Journal of Applied and Natural Science 8 (4): 1884-1892 (2016)

JANS

Study on genetic diversity in chilli (*Capsicum annuum*) based on multivariate analysis and isozyme analysis

Chandan Kumar Mondal[1,2*], Pinaki Acharyya[1] and Uttam Saha[1,3]

[1]Department of Horticulture, Institute of Agricultural Science, University of Calcutta, Kolkata – 700019 (West Bengal), INDIA
[2]Present Address: Ramkrishna Ashram KVK, Nimpith, South 24 Parganas -743338 (W.B), INDIA
[3]Present Address: Department of Agriculture, Govt. of Tripura, Amarendranagar- 799103 (Tripura), INDIA
[*]Corresponding author. E-mail: drchandanmondal@gmail.com

Received: February 10, 2016; Revised received: July 28, 2016; Accepted: October 24, 2016

Abstract: Thirty seven diverse chilli (*Capsicum annuum*) genotypes were studied for 22 growth, yield and fruit quality traits. Multivariate analysis grouped the genotypes into 7 clusters. Cluster IV was largest containing 14 genotypes. Inter cluster distance was maximum between cluster V and VII (248.09), and minimum between cluster I and II (57.80). Cluster VII was most heterogeneous (intra-cluster divergence value 191.25) and Cluster II was most homogeneous (30.25). Genotypes were also analyzed for peroxidase enzyme polymorphism using gel electrophoresis which resulted seven electrophoretic bands (Rf 0.19 to 0.59) and grouped the genotypes into 6 zymotypes. Zymotype P_4 included maximum (13) number of genotypes. Number of clusters in peroxidase and multivariate analysis were almost same but distribution of genotypes varied. 73% of total genotypes showed similar pattern of grouping suggesting that the two methods are complementary to each other and should be carried out simultaneously to determine genetic diversity more effectively. Considering variability and diversity analysis of the genotypes, CUCH-4 from Cluster-II (& Zymotype-P_2) and CUCH-31, CUCH-34 and CUCH-35 from Cluster-VII (& Zymotype-P4) were identified as promising genotypes which can be used in further crop improvement programme.

Keywords: Capsicum, Chilli, Diversity, Multivariate analysis, Peroxidase analysis

INTRODUCTION

Chilli is an important commercial crop cultivated exclusively in tropical and temperate zones of the world and grown on more than 1.5 million hectares worldwide (Anonymous., 2007). Chilli finds its place as vegetable, spice and condiment. It is useful in pharmaceuticals also. India is the largest producer, consumer and exporter of chili, which contribute around 25% of total world production. After India, China is the major producer of Chili in the world (Anonymous, 2012). However, productivity of chilli in India is almost half than that of China (Ali, 2006). Identification of a genotype better suited for a particular region as well as it's improvement in all aspects is of immediate task. Genetic improvement of any crop depends on magnitude of genetic variability and the extent of heritability of economically important characters. Critical assessment of nature and magnitude of variability in the germplasm stock is one of the important prerequisites for formulating effective breeding methods. The higher genetic distance between parents, the higher heterosis in progeny can be observed (Lahbib *et al*., 2012). Traditionally, characterization and variability evaluation of a genetic stock is done by a combination of morphological and agronomic traits. However, this approach is prone to environmental influences (Peeraullee & Ranghoo-Sanmukhiya, 2013). The isozyme markers have been useful in determining genetic relationships among closely related species and cultivars (Aniel Kumar *et al*., 2013). Although morphological traits and isozyme marker analysis have been used to distinguish cultivars, both systems have limitations, the first due to environmental effects (Bhat *et al*., 1992a,b) and the latter due to selection of markers. Isozyme electrophoresis is chosen for its relative simplicity because it provides direct visualization of gene products (Brewer and Sing, 1970) and potentially can provide a unique fingerprint for each genetically distinct clone (Lebot *et al*., 1991). In chili pepper such studies on isozymes are very limited (Gupta *et al*., 1997; Onus and Pickersgill, 2000; Barrera *et al*., 2005). Nitesh *et al*. (2010) reported varietal identification of chilli peppers by PPO isozyme profiles. However, in depth studies on polymorphism of isozyme for assessing intravarietal relationships in chilli peppers is very limited (Aniel Kumar, Subba Tata, 2013).

The present study was aimed at assessing 37 chilli genotypes based on 17 morphological and 5 biochemical traits, grouping of genetically similar genotypes and further verification of this diversity analysis using peroxidase isozyme polymorphism analysis through gel electrophoresis.

MATERIALS AND METHODS

Field trial: The 37 chilli genotypes were collected from different locations as mentioned in Table-2 and evaluated for three consecutive years (2007-08, 2008-09, 2009-10) following randomized block designs with three replications. Pre-soaked seeds of all the genotypes were sown in seedbed in the first week of November each year. 45 days old seedlings were transplanted in the main field in the individual plots with a spacing of 45cm × 45cm ensuring 40 plants in each plot. Transplanting was done by 2nd fortnight of December each year. Essential intercultural operations (weeding, staking, time bound irrigation, plant protection measures etc.) were carried out as and when required. Seventeen morphological and five biochemical traits were selected for study following descriptor of Capsicum (IPGRI, 1995).

Multivariate analysis: The genetic divergence was calculated following Mahalanobis D^2 statistics (Mahalanobis, 1936). Multivariate analysis or D^2 analysis is a method for grouping genetically similar germplasms. It has been observed that for varieties development programme, it is desirable to classify the germplasm on the basis of diversity of different characters and to make crosses between groups having maximum diversity (Narasimhayya and Venkatarao, 1974).

Peroxidase isozyme analysis: The analysis was conducted taking leaf extract of 2nd or 3rd leaf of 60 days old plant. 500 mg leaf sample was crushed in 2.5ml phosphate buffer (pH 6.8; K_2HPO_4 solution - 6.15 ml and KH_2PO_4 solution - 3.85 ml dissolved in 100 ml water) in a pre-chilled mortar & pestle. The crude homogenate was centrifuged at 14,000 rpm at 4°C for 20 minutes. The supernatant was transferred into fresh tubes and divided into two parts. One part was used for protein estimation.

Protein estimation: Dried test tubes, cleaned with distilled water and alcohol, were marked according to the sample accession numbers. One was marked as blank. In each test tube 10 µl cold tissue extract, 990 µl phosphate buffer and 5 ml G250 dye were added. In the blank, instead of tissue extract, 10 µl distilled water was added. The mixtures turned deep blue in colour immediately. Sample and blank were taken in two different cuvates and absorbance was estimated using a spectrophotometer (Makeup Jasco, Model-V630) at 595 nm. Protein content in each sample was calculated as per absorbance values against standard curve prepared using standard protein (BSA).

Peroxidase analysis was conducted in polyacrylamide gel (PAGE) system. The tissue extracts were taken in required amount after calculating its protein content. Vertical electrophoresis unit was used to run the gel.

Gel preparation: Gel was prepared in a dark conical flask having 12.2 ml water, 10 ml 30% Acrylamide mix, 7.5 ml Tris-HCl (pH 8.8), 300 µl 10% APS and 20 µl TEMED. It was 10% Tris-Glycine Polyacrylamide gel solution.

Electrophoresis: The gel was ready in 1 hr. The gel plate was placed in the tank after pouring the tank buffer (Tank buffer prepared with Tris (1.2 g) and glycine (5.8 g) dissolved in 100 ml distilled water and the volume was made upto 200 ml and diluted 10 times). Now, 32 µl of sample extract and 8 µl Bromophenol blue dye were mixed and added carefully at the comb places at top of the gel. Then electrophoresis was carried out at 15 Amp. & 150 volt, approximately for 5 hrs until bromophenol blue reached the gel end. The gel was stained for peroxidase.

Staining: Gel plates were taken out, the gel was separated carefully and it was dipped in staining solution (100 mg ortho-dianisidine dissolved in 1 ml of Acetic acid; 200 ml of water and 2 ml of H_2O_2 added to it) horizontally and kept in dark for 30 minutes with occasional shaking. The bands of peroxidase developed. After staining, the gel was washed with distilled water and photographed by a gel documentation system (Multidoc, UVP) for band identification and final calculation.

Band analysis: Isozyme banding patterns were analyzed on the basis of number and Relative front (Rf) values of the bands. The Rf value is the mobility of each band travelled from the origin divided by the migration of tracking dye (Powers *et al.*, 1988). Similarity coefficient values of electrophoretic pattern of each enzyme were subjected to cluster analysis employing the unweighted pair group method using arithmetic averages (UPGMA). For cluster analysis, value '1' was put for the presence and '0' for absence of the band for each genotype. Zymotypes were used for clustering and the Euclidean distance method (Nourish, 1993) was used for the dissimilarity.

RESULTS AND DISCUSSION

The present study aimed at determining the genetic divergence of the 37 genotypes employing 22 important characters pertaining to growth, fruit yield components, proximate compositions of leaf and fruit and per plant fruit yield (Table 1).

Multivariate analysis: Following D^2 statistics 37 genotypes were meaningfully grouped into 7 clusters (Table 2). It was found that the clustering pattern was, in general, not related to geographic origin of the genotypes which was in conformity with the earlier findings of Murty and Arunachalam (1966), Varalakshmi and Babu (1991), Pandey and Dobhal (1993) and Sreelathakumary and Rajamony (2004). However, some genotypes having similar place of origin were grouped in the same cluster like CUCH–22 and CUCH–23 in Cluster IV. Hence, implication of geographical origin on genetic diversity may not altogether be ignored.

Cluster-IV was the largest containing 14 genotypes followed by cluster VII and II having 8 and 7 genotypes in each respectively. Rest four clusters (cluster-I,

Table 1. Mean values of growth characters, yield components and yield over years (pooled).

Geno-types	Ch1	Ch2	Ch3	Ch4	Ch5	Ch6	Ch7	Ch8	Ch9	Ch10	Ch11	Ch12	Ch13	Ch14	Ch15	Ch16	Ch17	Ch18	Ch19	Ch20	Ch21	Ch22
CUCH-1	9.982	49.954	49.524	6.344	6.844	2.556	48.667	36.462	8.256	7.722	3.322	49.604	2.803	0.736	86.687	580.333	132.907	0.667	0.500	3.153	3.262	135.810
CUCH-2	15.857	50.771	45.512	6.789	8.500	2.622	57.444	36.623	8.744	8.889	2.867	49.310	2.464	0.724	95.989	504.111	93.731	0.349	0.649	3.339	1.912	118.442
CUCH-3	12.614	50.131	41.603	7.522	7.200	2.289	47.667	37.112	8.211	9.778	3.611	50.432	2.457	0.597	68.232	524.556	89.770	0.727	0.698	3.728	3.432	124.573
CUCH-4	15.821	60.241	41.134	6.067	8.333	3.311	57.667	39.508	8.456	8.644	3.278	54.444	3.127	0.788	89.733	591.222	104.699	0.552	0.699	3.553	2.698	168.460
CUCH-5	12.142	50.127	42.469	6.400	6.989	2.322	57.889	39.507	8.633	9.944	2.967	46.290	3.077	0.908	55.621	511.556	95.193	0.446	0.937	4.436	2.238	140.826
CUCH-6	11.137	56.581	43.702	9.589	8.722	3.700	53.111	39.000	8.067	8.222	3.056	52.974	2.972	0.736	70.002	541.889	120.510	0.566	0.666	3.448	2.748	155.473
CUCH-7	11.264	57.610	49.976	8.567	6.489	2.533	56.778	42.469	7.744	12.167	3.000	53.024	2.678	0.714	56.378	578.667	89.929	0.909	1.138	6.851	4.173	139.824
CUCH-8	9.426	52.778	44.994	6.678	8.200	2.589	47.556	28.511	9.256	16.056	3.856	40.363	2.932	0.733	41.622	518.556	75.372	0.614	0.963	4.951	3.028	113.990
CUCH-9	10.659	57.717	50.521	6.611	7.556	3.178	57.333	36.172	8.878	10.222	3.778	46.328	2.956	0.979	86.823	587.889	115.913	0.817	1.220	6.745	3.808	134.702
CUCH-10	9.300	48.168	52.431	7.522	8.378	3.667	53.444	38.073	7.867	7.833	2.556	45.889	2.927	0.734	68.296	536.445	108.766	0.600	0.671	3.458	2.972	132.903
CUCH-11	10.968	53.661	53.334	5.711	8.678	3.367	56.778	38.136	8.822	10.389	4.722	56.013	2.794	0.694	64.388	567.778	86.271	0.578	0.640	3.753	2.862	150.167
CUCH-12	11.101	51.109	39.443	6.189	10.856	3.600	64.667	36.524	6.778	11.944	3.111	37.141	2.936	0.894	69.552	568.333	90.564	0.583	0.672	3.973	2.909	105.329
CUCH-13	10.092	53.430	45.484	8.189	8.111	2.567	65.000	41.504	9.078	11.500	2.989	36.102	3.012	0.771	66.472	574.333	87.462	0.599	0.708	3.649	2.959	102.193
CUCH-14	11.942	52.500	49.483	6.078	7.211	2.444	58.778	30.488	8.133	10.889	2.944	36.190	3.126	0.802	62.561	570.556	92.173	0.554	0.723	4.648	2.758	109.464
CUCH-15	9.266	46.299	41.678	5.944	6.611	2.589	47.111	35.318	8.544	6.167	3.022	48.163	3.268	0.912	42.642	579.778	81.171	0.383	0.739	4.288	1.988	151.616
CUCH-16	10.810	55.520	49.977	7.422	8.656	2.878	57.444	32.702	8.744	8.889	3.078	47.806	3.321	0.908	58.511	591.333	91.490	0.602	1.226	7.257	2.972	154.386
CUCH-17	9.669	51.090	38.968	7.889	6.511	2.144	35.667	27.420	8.078	8.333	3.000	36.887	2.157	0.577	55.753	495.333	92.258	0.678	0.693	3.555	2.793	75.436
CUCH-18	9.732	49.790	40.036	5.244	6.600	2.067	44.556	41.349	8.156	7.333	3.078	38.210	2.993	0.839	65.358	508.111	80.404	0.321	0.739	3.754	1.708	110.266
CUCH-19	10.123	56.840	48.497	7.333	8.722	2.811	50.000	28.691	8.511	10.333	3.078	36.094	3.282	0.886	72.527	541.556	76.367	0.383	0.656	3.349	1.988	113.736
CUCH-20	10.092	59.348	39.033	6.233	10.711	4.278	68.333	35.444	7.356	5.644	5.878	38.651	2.151	0.534	35.756	581.222	74.380	0.922	0.441	2.539	2.892	80.396

Contd……..

CUCH-21	14.834	52.179	48.908	6.644	7.867	2.533	46.111	26.934	8.811	10.500	2.811	48.238	2.753	0.784	95.386	569.333	109.189	0.457	0.690	3.539	2.378	129.178
CUCH-22	12.159	39.901	47.272	8.656	8.756	3.456	68.556	37.992	8.156	10.611	3.056	40.554	3.023	0.646	89.499	570.556	88.382	0.211	0.572	2.948	1.532	119.897
CUCH-23	12.583	50.941	50.509	9.667	8.078	3.267	66.889	28.874	8.189	11.833	3.078	33.784	2.750	0.823	88.726	501.111	62.062	0.606	0.623	3.148	2.132	87.918
CUCH-24	9.692	39.687	38.392	7.400	7.178	2.333	47.111	27.350	6.089	17.111	2.922	47.880	1.963	0.510	40.434	521.333	90.757	0.346	0.807	3.651	2.318	89.523
CUCH-25	12.114	48.129	45.060	6.956	6.789	2.111	46.667	29.541	5.756	12.611	3.000	61.523	1.799	0.504	42.373	514.111	93.193	0.681	0.727	3.149	3.328	107.376
CUCH-26	10.108	35.899	46.151	5.289	5.922	2.033	38.778	26.184	5.856	14.389	2.778	57.389	2.042	0.611	69.578	514.889	78.473	0.671	1.222	7.657	3.282	112.651
CUCH-27	10.114	37.400	40.266	6.033	5.478	1.789	38.000	36.204	6.200	13.889	3.022	48.971	2.139	0.626	50.541	550.222	92.046	0.620	1.020	5.749	3.052	99.556
CUCH-28	10.711	40.524	33.468	5.267	5.922	2.233	47.778	28.537	7.211	12.278	2.978	34.498	2.120	0.573	36.244	517.000	70.527	0.619	0.816	4.242	2.528	72.346
CUCH-29	16.748	46.610	33.661	5.633	7.211	2.378	49.000	40.094	9.767	8.000	3.889	45.456	3.793	1.033	90.766	604.333	88.786	0.619	0.822	4.185	3.008	170.148
CUCH-30	12.093	50.050	37.149	5.344	7.156	1.911	63.444	35.367	9.044	7.056	3.144	45.726	2.496	0.678	67.690	492.889	108.604	0.547	0.649	3.316	2.722	109.809
CUCH-31	13.002	50.497	54.303	6.989	11.544	4.533	47.667	37.153	8.667	11.411	2.967	60.553	4.063	0.963	79.357	611.333	118.306	0.546	1.261	7.751	3.078	238.351
CUCH-32	12.271	50.442	45.098	6.733	7.611	2.322	37.889	38.262	7.822	8.033	3.011	40.591	2.714	0.740	39.630	551.333	79.598	0.256	0.612	2.938	1.432	105.532
CUCH-33	10.324	49.621	45.606	8.789	8.111	5.511	78.000	35.270	4.378	15.800	2.889	37.610	2.962	0.853	81.561	602.556	62.004	0.594	0.961	5.146	2.938	106.958
CUCH-34	12.659	51.213	45.774	6.633	8.411	2.867	56.889	30.117	7.122	16.389	4.078	48.636	4.089	1.074	40.713	589.445	87.973	0.682	0.753	3.851	3.332	195.476
CUCH-35	13.481	51.463	48.981	6.378	7.922	3.167	72.222	36.376	7.911	10.889	3.067	70.901	2.890	0.689	79.502	513.000	137.288	0.581	0.759	3.855	3.148	202.801
CUCH-36	9.558	49.898	40.620	8.544	7.833	3.122	68.667	30.473	7.822	8.167	3.122	43.082	2.164	0.706	50.001	517.667	103.916	0.969	0.541	2.752	2.102	89.089
CUCH-37	10.237	50.591	46.887	6.378	7.522	3.033	67.444	41.440	5.156	6.556	2.989	46.370	1.919	0.527	94.683	557.445	122.690	0.813	0.728	4.765	2.882	85.184
SEm	0.139	0.800	0.569	0.198	0.109	0.049	0.740	0.169	0.093	0.164	0.071	1.004	0.042	0.005	0.254	1.561	0.964	0.003	0.007	0.128	0.015	1.404
CD (0.05)	0.386	2.217	1.576	0.548	0.301	0.136	2.051	0.469	0.258	0.454	0.198	2.783	0.115	0.013	0.703	4.325	2.671	0.009	0.018	0.217	0.042	3.891

Table 2. Grouping of 37 genotypes in Clusters (Pooled analysis).

Cluster	No. of genotypes	Genotypes	Place of Collection
CLUSTER I	2	CUCH-11	Horticultural Research Station, Lam, Guntur, A.P. - 522034
		CUCH-13	Horticultural Research Station, Lam, Guntur, A.P. - 522034
CLUSTER II	7	CUCH-1	AICRP-Vegetable Crops, Directorate of Research, B.C.K.V., Kalyani, WB
		CUCH-2	AICRP-Vegetable Crops, Directorate of Research, B.C.K.V., Kalyani, WB
		CUCH-3	AICRP-Vegetable Crops, Directorate of Research, B.C.K.V., Kalyani, WB
		CUCH-4	AICRP-Vegetable Crops, Directorate of Research, B.C.K.V., Kalyani, WB
		CUCH-5	AICRP-Vegetable Crops, Directorate of Research, B.C.K.V., Kalyani, WB
		CUCH-6	AICRP-Vegetable Crops, Directorate of Research, B.C.K.V., Kalyani, WB
		CUCH-10	AICRP-Vegetable Crops, Directorate of Research, B.C.K.V., Kalyani, WB
CLUSTER III	2	CUCH-17	Local collection by KVK, Nimpith, South 24 PGS, W.B. - 743338
		CUCH-24	Local collection from Dalkhola, Uttar Dinajpur, W.B
CLUSTER IV	14	CUCH-7	AICRP-Vegetable Crops, Directorate of Research, B.C.K.V., Kalyani, WB
		CUCH-8	AICRP-Vegetable Crops, Directorate of Research, B.C.K.V., Kalyani, WB
		CUCH-9	AICRP-Vegetable Crops, Directorate of Research, B.C.K.V., Kalyani, WB
		CUCH-12	Horticultural Research Station, Lam, Guntur, A.P. - 522034
		CUCH-14	Horticultural Research Station, Lam, Guntur, A.P. - 522034
		CUCH-15	IARI, Pusa, New Delhi - 110012
		CUCH-16	G. B. Pant University Of Agriculture & Technology, Pantnagar, Udham Singh Nagar, Uttarakhand - 263145
		CUCH-18	Local collection by KVK, Nimpith, South 24 PGS, W.B. – 743338
		CUCH-19	Local collection by KVK, Nimpith, South 24 PGS, W.B. – 743338
		CUCH-20	A.A.U., Jorhat, Assam
		CUCH-21	A.A.U., Jorhat, Assam
		CUCH-22	Local collection from Nagaland
		CUCH-23	Local collection from Morigaon, Assam
		CUCH-25	Local collection from Dalkhola, Uttar Dinajpur, W.B
CLUSTER V	2	CUCH-32	Amtala Seed Stores, Amtala, South 24 PGS, W.B.
		CUCH-36	Local collection from Kuyemuri island, South 24 Pgs
CLUSTER VI	2	CUCH-30	Amtala Seed Stores, Amtala, South 24 PGS, W.B.
		CUCH-37	Local collection from Mandirbazar, South 24 PGS, W.B.
CLUSTER VII	8	CUCH-26	Local collection from Dalkhola, Uttar Dinajpur, W.B
		CUCH-27	Local collection from Dalkhola, Uttar Dinajpur, W.B
		CUCH-28	Local collection from Aminpur, Hasnabad, North 24 PGS., W.B
		CUCH-29	Amtala Seed Stores, Amtala, South 24 PGS, W.B.
		CUCH-31	Amtala Seed Stores, Amtala, South 24 PGS, W.B.
		CUCH-33	Amtala Seed Stores, Amtala, South 24 PGS, W.B.
		CUCH-34	Local collection from Canning, 24 Pgs.(S) W.B.
		CUCH-35	Local collection from Damkal island, South 24 PGS

Table 3. Intra and Inter cluster distance among 37 genotypes.

Cluster	CLUSTER I	CLUSTER II	CLUSTER III	CLUSTER IV	CLUSTER V	CLUSTER VI	CLUSTER VII
CLUSTER I	66.578	57.800	82.870	148.928	99.142	59.537	198.739
CLUSTER II		30.250	72.308	135.492	97.659	59.747	181.388
CLUSTER III			39.625	154.189	81.146	74.105	205.731
CLUSTER IV				184.468	182.851	150.558	197.269
CLUSTER V					58.612	95.233	248.096
CLUSTER VI						66.408	201.711
CLUSTER VII							191.254

III, V and VI) had two genotypes in each (Table 2). The intra-cluster divergence was highly variable ranging from 30.25 in cluster II to 191.25 in cluster VII (Table 3). So, it may be said that cluster II, having 7 genotypes, is the most homogeneous group and cluster -VII with 8 genotypes is the most heterogeneous group. High heterogeneity was also observed in cluster -IV showing high intra-cluster divergence (184.46). Inter-cluster distance was maximum (D^2 value 248.09) between cluster V and VII, suggesting wide diversity

Table 4. Cluster wise mean value for twenty two characters.

Cluster	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
CLUSTER I	10.53	53.54	49.40	6.95	8.39	2.96	60.88	39.82	8.95	10.94	3.85	46.05	2.90	0.73	65.43	571.0	86.86	0.58	0.67	3.70	2.91	126.18
	0	6	9	0	5	7	9	0	0	5	6	8	3	3	0	56	7	9	4	1	1	0
CLUSTER II	12.40	52.28	45.19	7.17	7.85	2.92	53.69	38.04	8.31	8.719	3.09	49.84	2.83	0.74	76.36	541.4	106.5	0.55	0.68	3.58	2.75	139.49
	8	2	6	6	2	4	8	1	9		4	9	2	6	6	45	11	8	9	8	2	8
CLUSTER III	9.681	45.38	38.68	7.64	6.84	2.23	41.38	27.38	7.08	12.72	2.96	42.38	2.06	0.54	48.09	508.3	91.50	0.51	0.75	3.60	2.55	82.480
		9	0	5	5	9	9	5	4	2	1	4	0	4	4	33	8	2	0	3	6	
CLUSTER IV	11.15	52.19	46.09	7.01	8.07	2.88	55.77	33.64	8.07	10.37	3.34	43.29	2.85	0.78	64.83	555.7	87.18	0.57	0.79	4.36	2.68	118.43
	0	0	9	6	9	1	0	4	2	1	1	1	5	3	7	94	5	5	5	7	5	4
CLUSTER V	10.91	50.17	42.85	7.63	7.72	2.72	53.27	34.36	7.82	8.100	3.06	41.83	2.43	0.72	44.81	534.5	91.75	0.61	0.57	2.84	1.76	97.311
	5	0	9	9	2	2	8	8	2		7	7	9	3	6	00	7	3	7	5	7	
CLUSTER VI	11.16	50.32	42.01	5.86	7.33	2.47	65.44	38.40	7.10	6.806	3.06	46.04	2.20	0.60	81.18	525.1	115.6	0.68	0.68	4.04	2.80	97.497
	5	1	8	1	9	2	4	4	0		7	8	8	3	7	67	47	0	9	1	2	
CLUSTER VII	12.14	45.40	43.52	6.37	7.56	3.06	53.54	33.74	7.13	12.88	3.20	50.50	3.01	0.80	66.03	562.8	91.92	0.61	0.95	5.30	3.04	149.78
	3	3	6	6	5	4	2	2	9	1	9	2	2	3	3	47	5	7	2	5	6	6

Characters:
1. Days to 50% germination
2. Plant height (cm)
3. Plant canopy width (cm)
4. No. of primary branches/plant
5.Leaf length (cm)
6. Leaf width (cm)
7. Days to 50 % flowering
8. Days from fruit set to fruit maturity
9. Fruit length (cm)
10.Fruit girth (mm)
11. Fruit pedicel length (cm)
12.No. of fruits per plant
13.Fresh fruit weight (g)
14.Dry fruit weight (g)
15.No. of seeds/fruit
16.100 seed weight (mg)
17.Vit C. content (mg/100g)
18.Capsaicin content (mg/100 g)
19. Total chlorophyll content in green mature fruit (µg/g of fruit)
20. Total carotenoid (µg/g of fruit)
21. Leaf phenol (mg/g of leaf)
22.Fruit yield per plant (g)

Table 5. Distribution of peroxidase bands among the zymotypes of 37 genotypes.

Zymotypes	Rf value of bands							No. of bands
	0.19	0.24	0.41	0.44	0.49	0.54	0.59	
P_1	√	√	√		√	√	√	6
P_2		√	√		√	√	√	5
P_3		√	√	√	√	√	√	6
P_4	√	√	√	√	√	√	√	7
P_5	√	√	√	√	√	√		6
P_6	√		√				√	3
Band frequency	51.35	97.30	100.00	62.16	97.30	97.30	97.30	
Total bands								33

Table 6. Similarity Matrix values of 6 peroxidase banding patterns

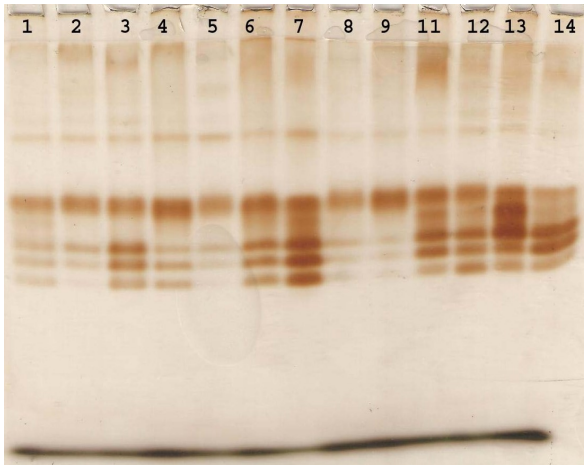
Zymotypes	P_1	P_2	P_3	P_4	P_5
P_2	0.833				
P_3	0.714	0.833			
P_4	0.857	0.714	0.857		
P_5	0.714	0.571	0.714	0.857	
P_6	0.50	0.333	0.333	0.429	0.286

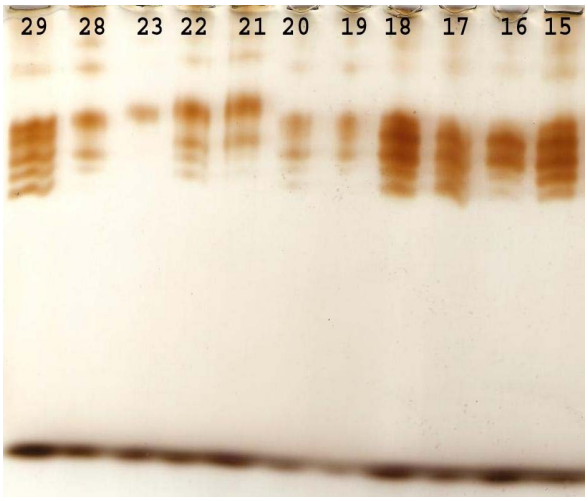
Table 7. Distribution of genotypes in the zymotypes.

Zymotype	Total no. of genotypes	% Genotypes	Genotypes
P_1	4	10.81	CUCH-1, CUCH-2, CUCH-3, CUCH-10
P_2	9	24.32	CUCH-4, CUCH-5, CUCH-6, CUCH-8, CUCH-9, CUCH-24, CUCH-25, CUCH-26, CUCH-27
P_3	9	24.32	CUCH-7, CUCH-11, CUCH-14, CUCH-16, CUCH-20, CUCH-29, CUCH-30, CUCH-32, CUCH-33
P_4	13	35.14	CUCH-12, CUCH-13, CUCH-15, CUCH-17, CUCH-18, CUCH-19, CUCH-21, CUCH-22, CUCH-31, CUCH-34, CUCH-35, CUCH-36, CUCH-37
P_5	1	2.70	CUCH-28
P_6	1	2.70	CUCH-23

among the members of these clusters. Minimal distance was noted between cluster-I and II (57.80) VI (59.53), indicating proximate relationship among the genotypes included in these two clusters.

Cluster-VII emerged as unique because it had the highest mean values for maximum number of yield contributing characters viz., leaf width, fruit girth, number of fruits per plant, fresh fruit weight, dry fruit weight and total fruit yield per plant (Table 4). This cluster also showed highest mean values for physiological and fruit quality characters like total chlorophyll content in





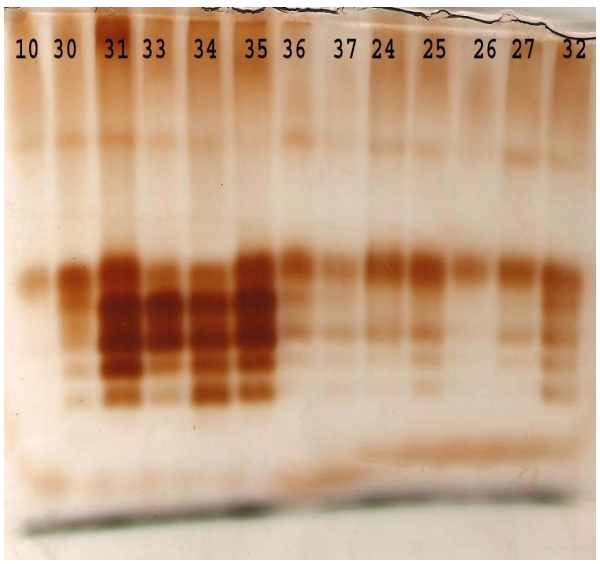


Fig. 1. *Peroxidase electrophoretic banding and zymotypes.*

Genotypes	1	2	3	4	5	6	7	8	9	11	12	13	14
Rf values													
0.19	-	-	-								-	-	
	-	-	-								-	-	
0.24	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-
0.41	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-
0.44							-			-	-	-	-
							-			-	-	-	-
0.49	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-
0.54	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-
0.59	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-

Genotypes	29	28	23	22	21	20	19	18	17	16	15
Rf values											
0.19		-	-	-	-		-	-	-		-
		-	-	-	-		-	-	-		-
0.24	-	-		-	-	-	-	-	-	-	-
	-	-		-	-	-	-	-	-	-	-
0.41	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-
0.44	-	-		-	-	-	-	-	-	-	-
	-	-		-	-	-	-	-	-	-	-
0.49	-	-		-	-	-	-	-	-	-	-
	-	-		-	-	-	-	-	-	-	-
0.54	-	-		-	-	-	-	-	-	-	-
	-	-		-	-	-	-	-	-	-	-
0.59	-		-	-	-	-	-	-	-	-	-
	-		-	-	-	-	-	-	-	-	-

Genotypes	10	30	31	33	34	35	36	37	24	25	26	27	32
Rf values													
0.19	--		--		--	--	--	--					
0.24	--	--	--	--	--	--	--	--	--	--	--	--	--
0.41	--	--	--	--	--	--	--	--	--	--	--	--	--
0.44		--	--	--	--	--	--	--					--
0.49	--	--	--	--	--	--	--	--	--	--	--	--	--
0.54	--	--	--	--	--	--	--	--	--	--	--	--	--
0.59	--	--	--	--	--	--	--	--	--	--	--	--	--

Fig. 2. *Zymogram of electrophoretic pattern of peroxidase isozyme of 37 genotypes.*

green mature fruit, total carotenoid content in red ripe fruit and leaf phenol content. Cluster-I though showed highest mean value for the growth and yield related characters i.e. plant height, plant canopy width, leaf length, fruit length, fruit pedicel length and 100 seed weight, but registered as third highest for fruit yield per plant after cluster-II. High mean values for earliness with respect to seed germination, days to 50% flowering and days from fruit set to maturity were recorded in cluster-III, though this cluster failed to score substantially high mean values for other yield related traits and at the same time, recorded the lowest yield per plant. Moderate expression of all the characters were recorded in Cluster-IV and V. Cluster-VI recorded the highest mean value for two fruit quality traits i.e. ascorbic acid content and capsaicin content but mean fruit yield per plant of this cluster was very low.

Crosses involving parents belonging to most divergent clusters are expected to give maximum heterosis and create wide variability in genetic architecture. However, for a practical plant breeder, the objective is not only obtaining high heterosis but also to achieve high level of production within the shortest possible time. In the present study maximum distance exists between cluster-V & cluster-VII. However, considering inter-cluster distance and other agronomic performances, crosses between the members of cluster-II and cluster-VII might exhibit high heterosis and is also likely to produce new recombinants with desired traits.

Isozyme analysis (analysis through poly-morphic banding): Study on genetic diversity in terms of isozyme poly-morphism have been extensively utilized in different vegetable crops viz., brinjal (Ali *et al*., 2011), tomato (Gunaseelan *et al.*, 2011; Evans and Allridge, 1965), potato (Beatriz *et al*., 2001), radish (Ivy *et al*., 2010), and field crops like cotton (Farooq and Sayyed, 1999), soybean (Buttery and Buzzel, 1968; Brim *et al,* 1969) etc. but in chili pepper, such studies on isozymes are very limited (Onus & Pickersgill, 2000; Barrera *et al*., 2005; Nitesh *et al.*, 2010).

In the present study, peroxidase electrophoretic banding, zymogram following electrophoretic banding pattern and zymotypes of the 37 genotypes have been presented in Figure 1& 2 and in Table 5. A total of seven bands of peroxidase were obtained ranging from Rf 0.19 to Rf 0.59. The number of bands varied with the genotypes. It is apparent from the Figure-2 and Table-5 that the band with Rf value 0.41 appeared in all the genotypes (100 % band frequency) while the band with Rf value 0.19 appeared in minimum (19) number of genotypes (51.25% band frequency). Only 23 genotypes showed the band with Rf value 0.44 (62.10% band frequency). Frequency of other 4 bands with Rf values 0.24, 0.49, 0.54 and 0.59 was 97.30%, however their appearance varied with the genotypes.

Similarity matrix values of 6 peroxidase banding pattern (Table 6) suggested that the Zymotypes P_1 and P_4; P_4 and P_3; P_4 and P_5 were close to each other in similar intensity (Similarity index value 0.857). The Zymotype P_6 was farthest from P_5 (Similarity index 0.286) followed by P_3 and P_2 (Similarity index 0.333).

Distribution of the peroxidase bands grouped the 37 genotypes into 6 zymotypes (Table 7). The Zymotype P_4 was having all the 7 bands and also maximum number of thirteen genotypes in it. Three Zymotypes (P_1, P_3 and P_5) were having 6 peroxidase bands, with variation in their banding pattern. Zymotype P_1 was devoid of band with Rf value 0.44 and contained 4 genotypes in it. Zymotype P_3 containing 9 genotypes had all bands except band with Rf value 0.19. Zymotype P_5 containing 1 genotype was lacking the last band with Rf value 0.59. Zymotype P_2 having 9 genotypes in it, had 5 bands excluding 2 bands with Rf 0.19 and 0.40. The last Zymotype P_6 containing only one genotype had only 3 bands i.e. Rf 0.19, Rf 0.41 and Rf 0.59.

Multivariate analysis and Isozyme analysis: It was clear from the present study that overall 7 clusters were formed with multivariate analysis employing 22 quantitative traits (Table 2) whereas 6 zymotypes were obtained with peroxidase isozyme analysis (Table 7). The number of clusters obtained by multivariate analysis and peroxidase isozyme analysis were almost same but the genotypes included in each individual cluster varied. Zymotype P_1 has been formed with 4 genotypes of Cluster II. Other members of Cluster II were grouped in Zymotype P_2, along with some members from Cluster III, IV, VI and VII. This phenomenon may be due to the environmental influence for the expression of quantitative traits. Almost similar result was recorded by Singh *et al*. (2010) in blackgram in which 8 clusters were formed with quantitative traits analysis whereas 9 clusters were obtained with SDS-PAGE analysis of total seed storage protein.

Conclusion

In the present study thirty seven genotypes of chilli were grouped into seven clusters and six zymotypes irrespective of their origin. From this study, it may be concluded that a wide range of variation for almost all the economically important traits are present in this crop. This implies a good possibility of breeding through hybridization programme or direct use of promosing genotypes as variety for successful chilli production. Distant parents are able to exert high heterosis. Considering this theme and variability and diversity analysis of the genotypes, CUCH-4 from Cluster-II (& Zymotype-P_2) and CUCH-31, CUCH-34 and CUCH-35 from Cluster-VII (& Zymotype-P4) were identified as promising genotypes. Further genetic studies may also be carried out using two or more promising genotypes, either through diallel or by line x tester analysis.

It also emerged that 72.97% of the total genotypes (i.e. 27 genotypes; 6 from C-IV to P_4, 4 each from C-II to P_1 and C-IV to P_3, 3 each from C-II to P_2, C-IV to P_2 and C-VII to P_4, 2 each from C-VII to P_2 and C-VII to P_3) grouped almost similarly under both multivariate analysis and isozyme polymorphism. So, it can be said that these two methods of determining diversity are complemen-

tary to each other and should be carried out simultaneously to determine genetic diversity in a more authentic manner. It is pertaining to say that isozyme polymorphism for chili cultivars could be used for cultivar registration which could be helpful for chili breeders.

REFERENCES

Ali, M. (ed.). (2006). Chili (Capsicum spp.) Food Chain Analysis: Setting Research Priorities in Asia. Shanhua, Taiwan: AVRDC – The World Vegetable Center, Technical Bulletin No. 38, AVRDC Publication 06-678. pp 15. In http://www.avrdc.org/pdf/TB38.pdf

Ali, Z., Xu, Z.L., Zhang, D.Y., He, X.L., Bahadur, S. and Yi, J.X. (2011). Molecular diversity analysis of eggplant (*Solanum melongena*) genetic resources. *Genet. Mol. Res*., 10 (2): 1141-1155

Aniel Kumar, O., Rupavathi, T. and Subba Tata, S. (2013). Genetic Variation of Isozyme Polyphenol Oxidase (PPO) Profiles in Different Varieties of *Capsicum annuum* L. *Notulae Scientia Biologicae,* 5(4):454-457

Anonymous (2007). *FAO Production Yearbook*, p: 333. Rome, Italy

Anonymous (2012). Crop report 2011/12, Oilseeds and Spices. P:6. http://www.agrocrops.com/ crop-report-2012.pdf

Barrera, L.Q., Garcia, M.C., Giraldo, M.C. and Melgarejo, L.M. (2005). Isozyme characterization of *Capsicum* accessions from the Amazonian Colombian collections. *Revista Colombiana De Biotecnologia,* 1: 59-65

Beatriz Helena Gomes Rocha, Eliane Augustin, João Baptista da Silva and Judith Viégas (2001). Isoenzymatic variability in wild potatoes. *Pesq. Agropec. Bras.*, 36 (5): 781-791

Bhat, K.V., Bhat, S.R. and Chandel, K.P.S. (1992a). Survey of isozyme polymorphism for clonal identification in Musa I. Esterase, acid phosphatase, and catalase. *J. Horti. Sci*., 67 (4): 501-508

Bhat, K.V., Bhat, S.R. and Chandel, K.P.S. (1992b). Survey of isozyme polymorphism for clonal identification in Musa II. Peroxidase, superoxide dismutase, shikimate dehydrogenase, and malate dehydrogenase. *J. Horti. Sci*., 67(6): 737-743

Brewer, G.J. and Sing, C.F, (1970). An Introduction to Isozyme Techniques, Academic Press, New York.

Brim, C.A., Usanis, S.A. and Tester, C.F. (1969). Organ specificity and genotype differences in isoperoxidases of soybeans. *Crop Sci.,* 9: 843-845

Buttery, B.R. and Buzzel, R.I. (1968). Peroxidase activity in seeds of soybean varieties. *Crop Sci.,* 8: 722-725

Evans, J.J. and Allridge, N.A. (1965). The distribution of peroxidases in extreme dwarf and normal tomato (*Lycopersicon esculentum*). *Phytochemistry*, 8: 499-503

Farooq, S. and Sayyed, H. (1999). Isozyme markers in cotton breeding-II: Inter and intra varietal variation in the activity of isozymes of the enzyme peroxidase as affected by area of cotton cultivation. *Pak. J. Bot.,* 31 (2): 347-359

Gunaseelan, C., Suganyadevi, P., Rajasabapathy, R. and Ruban, P. (2011). Differentiation of Hybrid (COTH2) and wild (CO3) varieties of tomato (*Lycopersicon esculentum*) using protein and peroxidase isozyme profile. *Res. & Rev.*, 1 (2): 1-5

Gupta, C.H.G., Lakshmi, N. and Srivalli, T. (1997). Analysis of isozyme patterns (peroxidase) of *Capsicum hybrids* through PAGE. *Capsicum and Eggplant Newsletter.* 16: 64-67

IPGRI, AVRDC and CATIE. (1995). Descriptors for Capsicum (Capsicum spp.). International Plant Genetic Resources Institute, Rome, Italy; the Asian Vegetable Research and Development Center, Taipei, Taiwan, and the Centro Agronómico Tropical de Investigación y Enseñanza, Turrialba, Costa Rica.

Ivy, N.A., Biswas, M.S., Rasul, G., Hossain, T. and Mian, M.A.K. (2010). Variations of genotypes of radish at molecular level using isozyme analysis for the identification of self-incompatible lines. *Global J. Biotech. Biochem,* 5 (1): 19-26

Lahbib, K., Bnejdi, F. and Gazzah, E.I. (2012). Genetic diversity evaluation of pepper in Tunisia based on morphologic characters. *Afr. J. Agric. Res*., 7 (23): 3413-3417

Lebot, V., Aradhya, M.K. and Manshardt, R.M. (1991). Geographic survey of genetic variation in kava (*Piper methysticum* Forst. F. and *P. wichmanii* C. DC.). *Pacific Sci*., 45 (2): 169-185

Mahalanobis, P. (1936). On the generalized distance in statistics. *Proc. Nat. Inst. Sci.,* (India), 12: 49-55

Murty, B.R. and Arunachalam, V. (1966). The nature of genetic divergence in relation to breeding system in crop plants. *Indian J. Genet*, 26A: 188-198

Narasimhayya, G. and Venkatarao, C. (1974). Classification of the varieties of cigarette tobacco using D2 statistic. *Indian J. Agric. Sci*., 44: 660 – 664

Nitesh, L., Dilraj, K., Patel, N.J. and Talati, J.G. (2010). Varietal identification of chilli (*Capsicum annuum* L.) by electrophoretic technique. *Indian J. Agric. Biochem.* 23 (1): 36-40

Nourish, M.J. (1993). SPSS for Windows-Professional Statistics; Release 6.0 SPSS Inc. 444N. Michigan Avenue, Chicago, Illinoise, 6061

Onus, A.N. and Pickersgill, B. (2000). A study of selected isozymes in *Capsicum baccatum*, *Capsicum eximium*, *Capsicum cardenasii,* and two interspecific F1 hybrids in Capsicum species. *Turk. J. Bot*, 24: 311-318

Pandey, G. and Dobhal, V. K. (1993). Multivariate analysis in chilli (*C. annuum* L.). *J. Spices Aromatic Crops*, 2 (1-2): 71-74

Peeraullee, N. and Ranghoo-Sanmukhiya, V.M. (2013). Assessment of genetic diversity in local chilli (*Capsicum annuum*) varieties in mauritius. *International Journal of Agriculture and Biology*, 12: 387

Powers, H.R., Lin, D. Jr. and Hubbes. (1988). Interspecific and intraspecific differentiation within the genus *Cronartium* by isozyme and protein pattern analysis. *Pl. Dis.*, 73: 691-694

Singh, A.K., Mishra, A. and Shukla, A. (2010). Genetic evaluation and identification of genetic donors in blackgram (*Vigna mungo*) revealed by agro-morphological traits and seed storage protein analysis. *World Appl. Sci., J.* 10 (4): 477-488

Sreelathakumary, I. and Rajamony, L. (2004b). Genetic divergence in chilli (*Capsicum annuum* L.) *Indian J. Hort*., 62 (2):137-139

Varalakshmi, B. and Babu, K. H. (1991). Genetic divergence, heritability and genetic advance in chilli (*C. annuum* L.). *Indian J. Genet*., 56(2): 174-178.