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Assessment of genetic diversity in different Chilli (C*apsicum annuum* L.) genotypes

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Abstract
Genetic divergence among 53 genotypes of chilli was assessed using Mahalanobis D^2 statistic for 20 characters at College of Horticulture, Venkataramannagudem, Dr. Y.S.R Horticultural University, Andhra Pradesh. The analysis of variance revealed significant differences among the genotypes for all the characters studied indicating considerable diversity in the material. (Based on Mahalanobis D^2 statistic) The fifty three genotypes were grouped into 9 clusters. The maximum contribution towards genetic divergence was by ascorbic acid content (24.67 %) followed by colour value (16.11 %), fruit weight (14.59 %), seed weight (9.80 %) and capsaicin content (8.56 %).The mutual relationships between the clusters revealed that inter-cluster distance values were greater than intra-cluster values. Among the clusters, cluster I was the largest group comprising of thirty eight genotypes, followed by cluster III with eight genotypes, whereas, the cluster II, IV, V, VI, VII, VIII and IX were monotypic or solitary. The highest inter cluster distance was observed between clusters VIII and IX (19458.80) whereas, the lowest was observed between clusters I and IV (478.13). Cluster III (445.93) has exhibited highest intra cluster distance and the lowest was observed in clusters II, IV, V, VI, VII, VIII and IX (0.00) D^2 cluster analysis revealed wide genetic distance (inter cluster) between the genotypes of cluster VIII (California Wonder) and IX (Bhut Jolokia) and the crossing between genotypes of these two clusters can be exploited for the development of heterotic hybrids in future breeding programmes.

Keywords: *Capsicum annuum*, D^2 analysis, clustering, genetic divergence

Introduction
Chilli (*Capsicum annuum* L.) is known as the universal spice of India and has diverse utilities as a spice, condiment, and culinary supplement, and medicine, vegetable and ornamental plant. The important chilli growing states are Andhra Pradesh, Karnataka, Maharashtra, Orissa, Tamil Nadu and Madhya Pradesh. A wide variability in chilli fruit morphology, pungency, bearing habit and crop duration is found throughout India (Asati and Yadav, 2004) [2]. It has originated in Mexico, Southern Peru and Bolivia. There are mainly five cultivated *Capsicum spp. viz., C. annuum, C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens* of which *Capsicum annuum* is the dominant species world over and could be broadly classified into non-pungent (sweet pepper) and pungent (chilli or hot pepper) based on their level of pungency (Bosland and Votava, 2000) [3]. Due to long history of cultivation, selection and popularity of crops, sufficient genetic variability has been generated. Rich variability in morphological traits in hot pepper occurs throughout India, particularly in southern peninsular region, North Eastern foot hills of Himalayas and Gangetic plains (Pradheep and Veeraragavatham, 2006) [10]. However, the high variability present in the crop has so far not been fully exploited in the crop improvement programmes. Genetic diversity is the basic requirement for any successful breeding programme. Assessment of genetic diversity among germplasm lines is a prerequisite for plant breeders in choosing potential parental lines because of two reasons: i.e., (i) In the hybridization programme, genetically diverse parents likely to produce high heterotic effect, and (ii) Genetically distant parents could produce a wide spectrum of variability in the segregating generation. Therefore, a clear characterization of germplasm is the first step to facilitate successful breeding efforts. The degree of genetic divergence can be quantified using Mohalanobis's D^2 statistic of multivariate analysis which is recognized as a powerful tool for assessing the relative contribution of different characters to the total divergence in self-pollinated crops (Golakia and Makne 1992 [6] and Shidhu *et al.*, 1989) [12]. Therefore, the present study was undertaken to assess the genetic diversity in 53 genotypes of chilli to identify suitable genotypes.

Materials and Methods

The experiment was carried out with 53 genotypes of chilli (Table 1) at College of Horticulture, Venkataramannagudem, Dr. Y.S.R Horticultural University, Andhra Pradesh, India during 2017 -2018 in a randomized block design with three replications. Each genotype was raised in 3.6 × 1.8 m plot size with a spacing of 60 × 45 cm accommodating 24 plants per plot. The crop was grown with standard package of practices. The observations were recorded five randomly selected competitive plants from each genotype and replication. The analysis of variance was carried out as per the procedure given by Panse and Sukhatme (1958) [9]. The character contribution towards genetic divergence was computed using the method given by Singh and Chaudhary (1977) [14]. Percentage contribution towards genetic divergence was calculated using the following formula.

Percentage contribution of the character = (N × 100) ÷ M

Where, N = Number of genotype combinations where the character was ranked first.

M = All possible combinations of number of genotypes considered.

The genetic divergence was worked out among the genotypes using Mahalanobis D^2 statistics (Mahalanobis, 1936) [8] and the D^2 values were calculated as

$$D^2_{ij} = \sum_{t=1}^{p} (Y_i^t - Y_j^t)^2$$

Where,

$Y_{it}$is uncorrelated mean value of i^{th} genotype for `t' characters

$Y_{jt}$is uncorrelated mean value of i^{th} genotype for `t' characters

D^2_{ij} is D^2 between i^{th} and j^{th} genotypes.

The genotypes were grouped into different clusters by employing Tocher's method as outlined by Rao (1952) [11]. For grouping of genotypes, D^2 values of all combinations of each genotype were arranged in ascending order of magnitude in a tabular form as described by Singh and Chaudhary (1977) [14].To start with, two populations having the closest distance from each other were considered, to which the third population having the smallest D^2 value from the first two populations was added. Similarly, the next nearest fourth population was considered and this procedure was continued. At certain stage when it was felt that after adding a particular population there was an abrupt increase in the average D^2, that population was not considered for including in that cluster. The genotypes of the first cluster were then eliminated and the rest were treated in a similar way. This procedure was continued till all the genotypes were included into one or other cluster.

The average intra and inter cluster distances were calculated by the formula given by Singh and Chaudhary (1977) [14].

Square of intra- cluster distance = $\Sigma Di^2 / n$

Square of inter- cluster distance = $\Sigma Di^2 / n_i n_j$

Where,

ΣDi^2= Sum of distance between all possible combinations.

n= Number of all possible combinations

n_i= Number of entries in cluster i

n_j= Number of entries in cluster j

Results and Discussion

The analysis of variance (ANOVA) revealed significant differences among 53 genotypes for quantitative and qualitative traits indicating the existence of variability among genotypes for 20 characters studied (Table 2). These findings are in accordance with the results of many earlier works (Kumar *et al.,* 2010 [7]; Shrilekha *et al.,* 2011 [13] and Yatung *et al.,* 2014 [17]). The per cent contribution towards genetic divergence by all the 20 contributing characters is presented in table 3 and figure 1. The results showed that the character, ascorbic acid content contributed maximum (24.67%) towards diversity by taking first rank 340 times followed by colour value (16.11 %) by taking 222 times first ranking, fruit weight (14.59 %) by 201 times, seed weight (9.80 %) by 135 times, capsaicin content (8.56 %) by 118 times, fresh to dry recovery (7.84 %) by 108 times, fruit length (5.22 %) by 72 times, oleoresin content (4.86 %) by 67 times, percentage of ChLCV disease incidence (4.06 %) by 56 times, disease severity (3.34 %) by 46 times, number of seeds per fruit (0.94 %) by 13 times. Whereas, remaining characters like plant height, plant spread, number of primary branches per plant, days to 50 per cent flowering, days to 50 per cent ripening, number of fruits per plant, fruit width, red ripe fruit yield per plant and dry fruit yield per plant had no contribution towards genetic divergence.

The 53 genotypes were grouped into 9 clusters (Table 4 and Figure 2). Out of nine clusters formed, cluster I was the largest group comprising of 38 genotypes, followed by cluster III with 8 genotypes, whereas, the cluster II, IV, V, VI, VII, VIII and IX were monotypic or solitary. The formation of distinct solitary clusters may be due to the fact that geographic barriers preventing gene flow and intensive natural and human selection for diverse and adoptable gene complexes must be responsible for this genetic diversity. The pattern of grouping of genotypes into different clusters was random and indicated that there is no parallelism between genetic divergence and geographical divergence of genotypes. Therefore, selection of genotypes for hybridization should be based on genetic diversity rather than geographical diversity. Vani *et al.* (2007) [16] reported fourteen clusters with 55 genotypes, Dutonde *et al.* (2008) [4] observed seven clusters with 40 accessions, Farhad *et al.* (2010) [5] reported six clusters with 45 chilli genotypes, Shrilekha *et al.* (2011) [13] reported seven clusters with 38genotypes and Yatung *et al.* (2014) [17] observed six clusters with 30 chilli genotypes and these findings support the results of this investigation.

The intra-and inter-cluster distance represent the index of genetic diversity among clusters (Table 5 and Figure 3). The inter cluster average D^2 value was maximum (D^2=19458.80) between cluster VIII and cluster IX indicating that the genotypes belonging to cluster VIII were far away from those of cluster IX. Hence heterosis could be exploited for the genotypes present in the distant clusters.Inter cluster average D^2 value (D^2=17484.30) of cluster V and VIII also recorded notable distance. However inter-cluster D^2 value was least (D^2=478.13) between cluster I and IV clearly indicating the closeness between the genotypes present in the clusters and this can be used for backcrossing programmes.

The intra-cluster D^2 values ranged from 0.00 to 445.93. Among the 9 clusters, cluster III with eight genotypes showed maximum intra cluster distance (D^2=445.93) followed by cluster I (D^2=269.36). Whereas, the presence of single genotype in remaining clusters (II, IV, V, VI, VII, VIII and IX) resulted in zero (0.00) in intra cluster distance. Several earlier reports (Ajjapplavara, 2009 [1]; Kumar *et al.,* 2010 [12]; Suryakumari *et al.,* 2010 [15] and Yatung *et al.,* 2014) [17] also indicate the presence of a high genetic divergence among

chilli genotypes in their respective experiments. The genotypes grouped into the same cluster presumably diverge very little from one another and crossing of genotypes belonging to the same cluster is not expected to yield desirable segregants. Consequently, a crossing programme should be conducted with putative parents. Thus, crosses between the members of clusters separated by inter-cluster distances are likely to be beneficial for further improvement. Selection of parents from these diverse clusters for hybridization would help in achieving the novel recombinants. Similar types of observations were reported by Ajjapplavara (2009) [1] and Suryakumari *et al.* (2010) [15]. The clusters with single genotype indicated their independent identity and importance due to various unique characters possessed by them.

Cluster II earned highest cluster mean value for fresh to dry recovery (33.87) and ascorbic content (195.56) (Table 6). Cluster III had the highest mean value for colour value (180.62) and seed weight (7.42) and lowest mean value for days to 50 per cent flowering. Cluster IV recorded highest mean value for oleoresin content (14.72) and lowest mean value for *ChLCV* disease incidence (6.14) and disease severity (0.67). Cluster V recorded the maximum plant height (109.33), plant spread (108.07), number of primary branches per plant (5.33) and number of fruits per plant (798.67). Cluster VI recorded the lowest mean value for days to 50% ripening (73.33) while, cluster VII recorded maximum number of seeds per fruits (153.62) and red ripe fruit yield per plant (852.11).Cluster VIII had the highest mean value for fruit length (14.48), fruit girth (22.42) and fruit weight (72.47) and cluster IX recorded the highest capsaicin content (1.84).

The genotypes in cluster III have flowered earlier and good for quality trait. Genotypes of clusters V, VII and VIII showed better performance for yield traits and cluster IV showed resistance to *chilli leaf curl virus*. These clusters can be used in breeding programme for introgression of their desired quality and resistant genes into the high yielding varieties.

Table 1: Germplasm accessions of chilli

Treatments	Accession number or Varieties	Source
T_1	IHR 1485	Indian Institute of Horticultural Research, Bangalore
T_2	IHR 1732	IIHR, Bangalore
T_3	IHR 2452	IIHR, Bangalore
T_4	IHR 2596	IIHR, Bangalore
T_5	IHR 2900	IIHR, Bangalore
T_6	IHR 3014	IIHR, Bangalore
T_7	IHR 3024	IIHR, Bangalore
T_8	IHR 3310	IIHR, Bangalore
T_9	IHR 3315	IIHR, Bangalore
T_{10}	IHR 3443	IIHR, Bangalore
T_{11}	IHR 3447	IIHR, Bangalore
T_{12}	IHR 3448	IIHR, Bangalore
T_{13}	IHR 3449	IIHR, Bangalore
T_{14}	IHR 3455	IIHR, Bangalore
T_{15}	IHR 3478	IIHR, Bangalore
T_{16}	IHR 3517	IIHR, Bangalore
T_{17}	IHR 3587	IIHR, Bangalore
T_{18}	IHR 3915	IIHR, Bangalore
T_{19}	IHR 4597	IIHR, Bangalore
T_{20}	IHR 4595	IIHR, Bangalore
T_{21}	IHR 4598	IIHR, Bangalore
T_{22}	IHR 4600	IIHR, Bangalore
T_{23}	IHR 4601	IIHR, Bangalore
T_{24}	IHR 4602	IIHR, Bangalore
T_{25}	IHR 4603	IIHR, Bangalore
T_{26}	IHR 4604	IIHR, Bangalore
T_{27}	IHR 4605	IIHR, Bangalore
T_{28}	IHR 4606	IIHR, Bangalore
T_{29}	IHR 4607	IIHR, Bangalore
T_{30}	IHR 4608	IIHR, Bangalore
T_{31}	IHR 4609	IIHR, Bangalore
T_{32}	IHR 4610	IIHR, Bangalore
T_{33}	IHR 4611	IIHR, Bangalore
T_{34}	IHR 4612	IIHR, Bangalore
T_{35}	IHR 4031	IIHR, Bangalore
T_{36}	IHR 4516	IIHR, Bangalore
T_{37}	IHR 4592	IIHR, Bangalore
T_{38}	IHR 4593	IIHR, Bangalore
T_{39}	IHR 4594	IIHR, Bangalore
T_{40}	G3	Horticultural Research Station, Lam, Guntur
T_{41}	G4	HRS, Lam, Guntur
T_{42}	G5	HRS, Lam, Guntur
T_{43}	LCA 206	HRS, Lam, Guntur
T_{44}	LCA 235	HRS, Lam, Guntur
T_{45}	LCA 305	HRS, Lam, Guntur

T_{46}	LCA 334	HRS, Lam, Guntur
T_{47}	LCA 353	HRS, Lam, Guntur
T_{48}	LCA 620	HRS, Lam, Guntur
T_{49}	LCA 625	HRS, Lam, Guntur
T_{50}	LCA 960	HRS, Lam, Guntur
T_{51}	Bhut Jolokia	Tura, Meghalaya
T_{52}	Meghalaya Local	Tura, Meghalaya
T_{53}	California Wonder	Namdhari seed company

Table 2: Analysis of variance for yield, yield attributes, quality traits and *ChLCV* disease incidence in chilli

S. No	Character	Mean sum of Squares		
		Replications	Treatments	Error
1.	Plant height (cm)	28.54	601.62 **	33.62
2.	Plant spread (cm^2)	43.86	336.19 **	30.03
3.	Number of primary branches per plant	0.34	0.87 **	0.12
4.	Days to 50 per cent flowering	11.84	74.03 **	6.29
5.	Days to 50 per cent ripening	31.13	104.82 **	21.56
6.	Number of fruits per plant	1830.81	41832.40 **	1706.66
7.	Fruit length (cm)	0.030	21.18 **	0.128
8.	Fruit width (cm)	0.26	34.01**	0.09
9.	Fruit weight	0.22	423.80 **	0.17
10.	Number of seeds per fruit	32.36	2023.27 **	19.28
11.	Seed weight (g/1000 seed)	0.03	4.19 **	0.03
12.	Red ripe fruit yield (g/plant)	10125.08	72208.23 **	13671.31
13.	Dry fruit yield (g/plant)	1144.87	6320.75 **	1502.76
14.	Fresh to dry recovery (%)	0.58	37.53 **	0.30
15.	Capsaicin content (%)	0.00	0.18 **	0.00
16.	Oleoresin content (%)	0.35	16.83 **	0.18
17.	Ascorbic acid content (mg/100g)	49.88	6718.75 **	21.65
18.	Colour value (ASTA units)	29.57	6589.61 **	26.99
19.	*ChLCV* disease incidence (%)	29.24	965.32 **	23.45
20.	Disease severity (%)	10.49	847.14 **	9.38

* Significant at 5 % level ** Significant at 1 % level

Table 3: Per cent contribution of different characters towards genetic divergence

S. No	Source	Times Ranked 1st	Contribution
1.	Plant height (cm)	-	0.00
2.	Plant spread (cm^2)	-	0.00
3.	Number of primary branches per plant	-	0.00
4.	Days to 50 per cent flowering	-	0.00
5.	Days to 50 per cent ripening	-	0.00
6.	Number of fruits per plant	-	0.00
7.	Fruit length (cm)	72	5.22
8.	Fruit width (cm)	-	0.00
9.	Fruit weight (g)	201	14.59
10.	No of seeds per fruit	13	0.94
11.	Seed weight (g/1000 seed)	135	9.80
12.	Red ripe yield (g /plant)	-	0.00
13.	Dry fruit yield (g / plant)	-	0.00
14.	Fresh to dry recovery (%)	108	7.84
15.	Capsaicin content (%)	118	8.56
16.	Oleoresin content (%)	67	4.86
17.	Ascorbic content(mg/100g)	340	24.67
18.	Colour value (ASTA)	222	16.11
19.	*ChLCV* disease incidence (%)	56	4.06
20.	Disease severity (%)	46	3.34

Table 4: Clustering pattern of 53 chilli genotypes

Cluster	No. of genotypes	Genotypes
I	38	IHR 4607, LCA 235, IHR 4594, IHR 2452, IHR 4605, IHR 4606, IHR 3517, IHR 3915, IHR 4593, IHR 3448, IHR 3447, IHR 4602, IHR 4598, IHR 3587, IHR 3443, IHR 4592, IHR 1732, IHR 4609, IHR 3014, G3, IHR 4516, IHR 2900, IHR 2596, IHR 3024, IHR 4595, LCA 353, LCA 625, G4, G5, LCA 960, LCA 206, IHR 4608, LCA 620, IHR 3310, IHR 4031, LCA 305, IHR 3315 and IHR 4597
II	1	LCA 334
III	8	IHR 3455, IHR 4600, IHR 4604, IHR 4610, IHR 3449, IHR 4611, IHR 4603 and IHR 4612
IV	1	IHR 1485
V	1	Meghalaya Local

VI	1	IHR 4601
VII	1	IHR 3478
VIII	1	California Wonder
IX	1	Bhut Jolokia

Table 5: Average intra (bold) and inter cluster D^2 values for nine clusters of various chilli genotypes

Cluster	I	II	III	IV	V	VI	VII	VIII	IX
I	269.36	481.21	636.72	478.13	1223.74	2407.76	7001.98	14808.83	4805.30
II		0.00	784.17	740.16	1582.66	3032.44	7428.37	15641.63	5981.28
III			445.93	962.79	1973.13	2057.76	5887.55	12895.89	5450.45
IV				0.00	1173.03	3043.24	7972.39	15997.31	4477.47
V					0.00	4170.58	8989.79	17484.30	2214.74
VI						0.00	1536.89	5713.91	7404.57
VII							0.00	1836.54	12008.48
VIII								0.00	19458.80
IX									0.00

Table 6: Mean values of clusters for 20 characters in various chilli genotypes

Character	I	II	III	IV	V	VI	VII	VIII	IX
Plant height (cm)	64.19	62.47	65.13	71.53	109.33	69.53	65.00	57.13	81.27
Plant spread (cm^2)	62.25	61.87	61.78	47.05	108.07	50.53	55.40	52.83	92.07
Number of primary branches per plant	3.45	3.13	3.16	3.87	5.33	3.60	3.27	3.60	3.13
Days to 50 per cent flowering	37.01	41.00	35.79	46.33	49.33	38.67	37.67	36.67	52.67
Days to 50 per cent ripening	80.78	87.67	79.21	85.67	97.67	73.33	77.67	76.00	95.33
Number of fruits per plant	213.56	218.78	112.33	264.55	798.67	20.00	16.56	11.44	102.00
Fruit length (cm)	7.49	6.94	11.90	4.96	2.15	7.53	9.23	14.48	5.30
Fruit width (cm)	3.68	3.45	5.28	3.17	2.09	12.00	15.57	22.42	7.50
Fruit weight (g)	3.13	2.53	7.27	2.12	0.52	27.73	50.07	72.47	3.33
No of seeds per fruit	74.66	65.20	96.04	54.47	24.40	80.39	153.62	118.27	28.27
Seed weight (g/1000 seed)	5.89	5.85	7.42	4.47	3.38	6.88	5.64	6.63	4.40
Red ripe yield (g/plant)	547.28	515.11	663.03	555.33	410.45	570.56	852.11	759.00	298.00
Dry fruit yield (g/plant)	155.19	172.72	189.93	192.89	109.25	127.36	175.06	145.95	65.87
Fresh to dry recovery (%)	28.47	33.87	28.61	36.33	27.07	22.47	21.00	19.87	22.67
Capsaicin content (%)	0.29	0.19	0.22	0.40	0.89	0.10	0.03	0.06	1.84
Oleoresin content (%)	10.85	13.12	12.87	14.72	6.80	8.81	12.27	8.87	8.37
Ascorbic content 100g)	89.05	195.56	103.89	49.07	177.67	57.78	140.00	71.11	66.67
Colour value (ASTA units)	87.90	56.52	180.62	95.21	91.71	91.20	119.50	129.70	64.46
ChLCV disease incidence (%)	84.82	90.00	90.00	6.14	57.29	90.00	90.00	90.00	90.00
Disease severity (%)	53.60	60.00	60.83	0.67	22.67	61.33	62.00	86.00	60.00

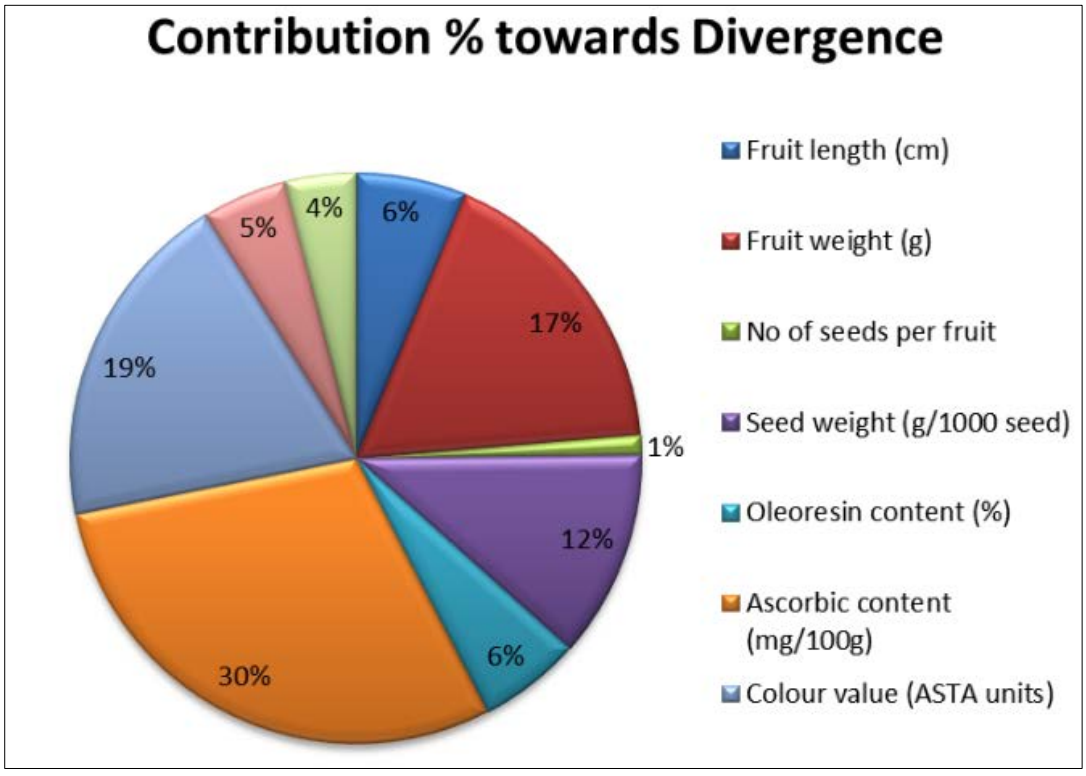

Fig 1: Per cent contribution of different traits towards divergence of Chilli genotypes

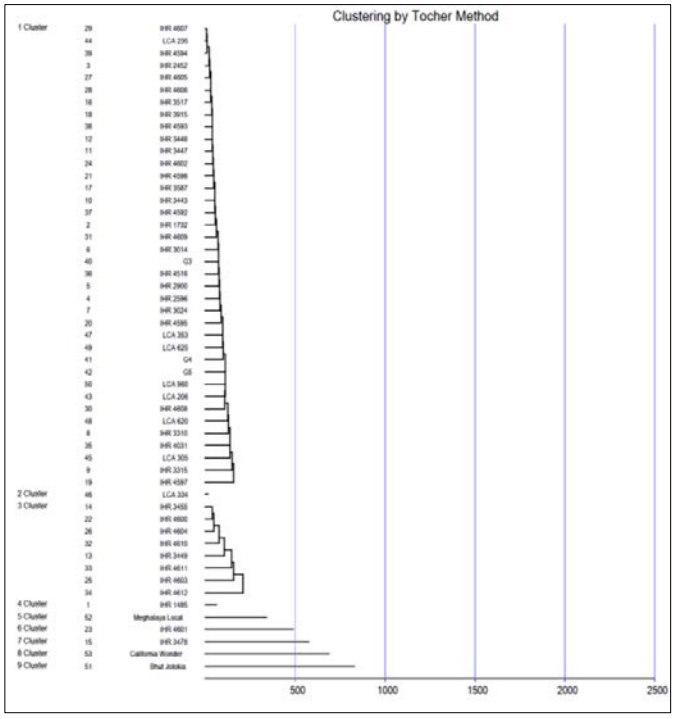

Fig 2: Dendrogram showing clustering pattern of 53 chilli genotypes by Tochermethod

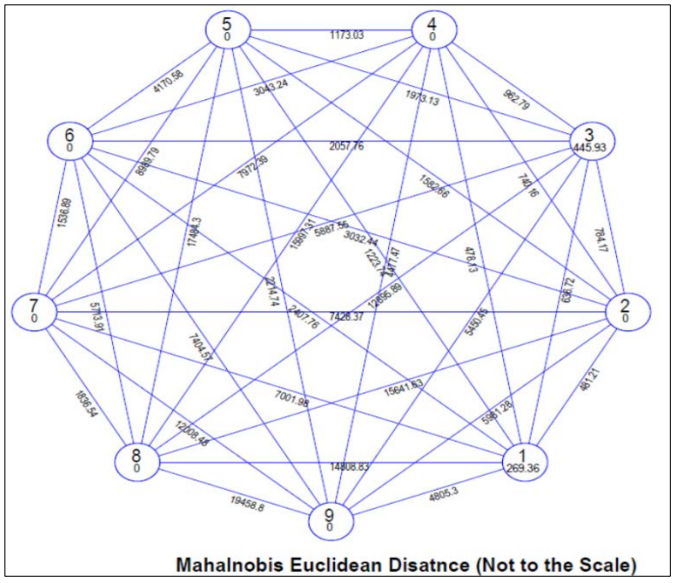

Fig 3: Diagram depicting the distances between different clusters of chilli genotypes

Conclusion

D^2 cluster analysis revealed wide genetic distance (inter cluster) between the genotypes of cluster VIII (California Wonder) and IX (Bhut Jolokia) and the crossing between genotypes of these two clusters can be exploited for the development of heterotic hybrids in future breeding programmes.

The clusters II, III, IV, V, VI, VII and VIII were found superior for one or more characters. Therefore, a multiple crossing programme can be proposed involving genotypes from these clusters for the development of superior segregants in advanced generations with high yield potential combined with chilli leaf curl virus resistance in chilli.

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