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Studies on genetic diversity in tomato (*Solanum lycopersicum* L.)

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Abstract
Sixty tomato genotypes were evaluated during Rabi, 2015 at Horticultural Research Station, Venkataramannagudem, West Godavari district, Andhra Pradesh to study the genetic diversity. Multivariate analysis following D^2 statistics revealed that considerable genetic diversity exists within and among the eight clusters. Genetic diversity studies for twenty attributes with respect to growth, earliness, yield and ToLCV resistance for sixty genotypes of tomato revealed that average fruit weight contributed maximum towards divergence followed by number of fruits per plant and number of locules per fruit. The sixty genotypes were grouped into eight clusters, among which cluster VII was the largest group comprising of 20 genotypes, followed by cluster II with thirteen genotypes, cluster I with ten genotypes, cluster V with eight genotypes, cluster VIII with six genotypes and cluster III, IV and VI were monotypic or solitary. The intra cluster distance varied from 0 to 382.80 and cluster VIII recorded highest intra cluster distance (382.80) followed by cluster V (271.17), cluster VII (215.88), cluster II (154.35) and cluster I (98.04).. Highest inter cluster distance (1296.49) was between cluster V and VIII, while the lowest (130.38) was between cluster I and VI. The inter cluster distance was minimum between cluster I and VI indicating narrow genetic diversity, where as the inter cluster distance was maximum between V and VIII followed by II and VIII (852.84) indicating wider genetic diversity between these groups. Selection of parents from these diverse clusters for hybridization would help in achieving novel recombinants.

Keywords: Tomato, genetic diversity, cluster analysis, D^2 statistics

Introduction
Tomato (*Solanumlycopersicum* L.) (2n = 24) belonging to the family Solanaceae is one of the most popular vegetable crops grown in India as well as all over the world. It originated in Peru Equador region of Latin America and has become one of the most widely grown vegetables with an ability to grow in diverse environmental conditions (Rice *et al*, 1987) [11]. Globally major tomato producing countries are China, India, USA, Turkey, Ezypt, Iran, Italy, Spain, Brazil and Mexico (FAOSTAT, 2015). In India, it occupies an area of 0.882 million hectares with a production of 18.74 million tonnes with a productivity of 21.20 tonnes per hectare. Tomato is widely grown in both Andhra Pradesh and Telangana states. In Andhra Pradesh tomato is grown in an area of 167.72 thousand hectares with a production of 3.35 million tonnes, with an average productivity of 21.20 t/ha (NHB, 2015) [8]. Even though tomato is cultivated in vast area, national average fruit yield is less compared to other countries like Japan, USA and Israel, because tomato crop is being cultivated under rain fed irrigation in several parts of the country. Moreover tomato is grown throughout the year and the varieties cultivated by many of the farmers are susceptible to biotic factors like ToLCV and abiotic factors like high temperatures during kharif and summer which goes beyond 35 ^{0} C in several parts of India. In India, tomato improvement programmes were initiated around the year 1950 when exotic cultivars were introduced by the Division of Botany, IARI, New Delhi (Bose *et al*. 2002) [2]. Improvement in yield and quality is normally achieved by selecting genotypes with desirable character combinations existing in the nature or by hybridization. Selection of parents on the basis of genetic diversity would be more promising for exploitation of hybrid vigour. It is well established fact that the more diverse the parents, greater the chances of obtaining high heterotic F_1s and broad spectrum of variability in the segregating generations (Arunachalam. 1981) [1]. Keeping this in view, present investigation was taken up to study the genetic divergence among the selected tomato germplasm.

Materials and Methods
The experimental material comprising of sixty genotypes of tomato were obtained from

NBPGR, Regional Centre, Rajendranagar, Hyderabad and AVRDC, Taipei, Taiwan. Evaluation of these accessions was carried out to estimate the variability and for grouping them into different clusters using D^2 statistics of Mahalanobis. The genotypes were evaluated in randomized block design with three replications during *Rabi*, 2015 at Horticultural Research Station, Dr. Y.S.R. Horticultural University, Venkataramannagudem, Andhra Pradesh, India. The seeds of sixty genotypes were sown in portrays September, 2015 and twenty one days old seedlings of selected genotypes were transplanted in the experimental field during October, 2015. In each replication, each genotype was grown in a single row plot of 4.5 m length at spacing of 60 x 45 cm in three replications accommodating 10 plants in each replication. The recommended package of practices was followed for raising all the genotypes. Necessary prophylactic plant protection measures were taken up to safe guard the genotypes from pests and diseases. Data were recorded on ten randomly selected plants from each genotype and each replication and their means were worked out for statistical analysis. The mean values of data were subjected to analysis of variance as described by Gomez and Gomez (1983) [4]. The observations were recorded on plant height (cm), number of primary branches per plant, number of fruits per cluster, number of clusters per plant, days to 50 per cent flowering, fruit set (%), days to fruit harvest, days to last harvest, number of fruits per cluster, number of fruits per plant, fruit length (cm), fruit width (cm), average fruit weight (g), fruit yield per plant, number of locules per fruit, titrable acidity (%), ascorbic acid content (mg/100 g), total soluble solids (° Brix), lycopene content (mg/100 g) and percentage incidence of ToLCV. The genetic diversity was estimated following Mahalanobis's (1936) [5] generalized distance (D^2) extended by Rao (1952) [10]. Based on the D^2 values, the genotypes were grouped into clusters following the method suggested by Tocher (Rao, 1952) [10]. Intra and inter cluster distances were calculated by the methods of Singh and Chaudhary (1985) [12].

Results and Discussion

Grouping of genotypes into various clusters

Sixty genotypes of tomato were grouped into eight clusters based on the estimated D^2 values based on square of the generalized distance as per the procedure suggested by Tocher (Rao, 1952) [10]. The pattern of distribution of sixty genotypes into different clusters is shown in Table.1. Out of eight clusters formed, cluster VII was the largest group comprising of 20 genotypes, followed by cluster II with thirteen genotypes, cluster I with ten genotypes, cluster V with eight genotypes, cluster VIII with six genotypes and cluster III, IV and VI were monotypic or solitary.

Table 1: Clustering pattern of variousgenotypes of tomato

Cluster	No. of genotypes	Genotypes
I	10	VRSL- 11,13,27, 30,45,51,52,57,60
II	13	VRSL- 9, 12, 21, 29, 32, 34, 38, 39, 44, 46, 48,55,58
III	1	VRSL- 53
IV	1	VRSL- 3
V	8	VRSL-19,23,26,35,36,37,41,49
VI	1	VRSL- 25
VII	20	VRSL- 1,3,5,7,8,10,14,17,18,20,22,24,28, 33,40,42,43,47,54,56
VIII	6	VRSL- 2,4,6,15,16,50

Average intra and inter cluster distances

The mean intra and inter cluster D^2 values among the eight clusters are presented in Table 2 (Fig.1) and the nearest and farthest cluster from each cluster based on D^2 values is given in the Table3. The intra cluster distance varied from 0 to 382.80 and cluster VIII recorded maximum D^2 value (382.80) followed by cluster V (271.17), cluster VII (215.88), cluster II (154.35) and cluster I (98.04). Intra cluster distances were not observed in cluster III, IV and VI as they were represented by single genotype.The inter cluster D^2 values revealed that highest inter cluster distance (1296.49) was between cluster V and VIII, while the lowest (130.38) was between cluster I and VI. The inter cluster distance was minimum between cluster I and VI indicating narrow genetic diversity, where as the inter cluster distance was maximum between V and VIII followed by II and VIII (852.84) indicating wider genetic diversity between these groups. Selection of parents from these diverse clusters for hybridization would help in achieving novel recombinants. Similar type of observations were reported by Omprakash and Vijay Bahadur (2013) [6], Rajasekhar Reddy *et al.* (2013 a) [9] and Meena and Bahadur (2015) [7]. The clusters with single genotype indicated their independent identity and importance due to various unique characters possessed by them.

Table 2: Average intra (bold) and inter cluster D^2 values for eight clusters of various genotypes

	I	II	III	IV	V	VI	VII	VIII
I	98.04	160.24	146.87	174.36	386.94	130.38	259.01	703.28
II		154.35	247.61	231.95	311.03	256.46	310.19	852.84
III			0.00	249.14	632.26	135.72	279.55	433.19
IV				0.00	447.75	171.10	304.57	796.79
V					271.17	490.45	527.83	1296.49
VI						0.00	222.71	484.79
VII							215.88	699.79
VIII								382.80

Table 3: Nearest and farthest clusters from each cluster based on D^2 values in tomato germplasm

Cluster No.	Nearest cluster with D^2 value	farthest clusters with D^2 value
I	VI (130.38)	VIII (703.28)
II	I (160.24)	VIII (852.84)
III	VI (135.72)	V(632.26)
IV	VI (171.10)	VIII (796.79)
V	II (311.03)	VIII (1296.49)
VI	I (130.38)	V (490.45)
VII	IV (222.71)	VIII (699.79)
VIII	III (433.19)	I (703.28)

Values in parentheses indicate D^2 values

Performance of characters in clusters

The cluster means for each of twenty traits are presented in Table 4. From the data it can be concluded that considerable differences exist for all the traits studied. The genotypes belonging to cluster VI recorded highest mean plant height (161.17 cm) followed by cluster V (158.63 cm), while genotypes belonging to cluster VII recorded lowest average plant height (130.42 cm) followed by cluster IV (133.47 cm). Number of primary branches per plant was highest in cluster IV (4.00) followed by cluster III and VIII (3.33), while least number of primary branches per plant was recorded in cluster VI (3.00). The trait, days to 50 per cent flowering recorded minimum value in the genotypes of cluster VIII (35.56 days) followed by cluster I (35.63 days), while genotypes of clusters V (39.96 days) exhibited maximum mean value for days to 50 per cent flowering. The genotypes belonging to cluster IV

recorded highest mean number of clusters per plant (58.67) followed by cluster VIII (56.72), whereas minimum number of clusters per plant was recorded in cluster I (23.97). Highest mean number of flowers per cluster was recorded in cluster VIII (6.18) followed by cluster II (6.05) with least number of flowers per cluster in cluster VI (4.22).The percentage of fruit set was highest in cluster III (66.29%) followed by cluster VI (56.71%), whereas least percentage of fruit set was exhibited by genotypes belonging to the cluster IV (43.60%). Days to first fruit harvest was least in cluster VIII (67.33 days) followed by cluster VI (69.00 days) with more number of days to first fruit harvest in cluster III and IV (75.00 days). More number of days to last fruit harvest was observed in cluster V (177.54 days) followed by cluster VII (172.65 days), while least number of days to last fruit harvest was reported in cluster VI (162.67).The genotypes belonging to cluster III (3.39) recorded highest mean number of fruits per cluster followed by cluster VIII (3.23), where as minimum number of fruits per cluster was recorded by cluster IV (2.08). Highest number of fruits per plant was recorded in cluster VIII (76.47) followed by cluster III (38.67) with least number of fruits per plant in cluster V (11.15).The maximum fruit length was recorded in genotypes of cluster III (6.17 cm) followed by cluster V (5.92 cm), where as minimum fruit length was registered in genotypes of cluster VIII (3.72 cm). Highest fruit diameter was noticed in cluster V (5.00 cm) followed by cluster I (4.73 cm) and least fruit diameter was recorded in the genotypes of cluster VIII (4.09 cm).The genotypes of cluster V recorded highest average fruit weight (122.19 g) followed by cluster II (78.17 g), while the genotypes of cluster VIII (17.08 g) recorded least fruit weight. Maximum mean value for fruit yield per plant was recorded in cluster VI (1.76 kg) followed by cluster VII (1.39 kg). Whereas, mean fruit yield per plant value was lowest in genotypes of cluster IV (0.73 kg).Number of locules per fruit was highest in cluster VI and VIII (3.00) followed by cluster VII (2.97), while the least number of locules per fruit was recorded in cluster III and IV (2.00).The highest mean value for total soluble solids was recorded in genotypes of cluster VIII (5.25 ^{0} Brix) followed by cluster III (4.87 ^{0} Brix), whereas the least mean value was exhibited in cluster IV (3.00). Titrabe acidity was highest in cluster V (0.58%) followed by cluster VIII (0.47%), whereas least value of titrable acidity was recorded in cluster III (0.22%).The genotypes belonging to cluster VI recorded highest ascorbic acid content (24.97 mg/100g) followed by cluster IV (23.92 mg/100g), where as the lowest ascorbic acid content was recorded in cluster VIII (19.80 mg/100g). Highest lycopene content was exhibited by the genotypes of cluster VIII (5.64 mg/100g) followed by cluster I (5.81 mg/100g) with least ascorbic acid content by cluster VI (4.33 mg/100g).Lowest percentage of tomato leaf curl virus (ToLCV) incidence in field screening was recorded in cluster III and IV (0.00) followed by cluster I (0.67%), where as cluster VII (14.33%) recorded highest percentage of tomato leaf curl virus (ToLCV). Mean values of different clusters indicated that a wide range of mean values existed for the traits studied indicating the presence of wide variation among the genotypes.

Relative contribution of characters towards diversity

The character that appears maximum number of times ranks first and greater is its contribution to genetic divergence. Number of times each of twenty traits appeared in first rank and its relative per cent contribution towards genetic divergence are presented in Table5 (Fig. 2). The results showed that the character, average fruit weight contributed maximum (25.31%) towards diversity by taking first rank 448 times, followed by number of fruits per plant (20.79%) by taking 368 times first ranking, number of locules per fruit (13.56%) by 240 times, fruit yield per plant (9.27%) by 164 times, plant height (4.35%) by 77 times, fruit length (4.29%) by 76 times, days to 50% flowering (3.90% by 69 times, TSS (3.84%) by 68 times, number of flowers per cluster (3.79%) by 67 times, percentage of ToLCV incidence (2.77%) by 49 times, percentage fruit set (2.03%) by 36 times, lycopene content (1.81%) by 32 times, fruit diameter (1.30%) by 23 times, titrable acidity (0.96%) by 17 times, number of fruits per cluster (0.73%) by 13 times, ascorbic acid content (0.62%) by 11 times, number of clusters per plant (0.57%) by 10 times and days to first harvest (0.11 5) by 2 times. The traits number of primary branches per plant and days to last fruit harvest did not contribute towards total diversity. Raja Sekhar Reddy *et al.* (2013 a) [9] and Ullah *et al.* (2015 a) [13] also reported similar kind of results.

Genetic divergence is one of the important factors for any crop improvement programme. The divergence study is important as the germplasm lines are of wide varied origin and are highly variable regarding their production potential and resistance to biotic and abiotic stresses. Divergence analysis generates valuable information on the nature and degree of genetic diversity, which is useful for the selection of desirable lines from germplasm for a successful breeding programme. Selection of parents/lines based on an individual attribute may not be as appropriate as the one based on a number of important traits collectively. Involvement of genetically diverse parents is essential to generate variability and to enhance yield, quality and resistance to biotic stresses. As breeding for resistance is the only solution to combat certain diseases, the study of diversity has become imperative to develop high yielding as well as disease resistant hybrids. The multivariate analysis using Mahalanobis D^2 static is a valuable tool for obtaining quantitative estimates of divergence between biological populations. Apart from high divergence, performance of the genotypes and characters with maximum contribution towards divergence should also be given due consideration which appears as desirable for inclusion in tomato improvement.Hence, apart from selection of genotypes from the clusters which have high inter-cluster distance for hybridization, one can also think of selecting parents based on extent of genetic divergence in respect of a particular character of interest. This means that if breeder's intention is to improve fruit yield or disease resistance. He can select the parents which are having high yield potential and disease resistance genes with high divergence to these characters.

Table 4: Mean values of clusters for 20 characters in various genotypes of tomato

Cluster	Plant height (cm)	No of primary branches	Days to 50% flowering	No. of clusters/ plant	No. of flowers/ cluster	Fruit set (%)	Days to first harvest	Days to last harvest	No. of fruits/ cluster	No. of fruits/plant	Fruit length (cm)	Fruit diameter (cm)	Average fruit weight (g)	Fruit yield /plant (kg)	No of locules/ fruit	TSS (^{O} Brix)	Titrable acidity (%)	Ascrobic acid content (mg/100g)	Lycopene content (mg/100g)	ToLCV incidence (%)
I	144.13	3.10	35.63	23.97	4.64	55.12	72.50	167.77	2.54	22.40	5.49	4.73	63.16	1.34	2.43	4.34	0.44	21.67	5.61	0.67
II	141.20	3.10	36.87	35.49	6.05	54.17	73.13	170.13	3.21	15.41	5.62	4.59	78.17	1.17	2.23	4.32	0.46	21.80	5.47	5.13
III	139.81	3.33	36.67	31.33	5.11	66.29	75.00	172.00	3.39	38.67	6.17	4.13	28.70	1.07	2.00	4.87	0.22	23.90	5.56	0.00
IV	133.47	4.00	36.67	58.67	4.78	43.60	75.00	171.67	2.08	16.95	3.33	4.50	50.70	0.73	2.00	3.00	0.32	23.92	4.86	0.00
V	158.63	3.08	39.96	43.50	5.75	50.30	74.50	177.54	2.77	11.15	5.92	5.00	122.19	1.28	2.88	4.10	0.58	21.60	5.28	2.08
VI	161.17	3.00	35.67	33.33	4.22	56.71	69.00	162.67	2.39	35.68	4.13	4.37	48.60	1.76	3.00	4.57	0.27	24.97	4.33	6.67
VII	130.42	3.20	36.97	36.00	5.37	56.66	69.35	172.65	2.95	26.47	4.52	4.28	53.20	1.39	2.97	4.54	0.41	22.16	5.10	14.33
VIII	139.33	3.33	35.56	56.72	6.18	52.15	67.33	171.28	3.23	76.47	3.72	4.09	17.08	1.29	3.00	5.25	0.47	19.80	5.64	11.67

Table 5: Percent contribution of different characters towards genetic divergence

S.No	Source	Times Ranked 1st	Contribution%
1.	Plant height (cm)	77	4.35
2.	Number of primary branches	0	0.00
3.	Number of clusters per plant	10	0.57
4.	Number of flowers per cluster	67	3.79
5.	Days to 50% Flowering	69	3.90
6.	Fruit Set (%)	36	2.03
7.	Days to First harvest	2	0.11
8.	Days to Last harvest	0	0.00
9.	Number of fruits per cluster	13	0.73
10.	Number of fruits per plant	368	20.79
11.	Fruit Length (cm)	76	4.29
12.	Fruit diameter (cm)	23	1.30
13.	Averagefruit weight (g)	448	25.31
14.	Fruit yield per plant (g)	164	9.27
15.	Number of locules perfruit	240	13.56
16.	TSS (^{o} Brix)	68	3.84
17.	Titrableacidity (%)	17	0.96
18.	Ascrobicacid content (mg/100 g)	11	0.62
19.	Lycopene content (mg/100g)	32	1.81
20.	ToLCV Incidence (%)	49	2.77

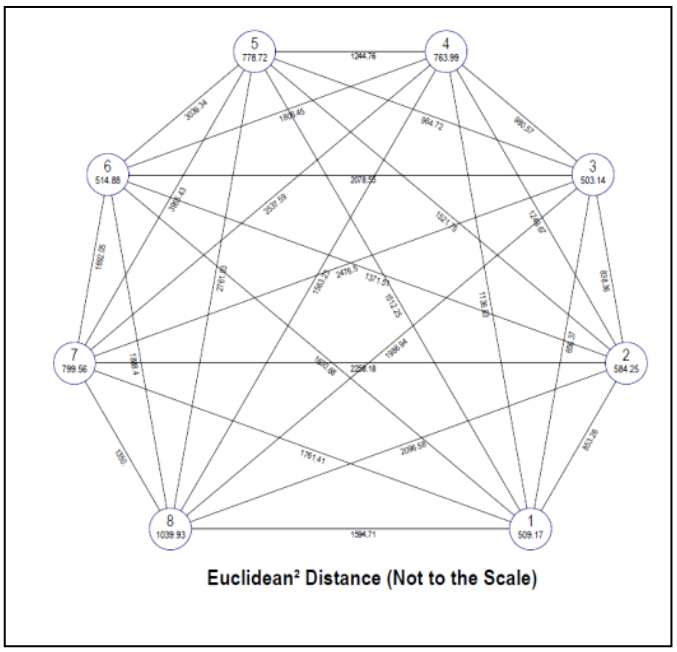

Fig 1: Diagram depicting the distances between different clusters of tomato germ plasm (Not to scale)

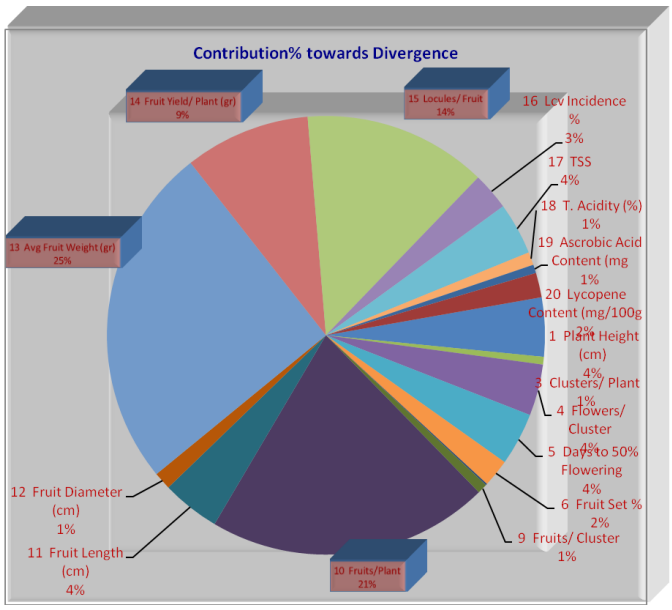

Fig 2: Percentage of contribution of different traits towards divergence of tomato germplasm

Conclusion

Highest inter cluster distance was recorded between cluster V and cluster VII which indicates that crossing genotypes belonging to cluster V with genotypes belonging to cluster VIII in hybridization programme will result in manifestation of higher magnitude of heterosis.

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