



BREEDING POTENTIAL OF INDETERMINATE TOMATO (*Solanum lycopersicum* L.) ACCESSIONS USING D^2 ANALYSIS

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SUMMARY

The exploration of genetic diversity is a pre-requisite in any breeding program for effective selection of superior accessions. Hence, a study was conducted on 19 accessions of tomato collected from Indian Institute of Vegetable Research, Varanasi and Vegetable Research Station, Junagadh Agricultural University, Junagadh, India, to assess the value and magnitude of genetic divergence using Mahalanobis D^2 statistics. Wide genetic diversity was observed among the accessions which were grouped into five clusters by Tocher's method based on D^2 values. The clusters III and IV contained highest number of accessions (6) followed by clusters I and II both had 3 accessions and cluster V had one accession. The clustering pattern indicated that there was no association between geographical distribution of accessions and genetic divergence. The diversity among the clusters was measured by inter-cluster distance. The maximum inter-cluster D^2 value was observed between the cluster I and IV (11347.2) followed by cluster I and III (10921.8). Therefore, selection of divergent parents based on these cluster distance would be useful in selecting accessions for hybridization and formulating a comprehensive strategy to develop superior hybrids or superior segregants in tomato. The information so generated can be effectively utilized for improving the specific traits in future breeding programs of tomato. Cluster mean analysis indicated cluster I showed maximum performance for fruit yield per plant (3270.6 g), cluster II recorded minimum days to flowering (57.3 days) and cluster V showed low mean for leaf curl incidence percentage (20.0). The accessions in cluster I, II and V could serve as direct source for development of high yielding varieties, early flowering and resistance to leaf curl disease, respectively.

Keywords: Breeding potential, genetic divergence, multivariate analysis, tomato, yield

Short statement summary: Information on the extent of genetic diversity among accessions is very important in crosses between groups with maximum genetic divergence that would be more responsive for improvement since they are likely to produce desirable recombination and segregation in their progenies after hybridization. To have this type of knowledge, research on genetic diversity is very essential.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.), a member of family Solanaceae is one of the most important vegetable crops in India as well as around the world (Cheema and Dhaliwal, 2005; Kumar *et al.*, 2010; Kumar and Dudi, 2011; Osekita and Ademiluyi, 2014). It has a chromosome number of $2n = 24$ (Rick, 1969). Tomato is native of West Coast of South America (Mexico and Peru) and was cultivated by Indians about 500 B.C. long before arrival of Spaniards (Rehman *et al.*, 2000; Tasisa *et al.*, 2012). Tomato is the most important vegetable crop next only to potato because of its wider adaptability, high yielding potential and multipurpose uses (Reddy *et al.*, 2013b). In India, tomato occupies an area of 0.87 million ha with a production of 17.50 million ton and productivity of 20.11 tons per hectare (FAO, 2012). Tomato is grown as annual or short lived perennial herbaceous plants. It has a taproot, and the growth habit of the plant is determinate, semi-determinate and indeterminate. It finds a very important role in every kitchen with enormous role in food and nutritional security. It also has a very important and significant position in the post-harvest industry (Kumar *et al.*, 2010). It is an important protective food because of its special nutritive value as it contains abundant and well balanced nutrition consisting of minerals, vitamins, dietary fiber, citric acid etc. (Thapa *et al.*, 2014). Ascorbic acid may play a key role in delaying the pathogenesis of a variety of degenerative diseases, such as cardiovascular disease, certain cancers, cataracts and it also prevents DNA mutation induced by oxidative stress (Byers and Guerrero, 1995; Marchioli *et al.*, 2001; Lutsenko *et al.*, 2002). Lycopene and β -carotene are the tomato carotenes which present the highest nutritional value (Tomlekova *et al.*, 2007; Glogovac *et al.*, 2010). Lycopene may alleviate chronic diseases such as cancer and coronary heart disease (Canene-Adams *et al.*, 2005; Omoni and Aluko, 2005; Kun *et al.*, 2006).

Looking at its commercial importance, there is utmost need to develop newer varieties/accessions/hybrids with higher yield, disease resistance, and processing traits. For this purpose the breeders choose genetically distant

parents, genetic diversity plays an important role in breeding vegetables, because hybrids derived from the lines of diverse origin display more heterosis than those between closely related strains (Lahbib *et al.*, 2012; Srivastava *et al.*, 2014). The greater is parental diversity, the greater is the chance of developing higher yielding breeding lines (Joshi and Dhawan, 1966; Singh *et al.*, 2012). Estimation of genetic divergence also allows breeders to eliminate some parents in downsizing the scale of hybridization activities and concentrate their efforts in a smaller number of combinations (Fuzzato *et al.*, 2002). Although tomato is a self-pollinated crop, there is genetic diversity not only in the morphological features but also in the quality attributes as reported by Abushita *et al.*, 1997. Among the various methods identified/developed to study the genetic divergence in the genotypes/accessions, the Mahalanobis D^2 (Mahalanobis, 1936) is reliable and most frequently used. For the first time use of this technique for assessing the genetic variability in plants was suggested by Rao (1952). It is a very useful technique of measuring genetic divergence (Meena and Bahadur, 2013; Sharma and Devi, 2013; Ramanjaneyulu *et al.*, 2014; Srivastava *et al.*, 2014). Further, grouping of the accessions based on Tocher's method will be more useful in choosing suitable parents for heterosis breeding (Prashanth *et al.*, 2008). D^2 analysis is a useful tool in quantifying the degree of divergence between biological population at genotypic level and to assess relative contribution of different components to the total divergence, both at the inter- and intra-cluster levels (Singh and Singh, 1980; Singh *et al.*, 2006a; Ara *et al.*, 2009). Genetic diversity analysis also reveals the redundancy of accessions with respect to a particular trait or combination of traits, which avoids wastage of resources. The progenies derived from diverse parents are expected to show a broad spectrum of genetic variability and provide better scope to isolate superior recombinants. Therefore, genetically diverse genotypes/ accessions should be used in a hybridization program to get superior recombinants. Keeping these points in mind, this study was undertaken to access and evaluate the genetic diversity in indeterminate tomato

accessions collected from diverse origin on the basis of yield and quality traits and to identify superior accessions for future use.

MATERIALS AND METHODS

Experimental location

A field study was carried out during the season 2012-13 at Vegetable Research Farm, Department of Horticulture, SHIATS, Allahabad, India. The city is situated in south-eastern part of the state Uttar Pradesh, India (25° 28' N latitude and 81° 54' E longitude) and at a mean altitude of 98 m above sea level. Geologically, the area forms a part of the Indo-Gangetic alluvial plains.

Climate and soil of experimental field

The climate of Allahabad is characterized as humid sub-tropical with an average annual rainfall of 1027 mm (40.4 inches). The rainfall is monsoonal in nature with around 75% received during July-September. The soil of the experimental field was loamy sand in texture, low in available nitrogen and organic matter,

comparatively rich in available phosphorus and medium in available potassium with slightly alkaline reaction. The mean monthly agro-meteorological observations were recorded during the crop season (Figure 1).

Experimental material

The experimental materials comprised of 19 indigenous accessions of indeterminate tomato collected from Indian Institute of Vegetable Research (IIVR), Varanasi and Vegetable Research Station (VRS), JAU, Junagadh, India. For raising good and healthy seedlings, the seeds were treated with carbendazim using 2.0 g per kg of seed. After that the seeds of 19 accessions of tomato were sown in the nursery bed on 30 September, 2012 and their seedlings were transplanted on 4th November, 2012 in small plots (2.0 m × 2.0 m) in open-field where row-to-row and plant-to-plant spacing was 60 cm x 60 cm that contained 16 plants. The experiment was laid out in a randomized complete block design (RCBD) with 3 replications. All the recommended agronomic package of practices were followed (like staking, earthing up, irrigation, weeding etc.), as recommended for commercial tomato production.

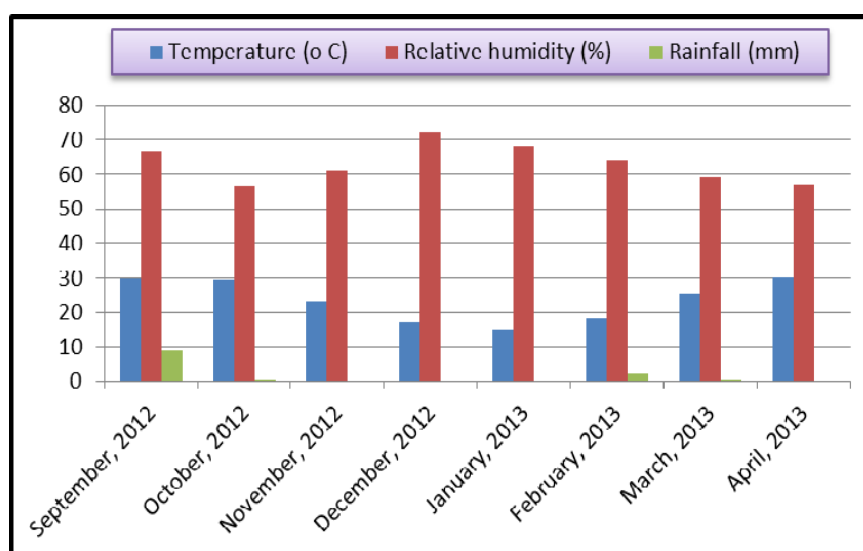


Figure 1. Mean monthly agro-meteorological observations recorded during crop season in 2012-13.

Experimental data

The observation were recorded on 5 randomly selected plants per replication for each accession on 15 quantitative characters i.e., [1] plant height (cm), [2] number of branches per plant, [3] number of leaves per plant, [4] days to flowering, [5] number of flower clusters per plant, [6] number of flowers per plant, [7] number of fruits per plant, [8] fruit set per cent, [9] fruit weight (g), [10] radial diameter of fruit (mm), [11] polar diameter of fruit (mm), [12] fruit yield per plant (g), [13] leaf curl incidence percentage (based on the scale given by Joshi and Choudhary, 1981), [14] TSS °Brix (by using a hand refractometer, Model: ATAGO, Tokyo, Japan) and [15] ascorbic acid (mg/100g) was estimated using 2,6-dichlorophenol indophenol method as illustrated by AOAC (1975).

Statistical analysis

Mahalanobis D^2 analysis

The data collected were subjected to multivariate analysis utilizing Mahalanobis D^2 statistic as suggested by Mahalanobis (1936) and Rao (1952) using statistical software WINDOSTAT 9.1 developed by INDOSTAT services Ltd. Hyderabad, India. Accessions were grouped into various clusters following Tocher's method as suggested by Rao (1952).

RESULTS AND DISCUSSION

On the basis of D^2 values, the 19 accessions were grouped into five highly divergent clusters

(Table 1 and Figure 2), indicating adequate genetic diversity for selecting superior and diverse parents which can be exploited for any breeding program. The cluster divergence was proved by the high inter-cluster and low intra-cluster D^2 values. The perusal of data (Table 1) depicted that clusters III and IV each had the maximum number of accessions (6) followed by cluster I and cluster II with 3 accessions in each cluster and cluster V is solitary consisting of only one accessions. The clustering pattern in the present study showed that accessions of different geographical areas were clubbed in one group indicating that there was no parallelism between genetic diversity and geographical origin. These results are similar to the findings of Peter and Rai (1976); Martin *et al.* (1981); Rai *et al.* (1998); Dharmatti *et al.* (2001); Mohanty and Prusti (2001); Parthasarathy and Aswath (2002); Joshi and Kohli (2003); Singh *et al.* (2006a); Mehta and Asati (2008); Singh *et al.* (2006b); Singh *et al.* (2008); Basavaraj *et al.* (2010); Kumar *et al.* (2010); Shashikanth *et al.* (2010); Kumar *et al.* (2013); Meena and Bahadur (2013). On the other hand, the accessions that originated in one region had been distributed into different clusters, indicating that accessions with same geographic origin could have under gone change for different characters under selection. This could be due to selection or genetic drift, which helps in creating more diversity rather than genetic distance. Therefore, selection of accessions for hybridization to generate diverse new gene combinations should be based on genetic diversity rather than geographic diversity. This finding is in conformity with the findings of Ganesh *et al.* (2007); Pawar *et al.* (2013).

Table 1. Clustering pattern of 19 accessions of indeterminate tomato based on D^2 statistics.

Cluster Number	Number of Accessions	Accessions Included
I	3	2012/TOINDVAR-2, 2012/TOINDVAR-3, 2012/TOINDVAR-4
II	3	EC 620430, EC 620432, EC 620434
III	6	2011/TOINDVAR-4, 2011/TOINDVAR-5, 2012/TOINDVAR-1, EC 620421, GT-1,
IV	6	2011/TOINDVAR-1, 2011/TOINDVAR-2, 2011/TOINDVAR-3, EC 620437, EC
V	1	ANGOORLATA

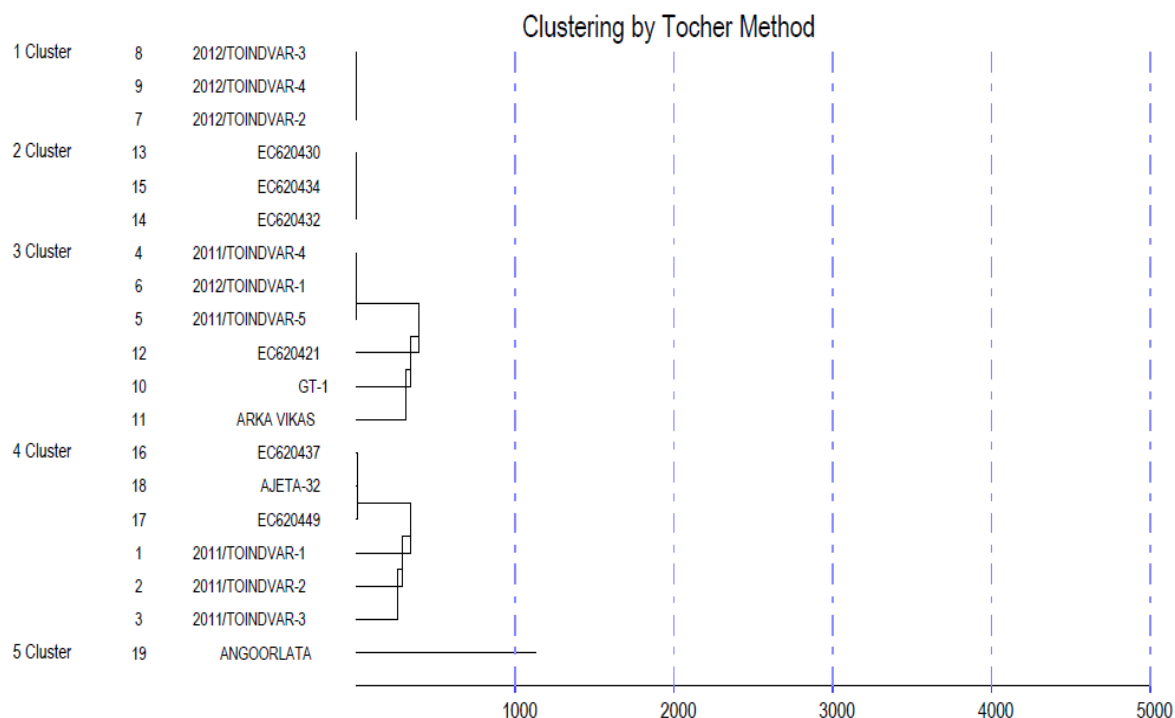


Figure 2. Dendrogram showing clustering patterns of 19 indeterminate tomato accessions grown under open field conditions (Tocher's method).

In line with this, Yashavantakumar *et al.* (2009) grouped 70 tomato genotypes into 7 clusters. Similarly, Chernet *et al.* (2014) clustered 36 genotypes into 6 distinct clusters using Mahalanobis distance; Iqbal *et al.* (2014) grouped 47 tomato genotypes into 5 clusters; Ghosh *et al.* (2009) clustered 40 genotypes into 6 clusters; Gonçalves *et al.* (2009) grouped 40 tomato accessions into 5 diverse clusters. Meena and Bahadur (2013) also employed Mahalanobis distance (D^2) to classify 30 tomato germplasm in to 6 clusters.

The divergence within the cluster (intra-cluster distance) indicates the divergence among the accessions falling in the same cluster. On the other hand, inter cluster divergence suggests the distance (divergence) between the accession of different clusters. The intra and inter cluster D^2 values among 19 accessions presented in Table 2 and Figure 3 revealed that cluster V showed minimum intra-cluster D^2 value (0) followed by

cluster II (7.0), whereas, maximum intra-cluster D^2 value (913.9) was shown by cluster III followed by cluster IV (779.3) and cluster I (9.9), revealing considerable genetic divergence among the accessions of this cluster and was due to both natural and artificial selection forces among the accessions (Rathi *et al.*, 2011). Minimum inter-cluster D^2 value was observed between the cluster III and IV (1870.3) followed by cluster II and IV (3097.5) indicated close relationship among the accessions included in these clusters. Maximum inter-cluster D^2 value was observed between the cluster I and IV (11347.2) followed by cluster I and III (10921.8), cluster I and II (6949.5), cluster II and V (6930.8), cluster IV and V (6757.2) and cluster I and V (4879.6) indicated that the accessions belonging to these groups were genetically most diverse and the accessions included in these clusters can be used as a parent

Table 2. Intra (Diagonal) and Inter-cluster distance (D^2) among indeterminate tomato accessions.

Clusters	I	II	III	IV	V
I	9.94	6949.50	10921.84	11347.25	4879.60
II		7.07	4095.74	3097.54	6930.82
III			913.93	1870.39	4185.23
IV				779.37	6757.26
V					0.00

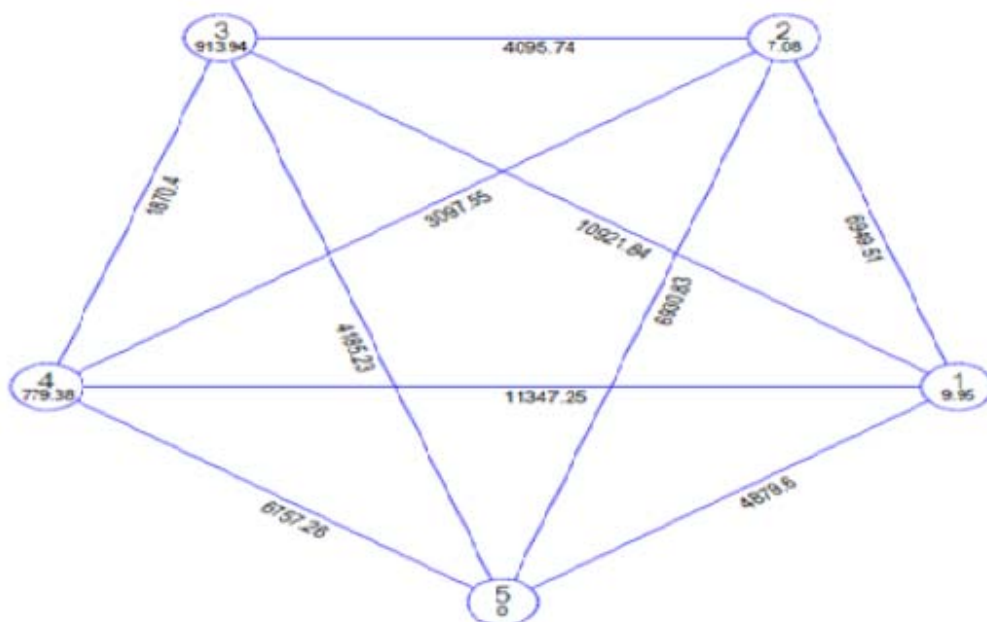


Figure 3. Mahalanobis Euclidean Distance (not to scale)

in hybridization program to get higher heterotic hybrids from the segregating population. Similar results were revealed by Babu and Patil (2004); Mehta *et al.* (2004); Mehta and Asati (2008); Meena and Bahadur (2013).

Several authors also reported diversity in the accessions of tomato by assessing genetic divergence on the basis of quantitative traits following Mahalanobis D^2 statistics (Guirgis *et al.*, 1994; Basavaraj *et al.*, 2010; Evgenidis *et al.*, 2011; Meena and Bahadur, 2013; Reddy *et*

al., 2013a). Average inter and intra-cluster distances revealed that, in general, inter-cluster distances were much higher than those of intra-cluster distances, suggesting homogeneous and heterogeneous nature of the accessions lines within and between the clusters, respectively. These results are in accordance with the findings of Mahesha *et al.* (2006); Sekhar *et al.* (2008); Meena and Bahadur (2013) in tomato. The percentage contribution of 15 characters for genetic divergence (Table 3) showed that leaf

Table 3. Percentage contribution of different characters to genetic divergence among indeterminate tomato accessions.

S. No.	Source	Time Ranked 1st	Contribution (%)
1.	Plant Height cm (at 120 DAT)	3	1.7
2.	Branches/ Plant (at 120 DAT)	0	0.0
3.	Leaves/ Plant (at 120 DAT)	2	1.2
4.	Days to flowering (50%)	0	0.0
5.	Flower Clusters/ Plant	2	1.2
6.	Flowers/ plant	27	15.8
7.	Fruits/ plant	0	0.0
8.	Fruit set (%)	1	0.6
9.	Fruit weight (g)	11	6.4
10.	Radial diameter of Fruit (mm)	3	1.7
11.	Polar diameter of Fruit (mm)	43	25.1
12.	Fruit yield/ plant (g)	1	0.6
13.	Leaf curl Incidence %	66	38.6
14.	TSS (°Brix)	3	1.7
15.	Ascorbic acid (mg/ 100 g)	9	5.3

DAT: Days after transplanting; mm: millimeter; g: gram.

curl incidence percentage contributed maximum (38.6%) towards genetic divergence followed by polar diameter of fruit (25.1%), number of flowers per plant (15.8%) and fruit weight (6.4%). Mohanty and Prusti (2001); Reddy *et al.* (2013a); Singh *et al.* (2008) also observed such maximum contribution for fruit weight to total divergence of tomato accessions. De *et al.* (1988) opined that traits contributing maximum towards the D^2 values needed to be given more emphasis for deciding the clusters to be taken for the purpose of choice of parents for hybridization.

Further, for crop improvement, intercrossing among accessions with outstanding mean performance was suggested by Roy and Sharma (1996); Kumar *et al.* (2013), and the reliable conformity for this can be known on the basis of cluster means. The cluster means of accessions (Table 4) revealed considerable genetic differences between the groups. Accessions in cluster I showed maximum performance for fruit yield per plant (3270.6 g) which indicates that the accessions included in this cluster could effectively be used for the crop improvement program for increasing yield. Cluster II recorded minimum days to flowering (57.3 days), whereas, maximum mean value for

radial diameter of fruit (59.8 mm) and polar diameter of fruit (50.2 mm). It reveals that if a breeding program is aimed at earliness, then accessions in cluster II can be selected (Meena and Bahadur, 2013). Cluster III showed high mean performance for TSS (4.9 °Brix) and ascorbic acid (43.9 mg/100g). Cluster IV recorded maximum performance for fruit weight (56.9 g) and leaf curl incidence percentage (24.5). Cluster V showed high mean value for plant height (171.7 cm), number of branches per plant (21.1), number of leaves per plant (221.2), days to flowering (63.0 days), number of flower clusters per plant (27.2), number of flowers per plant (136.0), number of fruits per plant (76.2) and fruit set percentage (56.0), whereas, low mean performance for leaf curl incidence percentage (20.0). Depending upon the breeding objective, the potential lines to be selected from different clusters as parents in a hybridization program may be based on genetic distance. In accordance to the findings, Edang *et al.* (1971); Hazra *et al.* (2010) reported that the clustering pattern could be utilized in choosing parents for cross combinations likely to generate the highest possible variability for various economic characters.

Table 4. Cluster mean of 19 indeterminate tomato accessions for 15 traits.

Character Clusters	Plant height at 120DAT	Branches / plant at 120DAT	Leaves/ plant at 120 DAT	Days to flowering	Flower clusters/ plant	Flowers/ plant	Fruits/ plant	Fruit set %	Fruit weight (g)	Radial diameter of fruit (mm)	Polar diamete r of fruit (mm)	Fruit yield/ plant (g)	Leaf curl incidence (%)	TSS °Brix	Ascorbic acid (mg/ 100g)
I	131.7	17.1	194.7	59.8	19.5	111.2	57.9	52.1	56.0	57.6	48.7	3270.6	24.4	4.2	33.6
II	158.8	19.6	204.1	57.33	22.33	126.5	56.7	45.4	48.8	59.8	50.2	2774.7	22.7	4.5	38.8
III	145.8	18.0	203.6	57.34	22.31	130.6	53.6	41.3	54.7	52.2	44.9	2920.7	22.5	4.9	43.9
IV	143.6	18.2	190.8	58.9	22.2	124.7	55.5	44.7	56.9	54.0	49.9	3119.3	24.5	4.3	33.7
V	171.7	21.1	221.2	63.0	27.2	136.0	76.2	56.0	34.6	34.7	41.2	2637.6	20.0	4.8	42.3

DAT: Days after transplanting; mm: millimeter; g: gram.

CONCLUSIONS

For generating wide spectrum of variability intercrossing of accessions of cluster I for fruit yield per plant; cluster II for minimum days to flowering, radial diameter of fruit and polar diameter of fruit; cluster III for TSS and ascorbic acid; cluster IV for fruit weight; and cluster V for plant height, number of branches per plant, number of leaves per plant, number of flower clusters per plant, number of flowers per plant, number of fruits per plant, fruit set percentage and low leaf curl incidence percentage. The accessions of the cluster I for highest mean yield per plant and cluster V for low leaf curl incidence percentage can be utilized as donor parents in hybridization program for enhancing the yield and minimum leaf curl incidence of other accessions grouped in a cluster and can be fixed by selecting transgressive segregants followed by continued selection in advanced generations which may lead to development of high yielding varieties with desired component characters. Accessions from highly divergent clusters may also be utilized in a breeding program for development of high yielding varieties with desirable attribute and can also be utilized in heterosis breeding program for development of F₁ hybrids with superior yield and quality characters. Hybridization between divergent parents is likely to produce wide variability and transgressive segregation with high heterotic effects. The above findings indicated that the smallest inter-cluster distance was observed between cluster III and IV (1870.3) followed by cluster II and IV (3097.5). The lines belonging to these clusters were relatively closer to each other, in comparison to lines grouped in other clusters. This analysis would be useful to avoid selecting parents from genetically homogeneous clusters, and maintain a relatively broad genetic base for breeding.

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