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Genetic divergence analysis in chilli (*Capsicum annuum* L.) under protected cultivation

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Abstract

A genetic divergence study on 24 chilli genotypes (*Capsicum annuum* L.) were conducted under protected condition in polyhouse at the North Instructional Farm of the School of Agricultural sciences, Karunya Institute of Technology and Sciences, Coimbatore. The variation among the genotypes for different traits were estimated by Anova. Mahalanobis D^2 distance was used to estimate the genetic distance between the genotypes. The genotypes were grouped into six clusters. Among the six clusters, cluster I, II and VI had seven genotypes each and the clusters III, IV and V had one genotype each. The maximum inter cluster distance was found between cluster IV and cluster V (44.68) and the minimum inter cluster distance was found between cluster IV and cluster VI (38.78). The maximum intra cluster distance was found in cluster II (36.35) and minimum intra cluster distance was found in cluster VI (30.74). Jwala chilli in cluster V had the maximum capsaicin content, Fat chilli in cluster IV had the maximum dry fruit yield per plant and Long chilli in cluster III had the maximum green fruit yield per plant. Selection of these genotypes as parents for hybridization will be more effective for the improvement of related characters having superiority. One of the most important applications of divergence analysis is the selection of parents for hybridization to produce superior hybrids. In the recombination breeding approach selection of genetically distant parents also facilitate isolation of transgressive segregants.

Keywords: *Capsicum annuum*, Mahalanobis D^2 , genetic divergence

Introduction

Chilli is one of the most important crops used extensively throughout the world as vegetable and as spices. There are around 27 species in the genus *Capsicum*, including 22 wild species and 5 cultivated species. According to Padilha and Barbieri (2016) [8], domesticated chilli species include *C. annuum*, *C. frutescens*, *C. baccatum*, *C. chinense*, and *C. pubescens*. It is believed to have originated in South America and has since expanded to the tropics, subtropics, and temperate areas of the globe (Pickersgill, 1997) [9]. Chilli performs well in warm humid tropical and sub-tropical regions extending from equator 45° latitude on both southern and northern hemisphere. It can grow well up to an altitude of 2000 m and temperature range of 15-35°C.

In the past few decades, protected cultivation, also known as polyhouse cultivation, has become one of the most popular methods for producing primarily horticultural crops both qualitatively and quantitatively. Protected farming, commonly referred to as controlled environment agriculture (CEA), is extremely productive, water and land-wise, as well as environmentally friendly (Jensen 2002) [4]. The technology comprises growing horticulture crops in a controlled environment where conditions like temperature, humidity, light, soil, water, fertilizers, etc. are adjusted to produce the maximum number of fruits and provide continuous supply of it even in the off-season. Due to their better production and commercial viability, chilli and other vegetables are extensively cultivated under protected agriculture. Vegetable production under a protected cultivation system leads to efficient use of the land resources. It significantly reduces biotic and abiotic stresses compared to open agriculture and can enhance the output of high-quality vegetables for both the domestic and export markets (Raju *et al.*, 2017) [11].

Multivariate analysis or D^2 statistics has been successfully employed for a quantitative assessment of genetic diversity. Mahalanobis's D^2 statistics is a useful tool for assessing the degree of genetic divergence and providing a quantitative measure of the relationship based on genotype distance. The maximum heterosis is expressed when the parents are genetically different.

The clustering of genotypes based on the genetic distance provide a scientific base for the selection of parents from the genetically distant clusters for the production of hybrids.

Materials and methods

The experiment was conducted in the polyhouse located at the North Instructional farm of the School of Agricultural sciences, Karunya Institute of Technology and Sciences, Coimbatore situated at 10.9362° N latitude and 76.7441° E longitude in the southern Western Ghats, in the Siruvani forest foothills. A total of 24 genotypes were used in this study (Table 1). These genotypes were collected from different parts of South India. The genotypes were grown under protected cultivation in a polyhouse in Completely Randomized Design. The seeds were sown in a seed bed of size 250 cm × 80 cm. The seedlings were then transplanted in a polybag of size 40 cm × 40 cm. Each genotype was transplanted in fifteen bags with each bag having one seedling and recommended package of practices were followed. Observations for 20 quantitative traits *viz.*, plant height (cm), number of primary branches, number of secondary branches, plant spread east west (cm), plant spread north south (cm), stem girth (cm), number of days to initiate flowering, number of days to 50% flowering, pedicel length (cm), green fruit girth (cm), green fruit length (cm), green fruit weight (g), number of green fruits per plant, green fruit yield per plant (g), dry fruit girth (cm), dry fruit length (cm), dry fruit weight (g), dry fruit yield per plant (g), number of seeds per fruit and 1000 seed weight (g) as well as two biochemical observations *viz.*, ascorbic acid content (mg/100g) and capsaicin content (SHU) were recorded. The genotypes were grouped into clusters based on their similarities and differences. Mahalanobis D² analysis developed by Mahalanobis (1936) were used to estimate the genetic distance between the genotypes.

Table 1: List of genotypes of chilli (*Capsicum annuum* L.) and their source of collection

S. No	Genotypes	Source
1	Samba chilli	Tirunelveli, India
2	Sankarankoil local	Tirunelveli, India
3	Long chilli	Thoothukudi, India
4	Fat chilli	Kerala, India
5	Mundu chilli	Ramanad, India
6	Naatu mundu chilli	Ramanad, India
7	Red mundu chilli	Ramanad, India
8	Jwala chilli	Kerala, India
9	Bydagi chilli	Hyderabad, India
10	Nanguneri local	Tirunelveli, India
11	Kashmiri chilli	Hyderabad, India
12	Malappuram local	Kerala, India
13	Bullet chilli	Kanyakumari, India
14	Mettupatti samba local	Salem, India
15	Mettupatti local	Salem, India
16	Anjugramam local	Kanyakumari, India
17	Alanthurai local	Coimbatore, India
18	Thondamuthur local	Coimbatore, India
19	Anaimalai local	Coimbatore, India
20	Mettupalayam local	Coimbatore, India
21	Annur local	Coimbatore, India
22	Sulur local	Coimbatore, India
23	Kangeyam local	Tiruppur, India
24	Dharapuram local	Tiruppur, India

Results and Discussion

Analysis of variance presented in (Table 2) showed significant differences for all the 22 characters studied among the chilli genotypes. This indicated that there is significant variation among the genotypes under study. From the results of D² analysis, the genotypes were grouped into six.

Table 2: ANOVA for 22 characters of chilli (*Capsicum annuum* L.)

S. No	Traits	Mean sum of squares	
		Genotypes	Error
1	Plant height (cm)	907.61**	26.98
2	No. of primary branches	0.84**	0.14
3	No. of secondary branches	5.01**	0.59
4	Plant spread EW (cm)	116.20**	12.82
5	Plant spread NS (cm)	134.07**	12.01
6	Stem girth (cm)	0.51**	0.07
7	Days to first flowering	751.98**	12.57
8	Days to 50% flowering	916.99**	13.16
9	Pedicel length (cm)	0.40**	0.08
10	Green fruit girth (cm)	6.52**	0.15
11	Green fruit length (cm)	19.50**	0.27
12	Green fruit weight (g)	1.64**	0.10
13	No. of green fruits/ plant	768.60**	36.51
14	Green fruit yield/ plant	8344.42**	378.75
15	Dry fruit girth (cm)	7.32**	0.16
16	Dry fruit length (cm)	20.25**	3.13
17	Dry fruit weight (g)	0.11**	0.01
18	Dry fruit yield/ plant (g)	910.96**	41.70
19	No. of seeds/ fruit	1407.72**	55.62
20	1000 seed weight (g)	1.99**	0.16
21	Ascorbic acid content (mg/100g)	602.80**	64.15
22	Capsaicin content (SHU)	0449167.15**	9163767.45

Clusters based on their similarity and differences (Fig 3). Among the six clusters, (Table 3) cluster I, III and VI had seven genotypes each and the clusters II, IV and V had one genotype each. From (Table 4) the maximum inter cluster D² distance was found between cluster IV and cluster V (44.68) followed by cluster V and cluster III (43.37), cluster I and cluster II (43.25), cluster III and cluster IV (41.66), cluster II and cluster V (41.02), cluster II and cluster IV (40.90), cluster I and cluster VI (40.75), cluster III and cluster VI (40.72), cluster V and cluster VI (40.68), cluster II and cluster VI (40.03) (Fig 1). Similar results were reported by Pujar (2017), Sathiyamurthy *et al.* (2019) [10, 12]. Minimum inter cluster D² distance was found between cluster IV and cluster VI (38.78) followed by cluster I and cluster IV (39.41), cluster I and cluster III (39.96). Singh *et al.* (2023), Nabarun *et al.* (2021) [13] also got similar results in their experiments. The maximum intra cluster distance was found in cluster II (36.35) followed by cluster I (34.77) and cluster VI (30.74). This was in accordance with the results of Singh *et al.* (2023), Dutonde *et al.* (2008), Chandan *et al.* (2016), Mamatha *et al.* (2017) [13, 3, 2, 6].

The variation among and within the clusters can be estimated using D² analysis. The genotypes Fat chilli and Jwala chilli present in the clusters IV and V had the maximum variation in the characters (Fig. 2). These genotypes were genetically different and can be utilized in breeding programme. Capsaicin content was reported to contribute the most to the variation followed by green fruit yield per plant (g), plant height (g) and ascorbic acid content (mg/100g). Sushmitha *et al.* (2019) also got similar results in their experiments. Selection based on these characters will produce superior

hybrids. The genotypes present in the clusters IV and VI showed the minimum inter cluster distance. This showed that the genotypes had less variation between them and selection of characters based on these genotypes will not be much effective. The cluster II had the maximum intra cluster distance showed that the genotypes although present in the same cluster, there was significant difference in one or two characters. The variation among the cluster II was due to maximum plant height (cm), number of primary and secondary branches in Nanguneri local, maximum green fruit yield per plant (g), minimum green and dry fruit length (cm) in Dharapuram local and the maximum capsaicin content (SHU) in Sankarankoil local. Such characters can also be considered for hybridization programme. The minimum intra cluster distance in cluster VI showed that the genotypes present in this cluster were closely related and showed minimum variation.

Table 3: Distribution of 24 genotypes among different clusters

Clusters	No. of genotypes	Name of genotypes
I	7	Samba, Mundu chilli, Naatu mundu chilli, Ramnad mundu chilli, Mettupatti local, Anaimalai local, Kangeyam local
II	7	Nanguneri local, Bydagi chilli, Sankarankoil local, Thondamuthur local, Mettupalayam local, Annur local, Dharapuram local
III	1	Long chilli
IV	1	Fat chilli
V	1	Jwala chilli
VI	7	Kashmiri chilli, Malappuram local, Bullet chilli, Mettupatti samba local, Anjigramam local, Alanthurai local, Sular local

Table 4: Average inter and intra cluster distance among six clusters

	C1	C2	C3	C4	C5	C6
C1	34.77	43.25	39.96	39.41	40.80	40.75
C2		36.35	40.55	40.90	41.02	40.03
C3			0.00	41.66	43.37	40.72
C4				0.00	44.68	38.78
C5					0.00	40.68
C6						30.74

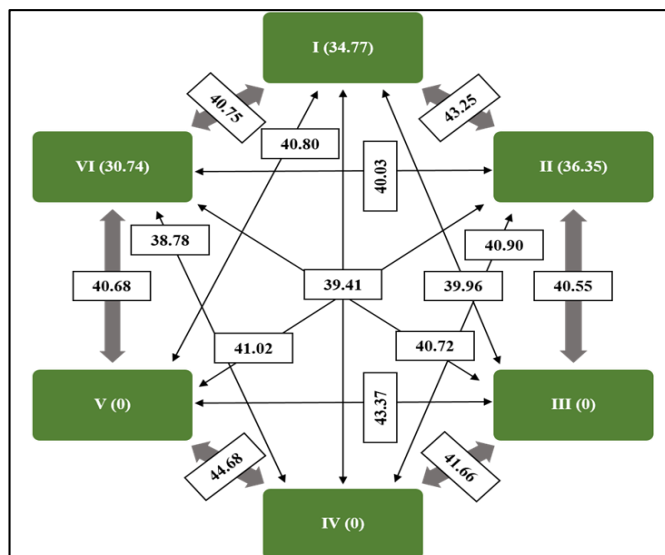


Fig 1: Mahalanobis D² distance between the clusters

On the basis of cluster means (Table 5), cluster III performed better for most of the observed quantitative traits. The genotype Long chilli in cluster III had maximum green fruit yield per plant (g), number of primary branches, number of secondary branches, minimum number of days to flowering initiation, maximum pedicel length (cm), green fruit length (cm) and dry fruit length (cm). Among the genotypes Sankarankoil local had the maximum number of fruits per plant and green fruit yield (g), Naatu mundu chilli had the minimum number of days for flower initiation and 50% flowering, Samba chilli had the maximum fruit length (cm), Bullet chilli had the maximum green fruit weight (g) and Jwala chilli had the maximum ascorbic acid content (mg/100g) and capsaicin content (SHU). Similar results in the cluster means were observed by Dutonde *et al.* (2008), Azrah *et al.* (2021), Chandan *et al.* (2016) [3, 1, 2]. Among all the genotypes, Sankarankoil local, Naatu mundu chilli, Samba chilli, Bullet chilli and Jwala chilli were found to be promising genotypes for different quantitative and biochemical traits. These genotypes can be utilized for the improvement of crops for specific traits and also for future breeding programmes.

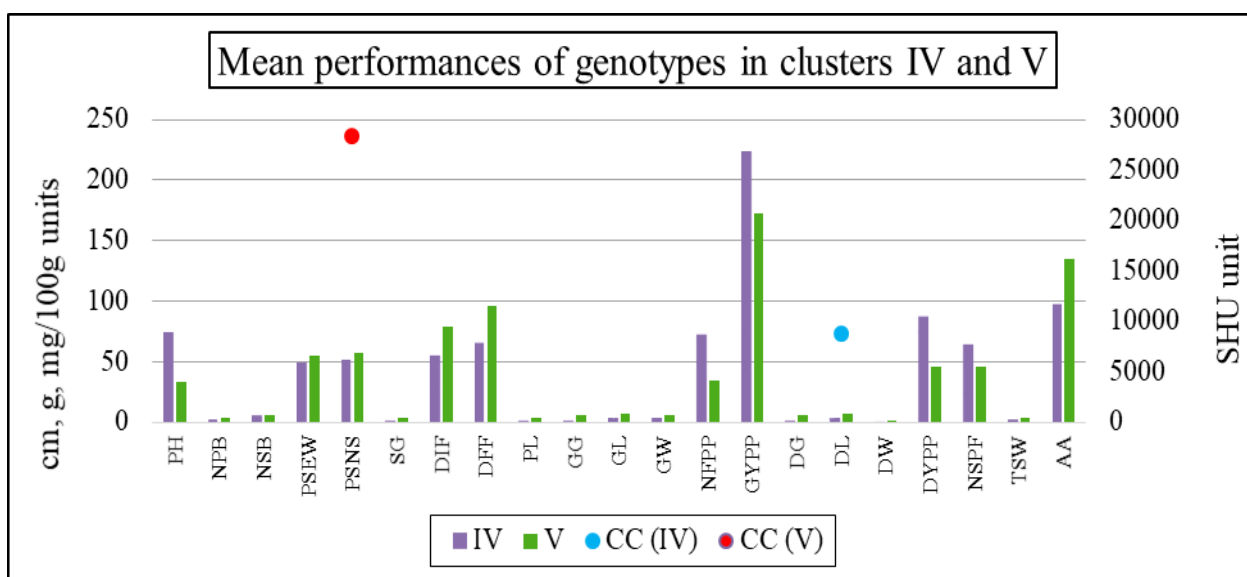


Fig 2: Graphical representation of mean performances of genotypes in the clusters IV and V having maximum inter cluster D² distance

Table 5: Cluster mean performances of 24 genotypes of chilli (*Capsicum annum* L.) for 22 traits

Traits	I	II	III	IV	V	VI
Plant height (cm)	81.04	78.19	87.87	75.05	32.57	81.80
No. of primary branches	2.63	2.96	3.92	3.00	2.92	2.99
No. of secondary branches	4.92	6.06	7.83	6.53	5.83	5.80
Plant spread East West (cm)	46.10	41.58	46.89	50.10	55.39	42.49
Plant spread North South (cm)	47.99	42.82	49.76	52.11	57.66	44.24
Stem girth (cm)	3.16	2.97	3.33	2.43	3.42	2.75
Days to flower initiation	57.96	59.45	54.73	55.83	78.92	70.56
Days to 50% flowering	72.12	75.45	66.47	66.25	95.92	84.42
Pedical length (cm)	3.02	2.79	3.46	2.82	3.06	2.88
Green fruit girth (cm)	5.23	3.86	3.65	2.28	6.21	4.48
Green fruit length (cm)	5.34	8.02	8.79	4.58	6.59	7.60
Green fruit weight (g)	3.50	3.47	4.14	4.82	5.46	4.13
Number of fruits per plant	50.72	67.03	63.27	73.17	34.42	63.90
Green fruit yield per plant (g)	178.83	217.30	262.45	223.67	172.38	229.31
Dry fruit girth (cm)	5.13	3.80	1.64	2.18	6.12	4.41
Dry fruit length (cm)	5.20	8.63	8.72	4.47	6.32	7.39
Dry fruit weight (g)	0.89	0.86	1.03	1.20	1.36	1.04
Dry fruit yield per plant (g)	45.21	60.82	64.95	88.40	45.82	65.98
No. of seeds per fruit	94.13	67.66	82.33	64.92	45.33	84.35
1000 seed weight (g)	4.54	4.29	4.45	3.86	3.44	3.95
Ascorbic acid content (mg/100g)	88.75	90.79	99.81	97.70	135.31	87.73
Capsaicin content (SHU)	8453.98	11088.43	25075.40	8801.92	28319.17	9622.82

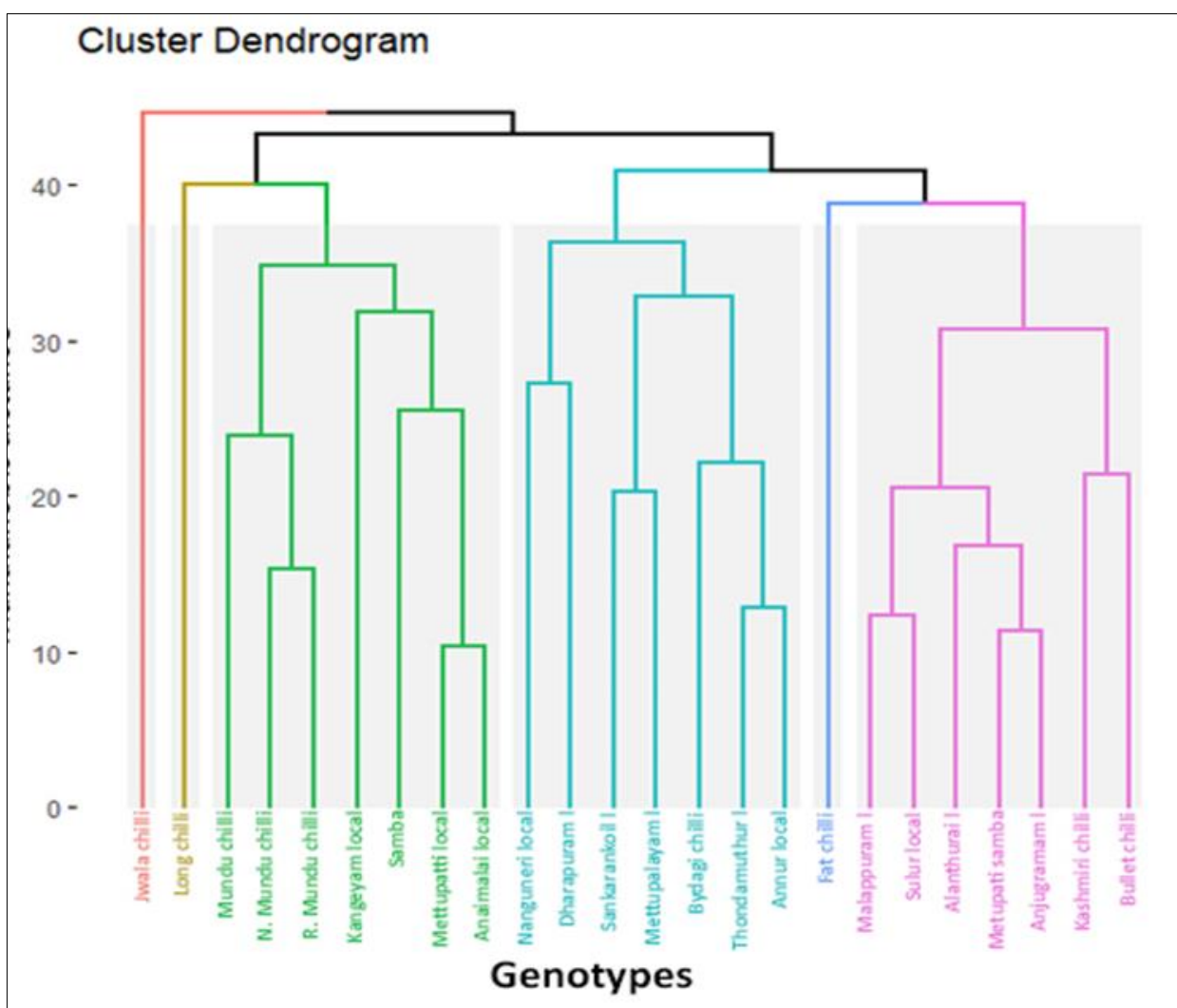


Fig 3: Dendrogram based on hierarchical clustering for chilli based on the observed traits

Table 6: Mean performances of 24 genotypes of chilli (*Capsicum annum L.*) for 22 traits

S. No	Variety	PH	NPB	NSB	PSEW	PSNS	SG	DIF	DFE	PL	GG	GL	GW	NFPP	GYPP	DG	DL	DW	DYPP	NSPF	TSW	AA	CC
1	Samba	80.08	2.53	4.80	41.92	44.58	3.21	57.13	68.13	3.49	3.26	11.50	3.54	69.20	241.00	3.13	10.93	0.90	61.61	79.53	5.32	112.74	6515.33
2	Sankarankoil local	96.77	2.47	5.27	37.80	39.70	3.33	50.73	62.47	2.20	3.36	11.16	3.99	93.60	302.00	3.29	11.08	1.00	93.81	79.73	6.26	100.20	18874.47
3	Long chilli	87.87	3.92	7.83	46.89	49.76	3.33	54.73	66.47	3.46	3.65	8.79	4.14	63.27	262.45	1.64	8.72	1.03	64.95	82.33	4.45	99.81	25075.4
4	Fat chilli	75.05	3.00	6.53	50.10	52.11	2.43	55.83	66.25	2.82	2.28	4.58	4.82	73.17	223.67	2.18	4.47	1.20	88.40	64.92	3.86	97.70	8801.92
5	Mundu chilli	70.73	2.73	5.53	45.43	47.74	3.46	48.07	60.07	3.41	7.81	2.28	4.12	50.87	208.37	7.78	2.16	1.03	52.08	127.80	5.26	72.85	8404.87
6	N. mundu chilli	85.45	2.47	4.27	44.96	46.40	3.86	35.20	45.93	2.96	7.53	2.95	3.96	38.47	153.50	7.35	3.01	1.07	40.95	119.67	4.16	83.58	7244.07
7	R. mundu chilli	60.77	2.33	3.73	54.83	57.58	2.94	40.80	52.07	2.60	7.10	3.26	3.26	33.07	116.33	6.98	3.18	0.82	27.17	103.27	4.82	90.51	7990.07
8	Jwala chilli	32.57	2.92	5.83	55.39	57.66	3.42	78.92	95.92	3.06	6.21	6.59	5.46	34.42	172.38	6.12	6.32	1.36	45.82	45.33	3.44	135.31	28319.17
9	Bydagi chilli	52.65	2.58	5.42	36.03	36.41	2.68	39.67	52.25	2.80	3.94	10.26	3.24	43.25	136.61	3.94	10.15	0.83	39.51	45.33	5.10	79.82	13912.33
10	Nanguneri local	105.66	4.27	8.60	58.11	60.32	2.47	76.07	92.20	3.13	3.95	9.34	3.95	73.27	231.67	3.85	9.24	0.99	72.37	73.47	5.03	83.41	8123.4
11	Kashmiri Chilli	61.86	3.42	6.25	39.79	41.84	2.33	56.00	69.25	3.23	4.59	9.30	5.25	48.92	185.67	4.50	8.44	1.46	70.60	113.75	3.96	93.32	10738.5
12	Malappuram local	76.36	2.58	4.83	42.58	44.86	2.93	83.83	98.50	2.28	3.69	6.30	3.53	55.58	183.32	3.59	6.22	0.88	49.52	77.75	3.14	84.9975	7853.83
13	Bullet chilli	101.26	3.42	7.25	42.93	45.83	2.52	61.17	74.17	3.30	6.94	6.46	5.58	75.58	271.00	6.85	6.36	1.30	98.75	74.00	4.53	77.34	13388.17
14	Mettupatti samba local	74.90	2.20	3.67	37.62	38.78	3.09	52.75	65.92	3.01	4.04	10.48	3.65	68.67	247.51	3.97	10.38	0.94	64.81	80.92	4.41	78.49	9137.33
15	Mettupatti local	101.24	3.25	6.67	52.99	54.87	2.68	87.25	104.50	2.66	3.58	5.18	2.98	35.92	107.52	3.50	5.09	0.79	28.42	83.25	4.59	76	8703.83
16	Anjugramam local	79.87	2.73	5.20	40.01	40.41	3.38	73.08	85.83	2.94	4.03	6.37	3.68	66.75	244.70	3.94	6.26	0.90	59.88	89.00	3.68	88.85	9284.67
17	Alanthurai local	101.24	3.40	7.13	48.74	50.01	2.43	87.67	103.47	2.60	3.88	6.91	3.75	59.53	219.75	3.87	6.76	0.94	55.00	62.47	3.79	104.93	8287.13
18	Thondamuthur local	60.37	2.83	5.92	37.15	37.61	2.47	67.00	86.75	2.99	4.10	5.78	3.24	68.50	222.20	4.04	5.71	0.82	55.90	74.17	4.04	108.10	9955.67
19	Anaimalai local	74.06	2.92	5.75	42.94	44.30	3.23	71.08	87.92	2.74	3.54	4.43	3.17	49.67	157.69	3.46	4.35	0.79	39.36	52.83	3.77	92.21	9194.92
20	Mettupalayam local	69.86	2.33	4.92	40.42	42.05	3.32	38.67	56.42	2.26	4.05	6.73	3.06	49.08	145.94	3.98	11.51	0.71	44.62	63.17	2.49	93.82	9380.33
21	Annur local	84.84	2.92	7.00	42.68	44.00	3.00	72.58	90.42	2.97	3.76	7.57	3.56	67.00	238.36	3.70	7.49	0.88	58.70	52.83	3.72	74.65	7244.33
22	Sulur local	77.10	3.17	6.25	45.78	47.94	2.55	79.42	93.83	2.82	4.21	7.38	3.50	72.25	253.23	4.14	7.30	0.87	63.28	92.58	4.13	86.17	8670.08
23	Kangeyam local	94.91	2.20	3.67	39.61	40.47	2.78	66.17	86.25	3.24	3.81	7.77	3.43	77.83	267.42	3.72	7.70	0.86	66.86	92.58	3.87	93.33	11124.75
24	Dharapuram local	77.16	3.33	5.33	38.89	39.66	3.53	71.42	87.67	3.18	3.89	5.31	3.27	74.50	244.31	3.83	5.24	0.82	60.86	84.92	3.42	95.53	10128.50
	CD	16.35	1.18	2.42	11.27	10.91	0.82	11.16	11.42	0.87	1.23	1.62	1.00	19.02	61.25	1.26	5.57	0.28	20.32	23.47	1.24	25.21	9526.93

PH-Plant height (cm), NPB-no. of primary branches, NSB- No. of secondary branches, PSEW- Plant spread East West (cm), PSNS-Plant spread North South (cm), SG- Stem Girth (cm), DIF-Days to first flowering, DFE- Days to 50% flowering, PL-Pedicel length (cm), GG-Green fruit girth (cm), GL-Green fruit length (cm), GW-Green fruit weight (g), NFPP-No. of fruits per plant, GYPP-Green fruit yield per plant (g), DG-Dry fruit girth (cm), DL-Dry fruit length (cm), DW-Dry fruit weight (g), DYPP-Dry fruit yield per plant (g), NSPF-Number of seeds per fruit, TSW-1000 seed weight (g), AA-Ascorbic acid content (mg/100 g), CC-Capsaicin content (SHU)

Conclusion

In the present study the 24 genotypes were grouped into six clusters, selection of one parent from each cluster is the one approach. However, the individual merit of the genotypes also has to be considered while choosing parents for specific improvement of desirable traits. In the present study clusters, I, II and VI had seven genotypes each, and three clusters were mono genotypic. Mono genotypic clusters need to be considered if it has desired traits to be improved. The genotype Jwala chilli coming under cluster V is having the highest capsaicin content so this will be an ideal parent for improvement of capsaicin content. When a large number of genotypes happen to fall in a cluster more than one parent can be selected based on the individual merit from the cluster having greater intra cluster distance.

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