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Studies on clustering of chilli (*Capsicum annum* L.) genotypes based on genetic distance

M. Karthikeyan [*1], P. Karthikeyan[*2], K. R. Saravanan[3] and S. Madhavan[4]

1-Ph.D Research Scholar, 2-Associate Professor, 3-Assistant Professor, 1,2&3-Department of Genetics and Plant Breeding, 4-Assistant Professor, Department of Horticulture, Faculty of Agriculture, Annamalai University,Annamalainagar-608002 (Tamil Nadu), India

*Author for correspondence : M. Karthikeyan & P. Karthikeyan, karthikechik123@gmail.com, karthikeyan06oct@gmail.com

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Abstract

Mahalanobis' D^2 statistics was used to analyse forty-five chilli (*Capsicum annum* L.) genotypes based on eighteen characters in order to pick out supreme potential parents for hybridization. Based on D^2 values, the genotypes were divided into eleven groups with extreme divergence. Cluster I had the majority of genotypes (sixteen), whereas the fewest genotypes were identified in clusters VII, VIII, X and XI (one). Cluster XI had the greatest distance within the cluster. Clusters V and XI had the maximum generalized distance between them, followed by clusters VII and XI, clusters IV and VII, clusters IV and V and clusters II and XI. This suggests that the genotypes in these groups had more genetic variation. Following cluster VII and VIII, cluster V showed the highest cluster mean for green, dry fruit yield (846g and 95.50g) and several yield-related features. At clusters I, II and VI, no observation for high cluster means but had fair trait performance. It may be suggested to directly advance the genotypes from clusters V, VII, VII in hybridization to obtain unique recombinants.

Keywords: chilli, clustering, genetic distance, complex traits

Introduction:

Chilli (*Capsicum annum* L.) is a significant vegetable and universal spice crop with enormous therapeutic and commercial value. Chilies make up 42% of the nation's total spice exports, and they are mostly export to nations like China, Thailand, Malaysia, Sri Lanka, Vietnam, and Indonesia. India tops the world in production of chilli with 1.98 million metric tons, followed by China, Ethiopia, Thailand, Pakistan, and Bangladesh (*3rd Advance Estimates, 2020-21*). Andhra Pradesh is at the top of the list for dried chili output in 2020–21, with 7.97 lakh MT grown on 1.77 lakh ha and 4489 kg/ha productivity, after that Telangana, Madhya Pradesh, Karnataka, and West Bengal stands. In 2021–22, all over India, chilli was grown on 2.29 lakh hectares of land, with a yield of 10.78 lakh MT and a productivity of 4707 kg per ha. Red chilli production for 2021-22 is estimated to be 13.84 lakh MT, up from 12.32 lakh MT of the previous year (*2nd Advance Estimates, 2021-22*).

Pungency and colour are the two key characteristics of chilli pepper fruits that impact the fruit's quality, aroma, flavour, and taste, as well as consumer preference (Indrabi *et al*. 2021). In addition to vitamins A, B_2, B_{12}, C, E and K, chillies are a good source of bioactive substances, including flavonoids (quercetin, luteolin, and phenolic acids), capsaicinoids, and carotenoids (lutein, carotene, cryptoxanthin, zeaxanthin, violaxanthin, and capsanthin and capsorubin). Depending on the genotype and cultivar of the chilli, the concentration of these beneficial chemicals might vary greatly (Villa-Rivera and Ochoa-Alejo, 2020). Chillies can be found in more than 400 diverse kinds worldwide. For a breeder to select the best kind of parents for deliberate hybridization in heterosis breeding, knowledge of the type and extent of genetic difference is crucial to avail transgressive segregation (Gayathri *et al.* 2022). Cluster analysis is an appropriate avenue to resolve familial links. In examining genetic diversity through cluster analysis, a variety of techniques have been employed, with Tocher's method being the most broadly utilized (Yatung *et al*., 2014).

With the following specific goals in mind, the current study was guided toward a genetic diversity analysis of chilies: to compare the yield contributing traits and yield potentiality of various chili genotypes; to screen out the suitable parents group that are likely to produce superior segregates on hybridization; and to quantify the degree of genetic divergence in genotypes for the purpose of spotting the genetically distinctive parents which could be used in future breeding programmes.

Materials and methods

The experiment was conducted at PLant Breeding Farm, Department of Genetics and PLant Breeding, Annamalai Univeristy, during the year 2021. The experimental farm is located at 11.60° N latitude and 78.13° E longitude with an elevation of 185.63 meters above sea level. A total of 45 genotypes of chilli (*Capsicum annum* L.), collected from Guntur, Manipur, Raichur and various regions of Tamil Nadu were included in this study. With three replicated trials, the experiment was set up using a randomized block design. After being sown in separate nursery plots, 45-day-old seedlings were transplanted onto the experimental plot. The recommended dosage for applying manures and fertilizers was followed. The plants received irrigation and weeding as required. Field observations were recorded as replication data for Days to 50 percent flowering, number of primary branches per plant, plant height(cm), plant spread(cm), stem diameter(mm), fruit stalk length(cm), fruit length(cm), fruit girth(mm), placenta length(cm), number of fruits per plant, number of seeds per fruit, fresh fruit weight(g), dry fruit weight(g), 1000 seed weight(g), days to green fruit maturity (days), days to red fruit maturity (days), green fruit yield (g/plant) and dry fruit yield (g/plant). To measure genetic distance and divergence, an agri-statistical package AGRISTAT was utilized which works under Mahalanobis D^2 statistical (1936) procedure and Tocher's approach (Rao, 1952) for cluster formation.

Results and discussion

The 45 genotypes were divided into eleven divergent clusters based on Mahalanobis D^2 values (Table 1), demonstrating sufficient genetic variation for choosing outstanding and diverse parents that can be used in any breeding programme. With 16 genotypes, cluster I was the most populous group, descended by cluster II (ten genotypes), cluster III and cluster IV with 4 genotypes, cluster VI with three members. While clusters VII, VIII, X and XI only had one genotype each, cluster V and IX had two. The findings are comparable with Yatung *et al*. 2014; Srinivas and Thomas, 2018; Singh *et al*. 2023.

Broad trait variation in field performance brought the genotypes into separate groups as it experienced variable response from own genetic base and plot surroundings (Haq *et al*. 2022). From the cluster separation, it is cleared that the separation is solely based upon genotypes genetic architecture not upon the collection source (Deepo *et al*. 2020; Rahevar *et al*. 2021). Clustering was made out to confer the parents for intercrossing from the most diverse groups which would produce a broad spectrum of variability for quantitative traits examined to enable further genotype selection and trait improvement (Ananya *et al*. 2020).

Table 1. Cluster grouping of forty-five chilli collections

Cluster groups	No. of genotypes	Genotypes name
I	16	LCA 657, BVC 42, LCA 353, LCA 235, Meitei morok, Raja, Edayuru, 273, Hebanaro chilli, White bird eye, Orange bird eye, Cream bird eye, Black kanthari, Violet kanthari, Karanam potti, Gusto purple
II	10	Varsha, Mecheri local, LCA 625, Jwala, LCA 684, 334, Thiruvanamalai local, Super 10, LCA 680, Teja
III	4	Syngenta 5531, Syngenta byadgi, Guntur local, 341
IV	4	Naga chilli, Ney mulaku, Chocolate chilli, Bhut jolokia
V	2	LCA 620, Bullets
VI	3	Black hungarian, Black long, Black shade.
VII	1	PKM 1
VIII	1	34
IX	2	Byadgi dabbbi, Ujwala
X	1	Violet shade
XI	1	Tomato chili

Table 2. Distance (D^2) of intra and inter clusters

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	**185.25 (13.61)**	820.30 (28.64)	318.68 (17.85)	397.71 (19.94)	1280.69 (35.79)	330.51 (18.18)	1279.04 (35.76)	839.52 (28.97)	339.33 (18.42)	260.90 (16.15)	581.30 (24.11)
II		**150.40 (12.26)**	420.59 (20.51)	1697.27 (41.20)	249.94 (15.81)	963.81 (31.04)	278.24 (16.68)	282.14 (16.80)	603.13 (24.56)	867.84 (29.46)	2078.77 (45.59)
III			**187.04 (13.68)**	832.53 (28.53)	697.30 (26.41)	427.12 (20.67)	847.55 (29.11)	595.64 (24.41)	308.97 (17.58)	292.38 (17.10)	1124.91 (33.54)
IV				**167.55 (12.94)**	2345.14 (48.43)	701.36 (26.48)	2445.27 (49.45)	1625.46 (40.32)	558.16 (23.63)	535.76 (23.15)	337.29 (18.37)
V					**169.40 (13.02)**	1266.50 (35.59)	306.78 (17.52)	536.06 (23.15)	1017.15 (31.89)	1260.14 (35.50)	2797.71 (52.89)
VI						**134.58 (11.60)**	1309.96 (36.19)	1183.34 (34.40)	680.00 (26.08)	355.60 (18.86)	645.23 (25.40)
VII							**0.00 (0.00)**	319.77 (17.88)	1204.99 (34.71)	1491.78 (38.62)	2711.78 (52.07)
VIII								**0.00 (0.00)**	639.63 (25.29)	922.78 (30.37)	1891.52 (43.49)
IX									**234.34 (15.31)**	381.14 (19.52)	917.83 (30.29)
X										**0.00 (0.00)**	681.94 (26.11)
XI											**0.00 (0.00)**

(The value in the parenthesis indicates D-value. The bold letters indicate intra cluster distance)

Intra and inter cluster distance:

Table 2 displayed the average distances within and between clusters. The clustering pattern established in this research was validated because the largest intra-cluster divergence (D=15.31) for cluster IX was less than the smallest inter-cluster divergence (D=15.81) between cluster II and cluster V. With cluster II showing the greatest value, cluster III (D=13.68), cluster I (D=13.61), cluster V (D=13.02), cluster IV (D=12.94), cluster II (D=12.26) and cluster VI (D=11.60) were in order of decreasing intra-cluster distance. Having a solitary genotype, clusters VI, VII, VIII and IX were prevented from displaying any intra-cluster distance (D=0.00). Cluster V and cluster XI had the greatest inter cluster distance (D), which was measured at 52.59, followed by cluster VII and cluster XI at 52.07, cluster IV and cluster VII at 49.45 and cluster IV and cluster V at 48.43.

Formation of new variable clusters evidenced the narrow genetic distance within the cluster that paves path for broader distanced distinct types to separate (Rahevar *et al*. 2021). The minimum and maximum D^2 values between clusters indicated a close and repel association, respectively. The genotypes that fell under distanced and distinct clusters were thus genetically diverse and ideal for the chilli hybridization programme to achieve the greatest amount of hybrid vigor. Results are in line with Farhad *et al*. 2010; Hasan *et al*. 2014; Sreenivas *et al*. 2019; Deepo *et al*. 2020; Indrabi *et al*. 2021, Binolin *et al*. 2023.

Table 3. Cluster means of 18 characters for 45 genotypes

Clusters Vs Traits	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
DFF	106.00	87.90	98.5	118.67	83.83	108.56	76.34	70.00	104.50	100.33	112.20
NPB	5.00	5.03	5.42	4.75	5.67	4.67	6.64	4.10	6.01	4.67	6.12

PH	74.31	73.97	66.92	67.42	94.50	63.89	69.11	67.67	67.64	87.33	31.33
PS	64.06	67.00	62.58	74.83	79.83	70.68	77.05	59.03	71.50	21.34	53.36
SD	11.48	11.86	11.54	10.41	17.05	15.72	12.50	9.77	11.62	15.41	8.47
FSL	2.66	2.83	2.93	2.88	3.47	2.61	2.40	2.90	3.07	2.72	2.03
FL	5.26	5.75	5.76	5.28	6.28	2.57	4.87	5.93	4.88	5.11	2.00
FG	8.91	9.78	8.18	12.2	14.56	13.23	8.71	11.18	13.27	11.97	21.72
PL	3.85	4.93	5.18	2.90	6.18	2.21	4.27	5.02	3.52	3.62	1.53
NF/P	78.25	153.40	122.42	28.67	187.50	152.11	193.34	93.02	74.83	45.08	55.67
NS/P	61.15	80.93	94.08	36.42	86.83	39.67	80.67	70.68	82.00	46.06	45.68
FFW	3.67	4.57	4.73	3.89	5.16	2.74	3.53	4.23	5.02	4.74	1.75
DFW	1.57	2.51	2.37	1.65	2.61	1.28	1.21	1.92	2.75	2.65	0.95
TSW	3.59	4.90	4.22	3.48	5.12	3.01	5.84	4.31	4.11	2.78	2.50
GFM	75.83	51.33	73.83	80.17	52.17	82.89	46.00	49.67	62.50	80.11	84.11
RFM	92.50	69.40	87.25	98.75	70.83	105.64	62.63	64.62	81.52	103.32	111.33
GFY/P	395.15	704.93	545.75	186.00	846.00	514.08	788.07	513.05	400.12	396.36	165.14
DFY/P	37.81	67.03	54.75	31.33	95.50	56.44	79.03	52.67	41.03	40.10	19.37

[DFF-Days to 50% flower (days); NPB-No. of primary branches; PH-Plant height (cm); PS-Plant spread (cm); SD-Stem diameter (mm); FSL-Fruit stalk length (cm); FL-Fruit length (cm); FG-Fruit girth (mm); PL-Placenta length (cm); NF/P-No. of fruits per plant; NS/P-No. of seeds per fruit; FFW-Fresh fruit weight (g); DFW-Dry fruit weight (g); TSW-Thousand seed weight (cm); GFM-Days to green fruit maturity; RFM-Days to red fruit maturity; GFY/P-Green fruit yield (g); DFY/P-Dry fruit yield (g)]

Cluster means: The distribution of genotypes over eleven clusters for which character-wise means were determined (Table 3). Maximum number of seeds and thin fruits were come under cluster III. Late flowers with lowest fruit and seed count mean were in cluster IV. Cluster V displayed the largest average for plant height, plant spread, stem diameter, fruit stalk length, fruit length, placenta length, fresh fruit weight, 1000 seed weight, green fruit yield and dry fruit yield. The character variables number of branches and number of fruits were high in cluster VII with earliest green and dry fruit maturity. Early flowering and lowest plant branches were averaged in cluster VIII. Highest mean value for dry fruit weight was noted in cluster IX and lowest plant spread by cluster X. late fruit maturity (green and red) with broader fruits was reported in cluster XI along with lowest average for plant height, stem diameter, fruit stalk length, fruit length, placenta length, fresh fruit weight, dry fruit weight, 1000 seed weight, green and dry fruit yield. Cluster I, II and VI were not registered for both extremes of mean values.

The group average could be an effective out frame to picturize the genotypes action for quantitative traits. The most remarkable clusters like V, VII and VIII could be directly selected as they will be fruitful in producing a broad array of variability and eventually bring about transgressive segregants for chilli. Many previous works in chilli such as Mondal *et al*. 2016; Negi and Sharma, 2019; Ananya *et al*., 2020; Jayanthi *et al*. 2023 gave importance to group average for proceeding parental selection.

Table 4. Contribution of characters to divergence

S.No.	Plant characters	Contribution (%)
1	Days to 50% flower (days)	5.86
2	No. of primary branches	0.12
3	Plant height (cm)	0.51
4	Plant spread (cm)	0.90
5	Stem diameter (mm)	0.40
6	Fruit stalk length (cm)	0.11

7	Fruit length (cm)	0.51
8	Fruit girth (mm)	2.42
9	Placenta length (cm)	0.05
10	No. of fruits per plant	7.67
11	No. of seeds per fruit	1.11
12	Fresh fruit weight(g)	2.22
13	Dry fruit weight(g)	7.77
14	1000 seed weight (cm)	0.61
15	Days to green fruit maturity	19.19
16	Days to red fruit maturity	8.88
17	Green fruit yield (g/plant)	42.12
18	Dry fruit yield (g/plant)	0.81

The percent contribution of each character towards genotypes diversity was tabulated in table 4. The trait green fruit yield alone contributes 42% towards diversity followed by green fruit maturity (19.19), red fruit maturity (8.88) number of fruits (7.67), dry fruit weight (6.77), days to 50% flower (5.86), fruit girth (2.42) registered their involvement as such. The other traits in the taken list had involved negligible (≤ 1) amount to divergence. Relative contribution by traits confesses the broad extremes of highly contributed traits to genotypes diversity which was endorsed by lot of researchers (Hasan *et al*. 2014; Akand *et al.* 2016; Negi and Sharma, 2019; Gayathri *et al.* 2022 and so many).

Conclusion

The study suggests that chilli genotypes examined had significant dissimilarity between them based on the eleven groups categorized. Considering group distance and appreciable agronomic features, the inter-genotypic crosses between clusters IV and VII, IV and V, IV and II & IV and VIII may be recommended as general. Cluster V genotypes had shown their excellent outcomes for yield and its fellow traits by marking the highest average among all which might be move on to the next level of breeding. For the objective of plant earliness, cluster VII and VIII genotypes would be a better breeding choice. Apart from high trait extreme means, genotypes from mid average clusters like cluster II and III can also be suggested for hybridization by recognizing their reasonable performance for most of the traits. Exploring genotypes from these groups may therefore expect elite vigorous recombinants upon multiple crosses. Inspite of having broad genetic distance, clusters IV and XI were not promoted as parents for crossing work because of their long duration traits, lack of prominent fruiting ability and most importantly low yielding capacity.

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