape and both are strongly correlated (van der Knaap & Tanksley, 2003). Fruit shape and size are also affected pleiotropically by locule number (Rodriguez *et al*., 2011).

Several breeders have studied genetic diversity in tomato germplasm for improvement of various growth and yield related traits (El-Awady *et al*., 2012; Iqbal *et al*., 2014; Saleem *et al*., 2015). But, characterization of germplasm for fruit morphology and its correlation with fruit composition are scarce (Fernandez-Ruiz *et al*., 2004). Therefore, this study was conducted to assess genetic diversity in the available tomato germplasm (35 genotypes) for fruit morphology, biochemical composition and yield so that tomato genotypes suitable for processing and less susceptible to transportation shock can be selected.

Materials and Methods

Seeds of 35 genotypes, comprising of 26 tomato accessions and 9 local varieties were sown in nursery. These accessions were collected from Tomato Genetic

Resource Center (TGRC), USA, and Institute of Agri-Biotechnology and Genetic Resources, National Agricultural Research Centre (NARC), Pakistan. Local varieties were Nagina, Naqeeb, Riogrande, CCHaus, Pakit, Roma, Lyallpur I, Money Maker and Tibrido. Nursery was transplanted in field after 35 days on both sides of raised beds at a distance of 50 cm × 150 cm. Four plants of every genotype were grown in each of three replications in a randomized complete block design. Standard cultural practices and plant protection measures were followed. Data were recorded for fruit morphological, biochemical and yield related traits. Morphological parameters included fruit length (FL) in cm, fruit diameter (FD) in mm, fruit circumference (FC) in cm, number of locules per fruit (NLPF), pulp to seed ratio (PSR), stem-end blockiness (SEBlK), blossom-end blockiness (BEBlK), heart shape (HRT), and elongated shape (ES). Stem-end blockiness was calculated as the ratio of the width closest to the stem-end (10% below the top) of the fruit to the mid (maximum) width (van der Knaap & Tanksley, 2003). Blossom-end blockiness was calculated as the ratio of the width close to the blossom-end (10% above the bottom) of the fruit to the mid (maximum) width (van der Knaap & Tanksley, 2003). Heart shape was calculated as the ratio of fruit diameter at a distance 10% below the stem-end and 10% above the blossom-end of the fruit (van der Knaap & Tanksley, 2003). Elongated shape (Fruit shape index) was calculated as the ratio of length to the diameter of the fruit at midpoint (van der Knaap & Tanksley, 2003). Among biochemical parameters, data were collected for total soluble solids (TSS) in °Brix, titratable acidity (Aci) in % (Hortwitz, 1960), vitamin C (VitC) in mg 100 mL^{-1} of tomato pulp (Ruck, 1961), reducing sugars (RS) in %, non-reducing sugars (NRS) in %, and total sugars (TS) in % (Lane & Eynon, 1923). Yield related traits included number of fruit per truss (NPT), number of inflorescence per plant (NIPP), Single fruit weight (SFW) in g, and yield per plant in kg. Data were analyzed using analysis of variance technique (Steel *et al*., 1997). Agglomerative hierarchical clustering and principal component analysis was performed by using XLSTAT (Version 2014.5.04).

Results and Discussion

Correlation patterns: Considerable variation was observed for all traits except number of locules per fruit, TSS and reducing sugars, for which very low range was recorded. While, moderate level of variation existed for stem-end blockiness, blossom-end blockiness and elongated shape (Table 1). Moderate level of variation in fruit shape related traits suggested that fruit of most of the varieties can belong to more than one category of fruit shape. Our this statement is in line with the conclusion drawn by Visa *et al*. (2014) that fruit of some tomato varieties were related to one category of fruit shape, while some varieties possessed fruit that fit to more than one categories of fruit shape. Moreover, very low range for TSS and number of locules per fruit as well as other morphological traits (except fruit shape related traits) was corroborated by the findings of Mehta & Asati (2008).

Table 1. Mean values of different characters of tomato genotypes used in cluster analysis.

Variable	Minimum	Maximum	Mean	Std. Deviation
FPT	4.133	9.133	5.530	0.992
NIPP	17.600	51.533	37.600	8.864
SFW	6.933	71.467	36.853	13.651
NLPF	2.000	4.400	2.493	0.543
FL	19.227	61.381	39.789	7.275
FD	21.243	72.595	39.691	9.697
FC	7.733	15.667	12.656	1.690
PSR	1.667	13.533	7.568	2.822
SEBlK	0.559	0.877	0.750	0.077
BEBlK	1.000	1.647	1.355	0.153
HRT	0.644	1.157	1.003	0.097
ES	0.897	1.297	1.009	0.076
TSS	4.050	5.700	4.759	0.414
Aci	0.180	0.406	0.291	0.040
VitC	4.010	10.835	5.569	1.118
RS	3.465	4.585	4.038	0.241
TS	8.380	13.855	10.967	1.176
NRS	4.870	9.370	6.928	1.014
Yield	0.776	5.038	2.673	1.112

Significant correlation existed between various traits that can be exploited in planning breeding programs (Table 2). Number of flowers per truss showed significant positive correlation with number of inflorescence per plant, while significant negative correlation with elongated fruit shape. Number of inflorescence per plant was positively correlated with vitamin C content and fruit yield per plant. Single fruit weight exhibited significant positive correlation with number of locules per fruit, fruit length, fruit diameter, fruit circumference and pulp to seed ratio. Fruit length displayed significant positive correlation with fruit diameter, fruit circumference, pulp to seed ratio, heart shape of fruit and acidity. Similarly, fruit diameter also showed significantly positive correlation with fruit circumference, pulp to seed ratio, blossom-end blockiness and heart shape of fruit. Like fruit length and fruit diameter, fruit circumference also showed significant positive correlation with number of locules per fruit, pulp to seed ratio, heart shape of fruit and acidity. Pulp to seed ratio was significantly positively correlated with heart shape and acidity. Stem-end blockiness of fruit was significantly negatively correlated with blossom-end blockiness and vitamin C content of fruit but had significantly positive correlation with heart shape of fruit. Blossom-end blockiness of fruit exhibited significant positive correlation with heart shape of fruit. Reducing sugars were significantly positively associated with total and non-reducing sugars. Total sugars also had significant positive correlation with non-reducing sugars. Total soluble solids and vitamin C contents of fruit did not correlate with any of the mentioned traits. Existence of significant positive correlation between number of inflorescence per plant and yield indicates that fruit yield per plant can be increased by increasing number of inflorescence per plant. Significant positive correlation between single fruit weight, fruit length, fruit diameter and fruit circumference reflects that breeders can determine a single trait to reliably estimate others. While, significant positive correlation of heart shape of tomato fruit with fruit length, fruit diameter and fruit circumference depicts that heart shape of tomato fruit can be better predicted by these parameters. Significant positive correlation of heart shape of fruit with stem-end and blossom-end blockiness indicates that tomato fruit with heart shape will be blocky. Therefore, it is suggested that heart shape of fruit can be used for selection of blocky fruit, because blocky fruit (with more length than width) can bear transportation shocks better than non-heart shaped fruit and thus are suitable for processing (van der Knaap & Tanksley, 2003). These results were corroborated by findings of van der Knap & Tanksley (2003) who reported correlation between stem-end blockiness and fruit size (r=0.66) because both were controlled by the same loci. Non-significant correlation of TSS with titratable acidity and total sugars was in conformity to the findings of Fernandez-Ruiz *et al*. (2004). Significant correlation between number of inflorescence per plant and fruit yield per plant was in accordance to results of de Souza *et al*. (2012).

Table 2. Correlation coefficients among various traits of different tomato genotypes.

Variables	FPT	NIPP	SFW	NLPF	FL	FD	FC	PSR	SEBIK	BEBIK	HRT	ES	TSS	Aci	VitC	RS	TS	NRS	Yield
FPT	1																		
NIPP	0.480**	1																	
SFW	-0.117	-0.180	1																
NLPF	-0.058	-0.026	0.445**	1															
FL	-0.016	-0.241	0.631*	-0.031	1														
FD	-0.042	-0.182	0.663**	0.257	0.668**	1													
FC	-0.023	-0.062	0.807**	0.370*	0.489**	0.493**	1												
PSR	-0.057	-0.008	0.461**	0.092	0.418*	0.416*	0.644**	1											
SEBIK	-0.004	-0.332	-0.080	-0.050	0.162	-0.110	0.189	0.286	1										
BEBIK	0.020	0.251	0.317	0.192	0.200	0.417*	0.157	0.272	-0.577**	1									
HRT	0.028	-0.065	0.219	0.171	0.376*	0.354*	0.351*	0.580**	0.388*	0.516**	1								
ES	-0.352*	-0.273	0.293	-0.270	0.327	0.088	0.168	0.094	0.230	-0.164	-0.030	1							
TSS	-0.082	-0.221	0.096	0.116	-0.169	0.013	0.178	-0.007	0.104	-0.115	0.018	-0.021	1						
Aci	0.081	0.022	0.167	-0.058	0.416*	0.246	0.402*	0.415*	0.188	0.059	0.298	0.040	0.053	1					
VitC	0.071	0.373*	0.128	0.100	-0.053	-0.011	0.035	0.118	-0.356*	0.268	-0.116	-0.166	-0.224	-0.164	1				
RS	-0.118	-0.188	-0.126	-0.019	-0.123	-0.061	-0.097	-0.125	-0.037	-0.183	-0.229	-0.032	0.158	0.052	0.066	1			
TS	0.031	-0.166	0.016	-0.116	0.082	0.102	0.053	-0.110	0.051	-0.170	-0.129	-0.020	0.057	0.198	-0.106	0.727*	1		
NRS	0.064	-0.147	0.049	-0.130	0.125	0.133	0.084	-0.098	0.067	-0.153	-0.095	-0.015	0.028	0.217	-0.139	0.605**	0.987**	1	
Yield	0.269	0.476**	0.117	0.211	-0.042	0.030	0.238	0.297	-0.064	0.223	0.185	-0.217	-0.228	0.177	0.225	-0.136	-0.042	-0.016	1

Table 3. Principal component analysis for various traits in different tomato genotypes.

	PC1	PC2	PC3	PC4	PC5	PC6
Eigen value	4.207	3.102	2.332	1.792	1.443	1.157
Variability (%)	22.140	16.327	12.273	9.430	7.593	6.091
Cumulative %	22.140	38.467	50.740	60.170	67.763	73.855
Eigen vectors:						
Variables	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
FPT	-0.031	-0.163	0.259	0.367	0.007	0.031
NIPP	-0.064	-0.363	0.289	0.208	-0.094	-0.154
SFW	0.401	0.027	0.023	-0.288	0.039	-0.256
NLPF	0.166	-0.121	0.068	-0.221	0.556	-0.120
FL	0.364	0.126	-0.038	-0.010	-0.354	0.045
FD	0.369	0.049	0.085	-0.213	-0.090	0.201
FC	0.404	0.051	0.036	0.015	0.185	-0.312
PSR	0.370	-0.043	-0.028	0.207	0.017	-0.089
SEBlK	0.073	0.242	-0.284	0.470	0.158	-0.154
BEBlK	0.218	-0.287	0.180	-0.223	-0.106	0.471
HRT	0.311	-0.068	-0.081	0.262	0.089	0.434
ES	0.097	0.202	-0.296	-0.103	-0.381	-0.313
TSS	0.012	0.159	-0.096	-0.081	0.533	0.120
Aci	0.233	0.127	0.115	0.337	-0.071	0.079
VitC	0.011	-0.259	0.244	-0.202	-0.116	-0.345
RS	-0.100	0.339	0.337	-0.122	0.102	-0.030
TS	-0.019	0.415	0.428	0.013	-0.027	0.028
NRS	0.002	0.400	0.416	0.044	-0.055	0.040
Yield	0.119	-0.248	0.273	0.272	0.077	-0.271

Principal component analysis (PCA): Principal component analysis revealed that PC-I to PC-VI had Eigen values >1 and showed 22.10%, 16.33%, 12.27%, 9.43%, 7.59% and 6.09% variability, respectively (Table 3). Contribution of PC-I to PC-VI in total cumulative variability among the genotypes was 73.86%. PC-I exhibited positive factor loadings for all the studied traits except number of fruit truss^{-1}, number of inflorescence plant^{-1}, reducing sugars and total sugars. Number fruit truss^{-1}, number of inflorescence plant^{-1}, number of locules fruit^{-1}, pulp-to-seed ratio, blossom-end blockiness, heart shape, vitamin C contents and yield contributed negative factor loading to PC-II. Negative factor loading in PC-III was due to fruit length, pulp-to-seed ratio, stem-end blockiness, heart shape, elongated shape and TSS. PC-IV showed negative factor loading for single fruit weight, number of locules fruit^{-1}, fruit length, fruit diameter, blossom-end blockiness, elongated shape, TSS, vitamin C and reducing sugars. Most of the studied traits showed negative factor loading in PC-V except number of fruit truss^{-1}, single fruit weight, number of locules fruit^{-1}, fruit circumference, pulp-to-seed ratio, stem-end blockiness, heart shape, TSS, reducing sugars and yield. It is obvious that number of fruit truss^{-1}, fruit length, fruit diameter, blossom-end blockiness, heart shape, TSS, acidity, non-reducing sugars and total sugars were positive factor loaders for PC-VI. Fruit related traits i.e. single fruit weight, fruit length, fruit diameter, fruit circumference, and pulp-to-seed ratio, had higher contribution to PC-I than all other traits and therefore, can be termed as fruit axis. Sugars (reducing, non-reducing and total) contributed to the maximum extent to PC-II and PC-III, which can be regarded as sugar axis. Stem-end blockiness contributed maximum share in PC-IV, while blossom-end blockiness followed by heart shape were the highest contributors to PC-VI, so these two collectively can be regarded as fruit shape axis. The highest contribution to PC-V was by number of locules per fruit followed by TSS. Our results are supported by the findings of Merk *et al*. (2012) and Iqbal *et al*. (2014) who observed 28% and 44.20% of the variance for PC-I that was greatly influenced by the values of traits related to fruit size and weight in tomato i.e. single fruit weight, fruit length, fruit diameter, fruit circumference and pulp-to-seed ratio. This study also revealed that PC-IV and PC-VI had maximum contribution of traits (stem-end blockiness and blossom-end blockiness), which determine the processing value and heart shape fruit (with more length than width) of tomato. So, breeders can use principal component analysis to assess the trait(s) which cause differences in the germplasm.

Association between PC-I and PC-II, which contributed 38.47% of total variability, was perceived by plotting PC-I on X-axis and PC-II on Y-axis (Fig. 1). It can be visualized that single fruit weight was positively correlated with fruit length, fruit diameter, fruit circumference, stem-end-blockiness, elongated shape, TSS, acidity and non-reducing sugars. While, number of locules per fruit was positively correlated with pulp-to-seed ratio, blossom-end blockiness, heart shape, vitamin C contents and yield. It is also obvious that number of fruit per plant, number of inflorescence per plant, reducing sugars and total sugars were negatively correlated with all other plant and fruit traits as well as yield. Previously, Iqbal *et al*. (2014) also reported positive correlation between single fruit weight and fruit diameter, which corroborates our results.

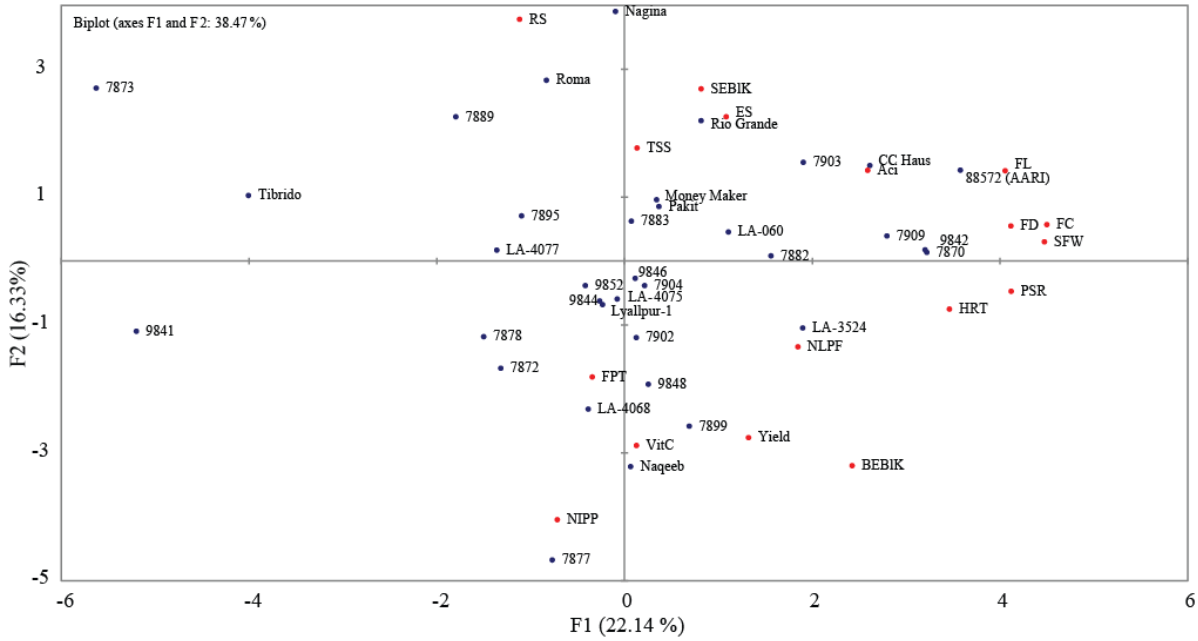

Fig. 1. Bi-plot of tomato genotypes for first two principal components.
(Note: Due to limited space and more number of genotypes, last 4-digits of genotypes from NARC have been mentioned; e.g. 7877 is actually NARC-017877. While, complete accession number or names of others genotypes have been mentioned)

Table 4. Distribution of different tomato genotypes in clusters.

Clusters	Genotypes
Cluster I	NARC-017870, NARC-017909, 88572(AARI)*, CC-Haus
Cluster II	NARC-017872, NARC-017873, NARC-017877, NARC-017878, NARC-017883, NARC-017889, NARC-017895, NARC-017899, NARC-017902, NARC-019841, NARC-019844, LA-4077, Lyalpur-1, Tibrido
Cluster III	NARC-017882, NARC-017903, NARC-017904, NARC-019842, NARC-019846, NARC-019848, NARC-019852, LA-3524, LA-4060, LA-4068, LA-4075, Money Maker, Roma, Naqeeb, Pakit, Rio-Grande, Nagina

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Cluster analysis: Cluster analysis was also performed for grouping of tomato genotypes because it has been regarded as an effective and reliable method to plan breeding program keeping in view the classification pattern of germplasm (Feng-Mei *et al*., 2006; Iqbal *et al*., 2014). Agglomerative hierarchical clustering grouped 35 genotypes in three clusters on the basis of studied parameters (Fig. 2). Cluster I consisted of four (11.4%) genotypes, while cluster II and cluster III comprised of 14 (40.0%) and 17 (48.6%) genotypes, respectively (Table 4). Cluster II contained genotypes which had higher number of inflorescence plant^{-1}, number of fruit truss^{-1}, non-reducing sugars, total sugars and yield but exhibited least fruit weight, fruit size (length, diameter and circumference) and pulp-to-seed ratio. Genotypes in cluster I were characterized by highest values of single fruit weight, fruit length, fruit diameter, fruit circumference, and pulp-to-seed ratio. Yield contributed maximum genetic divergence between cluster III and cluster II because all high yielding genotypes (3.490 kg plant^{-1}) were grouped in cluster II, while cluster III comprised of genotypes with the lowest yielding capacity (1.470 kg plant^{-1}). There was negligible difference in number of locules fruit^{-1}, stem-end blockiness, blossom-end blockiness, heart shape and elongated shapes of fruit in different clusters, although the least values of blossom-end blockiness, heart shape and elongated shapes of fruit was noted in cluster II, while maximum in cluster I. Moreover, it can be assumed from results that genotypes in cluster I possessed heart shaped fruit which are suitable for fresh consumption, while cluster II and cluster III had blocky fruit that are suitable for processing. So, it is suggested that suitable genotypes from these two clusters (I and II), which exhibited maximum genetic divergence for fruit shape related traits as well as for yield, should be selected while breeding for fruit shape, because Iqbal *et al*. (2014) and Zia-ul-Qamar *et al*. (2012) stated that traits contributing more in genetic divergence should be given priority over others while selecting clusters suitable for selection of parents for hybridization (Table 5). These two clusters (I and II) also showed the highest genetic divergence for fruit weight, fruit size (length, diameter and circumference), number of inflorescence plant^{-1} and number of fruit truss^{-1} and thus crossing genotypes of these two clusters may show more hybrid vigor in F_1. Moreover, gene action studies are also possible on the basis of these results by employing genotypes expressing any specific trait at very high level, as suggested earlier by Saleem *et al*. (2009). It can also be assumed from high value of number of inflorescence plant, number of fruit truss^{-1} and yield in cluster II that these two former traits are positively affecting the yield of tomato as concluded by de Souza *et al*. (2012). It can be visualized form results of D^2 statistics that cluster II was more close to cluster III with genetic distance of 19.78, while the greatest genetic distance (46.59) was observed between cluster I and cluster II (Table 6).

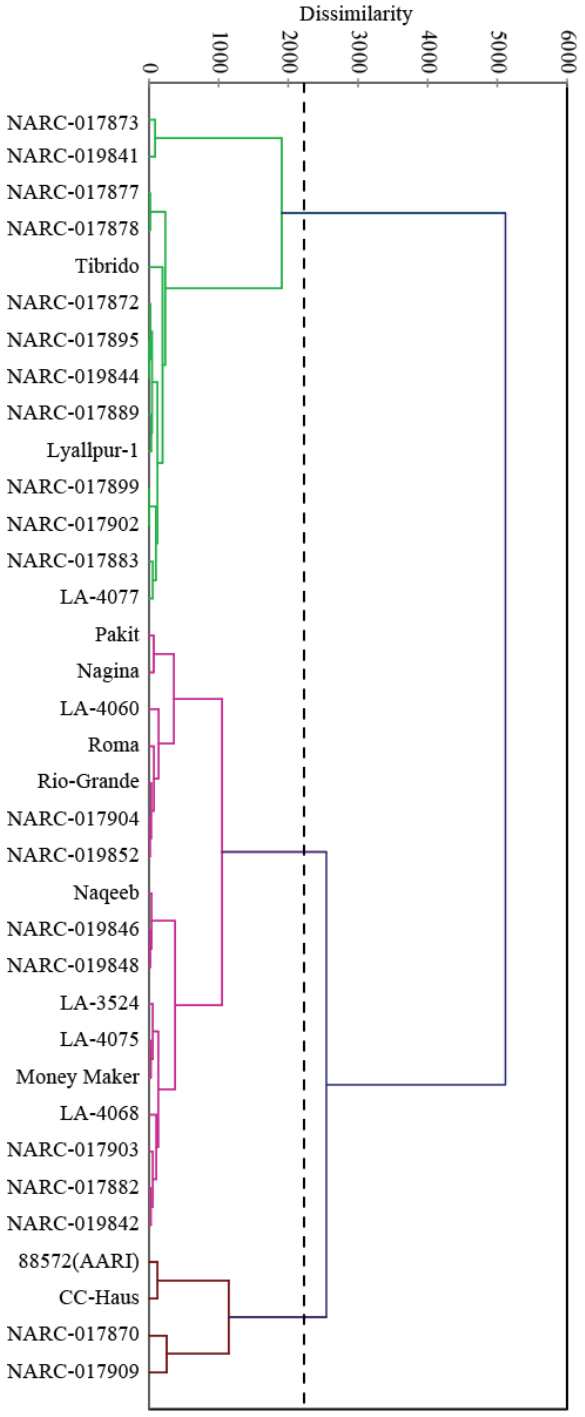

Fig. 2. Dendrogram on the basis of 19 traits of various tomato genotypes.

Table 6. D^2 statistics showing genetic divergence among the clusters of tomato genotypes.

	Cluster 1	Cluster 2	Cluster 3
Cluster 1	0		
Cluster 2	46.594	0	
Cluster 3	28.047	19.777	0

Table 5. Average values of various characters of different tomato genotypes in cluster analysis.

Traits	Clusters		
	1	2	3
FPT	4.400	6.800	6.733
NIPP	28.200	44.200	34.600
SFW	55.200	26.933	43.067
NLPF	2.400	2.667	2.333
FL	61.381	36.175	41.765
FD	69.162	32.320	41.647
FC	13.900	11.800	12.200
PSR	8.000	6.067	7.400
SEBlK	0.706	0.792	0.721
BEBlK	1.483	1.149	1.421
HRT	1.046	0.908	1.012
ES	1.077	0.973	1.060
TSS	4.300	4.650	4.950
Aci	0.320	0.320	0.240
VitC	5.215	6.020	6.020
RS	4.120	4.485	4.013
TS	11.120	13.855	10.225
NRS	7.000	9.370	6.212
Yield	1.541	3.490	1.470

Conclusion

It can be concluded from results of this study that fruit length and diameter were positively correlated with heart shape of tomato, while fruit diameter was also correlated with blossom-end-blockiness. Heart shape of tomato fruit was also correlated with stem-end blockiness. While, number of inflorescence plant^{-1} can be reliably used to predict tomato yield. Genotypes of cluster I and cluster II can be crossed to find heterosis for yield and fruit shape related traits. Alternatively, the best performing genotypes in cluster II can be included in national uniformity yield trial for release as a variety.

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