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Assessment of genetic divergence through cluster analysis of chilli varieties

Nabarun Sen, Karabi Biswas and Sankar Narayan Sinha*

Environmental Microbiology Research Laboratory, Department of Botany, University of Kalyani, Kalyani, West Bengal, India

Corresponding author (Email, sinhasn62@yahoo.co.in)

Our study on genetic diversity was conducted with six chilli genotypes with inter- and intra-specific cross varieties of the three most popular chilli species (*Capsicum annuum* L., *Capsicum chinense* Jacq., and *Capsicum frutescens* L.). Twelve quantitative characters, *viz.* plant height (cm), primary branches, secondary branches, number of flowers, number of fruits/plant; dry fruit weight (g), fruit length (cm), fruit diameter (cm), pedicel length (cm), pericarp thickness (mm), seeds/fruit, and seed weight (g), were taken into consideration. The analysis of variance revealed considerable variability among the genotypes for the character studied. Cluster analysis was used for grouping of parental and hybrid chilli genotypes under the study grouped into five clusters. Cluster I had the maximum (12) and clusters IV and V had the minimum number (1) of genotypes. The inter-cluster D^2 values ranged between 40.404 and 127.530. The minimum inter-cluster distance was between clusters III and IV (40.404). The maximum inter-cluster distance was observed between clusters II and V (127.53). Diversity pattern and other horticultural performance among the genotypes of new varieties were generated due to the cross between Local Line Mahadev Pramanick and Sukhia bullet and also the cross between Local Line Mahadev Pramanick and Habanero orange (cluster II) as well as single parental variety i.e. Habanero orange. These genotypes were identified as promising varieties with respect to various characters may be taken into consideration as better parents for an efficient hybridization programme of chilli.

Keywords.   Chilli; cluster analysis; genetic diversity; variance

1. Introduction

Capsicum is a genus from Solanaceae family. It is commonly known as 'chilli'. Red pepper is mainly used as vegetable, while green is used as spices when dried and processed. Its use in food, natural plant color, and as a pharmaceutical ingredient is extensive throughout the world. Recently use of hottest chilli *Naga Jolokia* has been recommended by Indian army as a spray for riot control and self-defense. The pungent flavor of chillies is due to the presence of capsaicinoids , which are closely related alkaloids. Among capsaicinoids, capsaicin and dihydrocapsaicin together account for about 90% of the pungency. The most common chilli species found in India are *Capsicum annuum, C. frutescens,* and *C. chinensis*. From ancient times various chilli varieties have already been used for curing several diseases. Tribal people of Nepal and in Himalayan range consume *Dolle khursani*, a local chilli variety, for curing gastroduodenal diseases. In folk medicine, chilli fruits have been used for the relief of anxiety and insomnia. The principal active component, capsaicin, of chilli was used as effective tropical analgesic agent and is used in arthritis pain, herpes-zoster-related pain, diabetic neuropathy, headache, etc. The chilli genotypes of the northeastern region have huge divergence and serve as a reservoir of genetic variability for chilli breeding. The chilli genotypes are selected by farmers for agronomic and horticultural traits important to them (e.g., fruit size, fruit weight, pungency level, color), and as a result of natural selection, are well adapted to the environment of the region. Genetic divergence in the chilli population accelerates the selection of suitable parents for utilization in breeding programs, leading to reduction in the number of crosses (Guerra

et al. 1999). Selection of the oldsters depends on the specific objective of the research program and their performance. Various statistical methods are used to select suitable parents. Complete information on the nature and degree of genetic divergence is helpful to the breeder to choose the right type of parents for purposeful hybridization in heterosis breeding (Patel *et al.* 1989; Farhad *et al.* 2010; Khodadabi *et al.* 2011). In order to learn transgressive segregation, the knowledge of genetic distance between parents is important (Joshi *et al.* 2004; Khodadabi *et al.* 2011). The standardization of variables is additionally essential towards determining the genetic distance in order that all variables are of comparable importance in determining the distance. Various methods have been used in studying of genetic diversity through cluster analysis, of which Mahalanobis' method is the most popular approach. The main objective of this study is to capture the potential genetic diversity between chilli genotypes grown in India by using cluster analysis and selection of suitable genotypes for future chilli hybridization programs.

2.   Material and methods

The breeding program of chilli varieties were performed in the experimental garden of Bidhan Chandra Krishi Viswavidyalaya (BCKV), Directorate of Research, Kalyani, Nadia, West Bengal, India. Seeds of following six pure chilli varieties were procured from the germplasm bank of BCKV. Seeds were sown in the experimental field and greenhouse of BCKV and fruits were harvested after 3–4 months of growth. The following chilli varieties were selected for breeding program.

1.   Local Line Mahadev Pramanick (MP) (*C. annuum* L.)
2.   Sukhia bullet (SB) (*C. annuum* L.)
3.   Kohima Jolokia (KJ) (*C. chinense* Jacq.)
4.   Habanero orange (HO) (*C. chinense* Jacq.)
5.   Dolle khursani (D) (*C. frutescens* L.)
6.   Mousinram chilli (M) (*C. frutescens* L.)

The 2- to 3-week-old seedlings were transplanted row to row on the basis of a randomized complete block design with four replications. Cow dung was applied. The observations were recorded on four randomly selected plants of each genotype based on qualitative and quantitative characters.

The statistical package for agricultural research (SPAR) version 1.0 program and SPSS were used to analyses chilli variability. The genetic divergence was calculated according to Mahalanobis' D^2 statistics (1936).

3.   Results

Analysis of variance exhibited significant differences among the varieties for all the traits under study, which indicated considerable amount of genetic variability, and were then subjected to further analysis. The computation from covariance matrix gave non-hierarchical clustering based on Mahalanobis' D^2 values (Mahalanobis 1936) among 19 chilli varieties (parental and hybrids) and grouped them into 5 clusters (table 1). Cluster I contained the highest number of varieties (12) followed by cluster II contained 3 varieties, cluster III contained 2 varieties, and clusters IV and V contained 1 variety each. SB x KJ, M, MP x KJ, SB x D, KJ x SB, D, HO x M, SB x HO, SB x M, MP x D, D x M, and D x SB varieties were classified in first cluster (cluster I) including 63.16% of the total varieties. The average value of plant height in different varieties in cluster I is higher (119.39 cm) than the mean of all varieties included in cluster s II, III, IV and V (table 2). MP x SB, SB x MP, MP x HO cross varieties were classified in II cluster including 15.79% of the total varieties. The average values for number of flowers, dry fruit weight, length of fruit, pedicel length, fruit yield per plant, and seeds per fruit in this cluster were higher than the mean of each trait included in clusters I, III, IV and V; for other traits, the average values were in the range of total mean (table 2). It is interesting to note that varieties generated as a result of crosses between MP × SB, SB × MP (reciprocal cross), and MP × HO showed the highest values for traits such as number of flowers, dry fruit weight, length of fruit, pedicel length, fruit yield per plant, and seeds per fruit. The varieties KJ and SB were classified into cluster III accounting for 10.53% of the total varieties. The average value of pericarp thickness in cluster III was greater than the average values of remaining clusters (table 2), and most of the other traits in cluster III were lower than the average value of pericarp thickness. Only one variety MP belonged to cluster IV accounting for 5.26% of the total varieties. In this cluster the number of secondary branches was more than other average values in the remaining cluster (table 2). Similarly, parental variety HO was classified into cluster V, accounting for 5.26% of the total varieties. There was a positive difference for the number of primary branches per plant, fruit diameter, and seed weight with the average values among

Table 1. Cluster analysis of chilli varieties

Cluster	No. of varieties	Name of varieties
CLUSTER I	Total: 12	SB x KJ, M, MP x KJ, SB x D, KJ x SB, D, HO x M, SB x HO, SB x M, MP x D, D x M, D x SB
CLUSTER II	Totals: 3	MP x SB, SB x MP, MP x HO
CLUSTER III	Totals: 2	KJ,
CLUSTER IV	Total: 1	MP
CLUSTER V	Total: 1	HO

the clusters, and other traits were not significant (table 2).

On the basis of cluster average value (table 3), it was observed that the genotypes in cluster V exhibited the lowest plant height (62.33), while those in cluster I exhibited the highest (119.39). Maximum number of primary branches per plant was observed in cluster V (12.33) and minimum number in cluster I (4.28). Cluster IV reported maximum secondary branches (6.33), while cluster III exhibited minimum (2.83).Number of flower(s) varied from 12.84 in cluster III to 110.78 in cluster II. Similarly, maximum number of fruits was in cluster II (72.78) and minimum in cluster I (7.95). Maximum weight of dry fruit was shown by cluster II (1.06) and minimum by cluster V (0.26). Maximum length of fruit was recorded in cluster II (4.21) and minimum in clusters III (2.38). Highest fruit diameter was observed for cluster V (3.17) and lowest in cluster II (1.01). Longest pedicel was recorded for cluster II (3.35) and shortest in cluster IV (1.14). Thick pericarp was recorded for clusters III (1.12) and II as exhibited thin pericarp by the values 0.18. Cluster II recorded highest seeds per fruit (70.89)s while cluster III recorded lowest seeds per fruit (21.50). Maximum seed weight was reported in cluster V (1.78)s while cluster I had minimum (0.40). Thus, varieties generated due to cross between MP and SB, and MP and HO (cluster II) as well as parental variety HO were identified as promising varieties with respect to characters like number of flower, fruit yield, fruit length, pedicel length, and seeds per fruit.

3.1 *Relative contribution of different characters*

The percentage contribution towards genetic divergence by 12 contributing characters is presented in table 4.The maximum contribution towards genetic divergence was by the number of primary branches (12.72%) followed by secondary branches (12.03 %), number of fruit per plant (10.83%), dry fruit weight (9.15 %), plant height (8.97 %), fruit diameter (8.15%), number of flower (7.65 %), pedicel length (7.45 %), seeds/fruit (6.93 %), seeds weight (6.36 %), and fruit length (5.88 %). The minimum contribution towards genetic divergence was by the pericarp thickness (3.88 %).

4. Discussion

The intra- and inter- cluster distance (D^2) values according to Mahalanobis' D^2 statistics (Mahalanobis 1936) are presented in table 3. The inter-cluster D^2

Table 2. Mean performance of yield per plant and its component characters in various clusters of chilli

CLUSTER	Plant Height (cm)	Primary Branches	Secondary Branches	Number of Flower	Number of Fruit/Plant	Dry fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Pedicel length(cm)	Pericarp thickness (mm)	Seeds/ fruit	Seeds weight (g)
I	**119.39**	4.28	3.72	14.89	7.95	0.61	2.87	1.02	2.73	0.25	28.97	0.40
II	99.67	9.44	3.22	**110.78**	**72.78**	**1.06**	**4.21**	1.01	**3.35**	0.18	**70.89**	0.41
III	78	6	2.83	12.84	10.33	0.95	2.38	1.60	2.43	**1.12**	21.50	0.51
IV	103	6	**6.33**	69.33	61	1.05	3.67	1.75	1.14	0.85	38.33	1.36
V	62.33	**12.33**	3.67	33	23.33	0.26	2.86	**3.17**	2.36	1.07	42.67	**1.78**

Bold values indicate maximum mean performance.

Table 3. Average intra- and inter-cluster distance (D^2) values

	I	II	III	IV	V
I	30.914				
II	64.139	**27.179**			
III	63.797	105.589	**32.345**		
IV	54.414	85.870	**40.404**	0.000	
V	91.997	**127.530**	56.138	51.039	0.000

Boldface values are maximum and minimum inter-cluster distances.

values ranged between 40.404 and 127.530. Minimum inter-cluster distance between clusters III and IV (40.404) indicated that varieties were genetically close to each other. Maximum inter-cluster distance was observed between clusters II and V (127.53), which indicated that these varieties were highly divergent. The intra-cluster divergence varied from 27.179 to 32.345. Maximum intra-cluster distance was achieved in cluster III (32.345), which comprised two varieties, while minimum divergence was observed in cluster II (27.179). Clusters IV and V showed zero intra-cluster distance due to the presence of only one genotype.

The highest genetic distance was observed between the clusters II and V (127.53) in the present study. According to Kumar *et al.* (2010), the hybrids of genotypes with maximum distance resulted in high yield and thus the cross between the genotypes from clusters II and V can be used in chilli breeding to achieve maximum heterosis. Minimum distance was noted between the genotypes of clusters III and IV (40.404), which can be used for backcrossing programs. Similar findings by Sundaram *et al.* (1980) revealed that cluster analysis can prove useful for finding high-yielding chilli genotypes. Such results were also supported by other authors (Indira 1994; Roy and Sorma 1996; Mishra *et al.* 2004), which indicated the presence of a high genetic divergence among chilli genotypes. Some of the desirable varieties identified in the present study included MP × KJ, KJ × SB, SB × HO in cluster I, MP × SB, SB × MP in cluster II, parental variety SB in cluster III, parental variety MP in cluster IV, and parental variety HO in cluster V. D^2 statistics has been found as a genetic divergence estimation tool, and being a numerical estimate, it has the added advantage for comparison among all possible pairs of populations in any group. Plant improvement is made through

Table 4. Percentage contribution of different characters towards genetic divergence in chilli

Source	Character-wise rank totals	Contribution %
1. Plant height	1197	8.97
2. Primary branches	1696	12.72
3. Secondary branches	1605	12.03
4. Number of flower	1020	7.65
5. Number of fruit/plant	1445	10.83
6. Dry fruit weight	1221	9.15
7. Fruit length	784	5.88
8. Fruit diameter	1087	8.15
9. Pedicel length	993	7.45
10. Pericarp thickness	518	3.88
11. Seeds/fruit	924	6.93
12. Seeds weight	848	6.36

generating variability in desired traits followed by selection. Continued success in plant improvement can only be realized when new substantial variability is found and used in a population. Divergence between any two parents expresses the allelic differences between them (Dias *et al.* 2003). The varieties grouped into the same cluster presumably diverge very little from one another. Crossing between varieties belonging to the same cluster is not expected to yield desirable traits. Therefore, crosses between the members of clusters separated by inter-cluster distances are likely to be beneficial for further improvement.

5. Conclusion

The 19 genotypes of the 3 most popular chilli species (*C. annuum* L., *C. chinense* Jacq. *C. frutescens* L.) under study were grouped into 5 clusters irrespective of their origin. Distant parents were ready to exert high heterosis. Considering this theme and variability, the varieties generated due to cross between MP and SB, and MP and HO (cluster II) as well as parental variety HO were identified as promising varieties with respect to characters like number of flower, fruit yield, fruit length, pedicel length, and seeds per fruit, which may be taken into consideration as better parents for an efficient hybridization program of chillis. Further, one or two promising genotypes from different clusters may be chosen for further genetic studies either by way of diallel or line x tester analysis.

Appendix

D^2 analysis

Mahalanobis (1936) D^2 analysis was used for assessing the genetic divergence among the test entries involving quantitative characters. Chilli genotypes are classified into 5 clusters based on D^2 values

ANOVA TEST OF EQUALITY OF GROUP/TREATMENT MEANS WILKS CRITERION 5.029×10^{-20}

V-STATISTIC=1710.804 D.F. = 216.

References

Dias LAS, Marita J, Cruz CD, Barros EG and Salomao TMA 2003 Genetic distance and its association with heterosis in cacao. *Braz. Arch. Bioltech.* **46** 339–347

Farhad M, Hasanuzzaman M, Biswas BK, Ariifuzzaman M and Islam MM 2010 Genetic divergence in chilli (*Capsicum annuum* L.). *Bang. Res. Pub. J.* **3** 1045–1051

Guerra EP, Destro D, Miranda LA and Montalvan R 1999 Parent selection for intercrossing in food type soybean through multivariate genetic divergence. *Acta Sci.* **21** 429–437

Indira P 1994 Diversity interrelationship among *Capsicum* spp. and forms and development of paprikas, Ph.D. Thesis, Kerala Agricultural University, Thrissur, India

Joshi BK, Mudwari A, Bhatta MR and Ferrara GO 2004 Genetic diversity in Nepalese wheat cultivars based on agromorphological traits and coefficients of parentage. *Nep. Agric. Res. J.* **5** 7–17

Khodadabi M, Fotokian MH and Miransari M 2011 Genetic diversity of wheat genotypes based on cluster and principal component analysis for breeding strategies. *Aust. J. Crop Sci.* **5** 17–24

Kumar BMD, Anand K and Mallikarjunasiah H 2010 Genetic divergence in chilli accessions. *Electron. J. Pl. Breed.* **1** 1363–1366

Mahalanobis PC 1936 On the generalized distance in statistics. *Proc. Nat. Inst. Sci. (India)* 49–55

Mishra AC, Singh RV and Ram HH 2004 Studies on genetic divergence in capsicum (*Capsicum annuum* L.) in Uttaranchal. *Capsicum Eggplant Newslett.* **23** 45–48

Patel M, Reddi M, Rana B and Reddy B 1989 Genetic divergence in safflower (*Carthamus tinctorius* L.). *Ind. J. Gen. Pl. Breed.* **49** 113–118

Roy A and Sorma RN 1996 Multivariate analysis in chilli (*Capsicum annuum* L.). *Agric. Res.* **17** 130–132

Sundaram A, Ramakrishnan A, Renganathan CR and Ramalingam S 1980 Genetic divergence in chilli. *Indian J. Agric. Sci.* **50** 391–398

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