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Studies on seed protein profiling in chilli (*Capsicum annuum* L) genotypes of Northeast India

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Abstract

North eastern region of India exhibits wide variability in fruit morphology, pungency, bearing habit and crop duration of chilli. In the present study, thirty chilli (*Capsicum annuum* L) genotypes from different areas of North eastern hill and other part of India were collected and evaluated for genetic diversity using morphological characters and SDS-PAGE analysis. Estimation of protein was done in seeds of thirty genotypes of chilli collected for the study. The analysis showed considerable variation in banding pattern of total protein which ranged from 7-19 numbers of bands. On the basis of presence (+) or absence (-) of bands, similarity index was calculated and genotypes were grouped in three major clusters which were further sub divided in 9 sub-clusters. The genotype CHFC9 was most distantly related to CHFC18. Hence, it was recommended that genotype CHFC9 and CHFC18 could be utilised for crossing programme to create more genetic diversity or segregants of desired characteristics through chilli breeding programmes. Hence, overview of diversity of chilli genotypes from North eastern hill region of India paves the way for conservation and utilization of genotypes and contributes to the development of systematic breeding programme.

Keywords: *Capsicum annuum*, Genetic diversity, PAGE electrophoresis, SDS.
Abbreviations: NEH_North eastern hills, SDS_Sodium dodecyl sulphate, PAGE_Polyacrylamide gel electrophoresis, SI_similarity index.

Introduction

Chilli (*Capsicum annuum* L), a member of Solanaceae is a major vegetable cum spice crop with considerable economic importance grown in tropical and sub tropical regions of the world (FAO, 2007). It is valued for its pungency which is due to crystalline acrid volatile alkaloid capsaicin, having diverse prophylactic and therapeutic uses in allopathic and ayurvedic medicine (Sumathykutty and Mathew, 1984). Apart from rich source of vitamin C, it has vitamin A and E, small quantity of proteins, fats, carbohydrates and traces of minerals (Hosmani, 1993). The genus has been enshrouded with controversy relating to its taxonomy, origin and phylogenetic affinities. Members of the genus are diploids (2n=24) and most of the varieties or cultivars within a species show resemblance with their morphometric. A critical estimate of genetic variability in the population is a pre-requisite for the effective selection of promising genotypes. Investigation on classification of chilli accessions based on fruit characteristics including pungency, colour, shape; flavour, size, and use etc. (Smith et al**.,** 1987; Bosland, 1992) have been reported. Genetic diversity studies carried out on chilli germplasm from India are not comprehensive (Sanatombi et al., 2010; Patel et al., 2011). Most of these studies have only used phenotypic traits (Thul et al., 2009).

Although phenotypic traits are important for diversity studies, they need to be supported by molecular markers to give robust genetic diversity estimates. Genetic diversity studies in *Capsicum* using morphological, cytological and biochemical marker systems (Kaur and Kapoor, 2001; Gopinath et al., 2006) are also conducted. The data on agronomic, morphological and physiological plant traits are generally used to estimate the magnitude of genetic diversity present in the germplasm. However, such data may not provide an accurate indication of genetic diversity because of environmental influences upon the expression of observed traits and also the time consuming and laborious field evaluation procedures. The introduction of biochemical techniques like Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), isozyme markers has been particularly helpful in deducing systematic relationships between groups where morphological and cytological data were not corollary. SDS-PAGE is an economical, simple and extensively used technique for describing the seed protein diversity of crop germplasm (Fufa et al., 2005; Iqbal et al., 2005). Furthermore, seed proteins, used as genetic markers convey greater precision to measures of genetic diversity be-cause they are the primary products of structural genes (Srivalli et al., 1999). Seed protein electrophoresis for the study of phylogenetic relationship in *Capsicum annuum* was performed by Panda et al. (1986) and of diploids and tetraploid hybrids of *Capsicum* was initiated by Srivalli et al. (1999). Characterization of Nigerian varieties of *Capsicum annuum* and *Capsicum frutescens* by SDS- PAGE of seed proteins was conducted by Odeigah et al. (1999) and of pepper cultivars is reported by Lucchese et al. (1999). Dubey and Ram (2008) also used SDS-PAGE for assessment of genetic diversity in bottle gourd. India is one of the leading chilli producing countries of the world. Though chillies are grown all over India, the North eastern hill states contribute for 51.72% of its annual production while having only 8.0% area under chilli cultivation (Spice Board, 2004). NEH region comprising the states of Arunachal Pradesh, Assam Nagaland, Meghalaya, Manipur, Tripura, Mizoram and Sikkim. Chilli is believed to have been introduced to India by Portuguese explores (Basu and De, 2003) and to North eastern region of

Table1. List of chilli (*Capsicum annuum* L) genotypes with their source and morphological traits.

Genotypes	Source	Flower colour	Fruit fresh colour	Ripe fruit colour	Seed colour	Fruit shape	Position of fruit	Fruiting behavior
CHFC1	East Siang, Arunachal Pradesh	White	Green	Red	Straw	Elongate	Erect	Single
CHFC2	East Siang, Arunachal Pradesh	White	Green	Dark red	Straw	Almost round	Erect	Single
CHFC3	Bishnupur, Manipur	White	Green	Red	Straw	Blocky	Pendant	Single
CHFC4	East Siang, Arunachal Pradesh	White	Green	Red	Straw	Elongate	Pendant	Single
CHFC5	East Siang, Arunachal Pradesh	White -purple margin	Deep purple	Dark red	Straw	Triangular	Intermediate	Single
CHFC6	East Siang, Arunachal Pradesh	Yellow-green	Green	Dark red	Straw	Elongate	Erect	Single
CHFC7	East District, Sikkim	Yellow-green	Green	Dark red	Straw	Almost round	Intermediate	Single
CHFC8	East Siang, Arunachal Pradesh	Yellow-green	Yellow	Light red	Straw	Elongate	Erect	Single
CHFC9	East Siang, Arunachal Pradesh	White	Green	Red	Straw	Elongate	Pendant	Single
CHFC10	East Siang, Arunachal Pradesh	White	Green	Red	Straw	Elongate	Pendant	Single
CHFC11	East Siang, Arunachal Pradesh	Purple -white base	Green	Red	Straw	Elongate	Pendant	Single
CHFC12	Lower Subansiri, Arunachal Pradesh	White	Green	Red	Straw	Elongate	Pendant	Single
CHFC13	East Khasi hill, Meghalaya	Yellow-green	Green	Dark red	Straw	Almost round	Intermediate	Single
CHFC14	East Siang, Arunachal Pradesh	White	Green	Red	Straw	Elongate	Pendant	Single
CHFC15	East Siang, Arunachal Pradesh	Purple	Deep purple	Dark red	Straw	Triangular	Erect	Single
CHFC16	Bishnupur, Manipur	Yellow-green	Green	Red	Straw	Blocky	Intermediate	Single
CHFC17	East Siang, Arunachal Pradesh	White	Green	Red	Straw	Elongate	Erect	Cluster
CHFC18	East Siang, Arunachal Pradesh	White	Green	Red	Straw	Triangular	Pendant	Single
CHFC19	East Khasi hill, Meghalaya	White	Green	Dark red	Straw	Blocky	Intermediate	Single
CHFC20	East Khasi hill, Meghalaya	Yellow-green	Green	Light red	Straw	Campanulate	Intermediate	Single
CHFC21	East Siang, Arunachal Pradesh	White	Green	Red	Straw	Elongate	pendant	Single
CHFC22	East Siang, Arunachal Pradesh	White - purple margin	Green	Dark red	Straw	Blocky	Pendant	Single
CHFC23	Jaunpur, Uttar Pradesh	White	Green	Red	Straw	Elongate	Erect	Cluster
CHFC24	East Khasi hill, Meghalaya	Yellow-green	Green	Dark red	Straw	Elongate	Erect	Single
CHFC25	East district, Sikkim	Yellow-green	Green	Dark red	Straw	Almost round	Intermediate	Single
CHFC26	East Siang, Arunachal Pradesh	White	Green	Red	Straw	Elongate	Erect	Single
CHFC27	East Siang, Arunachal Pradesh	Yellow-green	Green	Red	Straw	Campanulate	Intermediate	Single
CHFC28	East Siang, Arunachal Pradesh	White	Green	Red	Straw	Elongate	Pendant	Single
CHFC29	East Siang, Arunachal Pradesh	White	Green	Red	Straw	Blocky	Intermediate	Single
CHFC30	Pune, Maharashtra	White	Green	Red	Straw	Elongate	Erect	Single

India by Christian missionaries (Dhaliwal, 2007). Genetic resources of chilli landraces in North eastern region of India have not been well documented, however, a wide range of variability for several attributes viz., fruit shape, size, colour, bearing habit, perennial and pungency are observed. A few local names mentioned include Naga Jolokia, Bhut Jolokia and Bih Jolokia which are paprika type, medium in size with strong pungency (Asati and Yadav, 2004). Studies using molecular markers have either taken limited samples from one geographical location (Sanatombi et al., 2010) or have focused only on cultivated accessions (Patel et al., 2011). Therefore, a need exists for proper documentation and analysis of chilli genotypes from different geographical locations in India. The present study generated data on the genetic diversity of chilli genotypes from different locations which will be useful for breeding programmes and also for conservation of germplasm. To use genetic resources adequately, it is necessary to understand the extent and pattern of genetic diversity. In NEH region of India, chilli has long been cultivated in a traditional manner and there exists a considerable amount of genetic variability among the landraces (Bhagowati and Changkija, 2009; Yumnam et al., 2012) which is yet to be characterised and utilized. Therefore, an attempt with the present investigation was undertaken to evaluate the extent of variability existing in thirty chilli genotypes of North eastern region of India through morphometric and seed protein analysis to provide a scientific basis for future selection and crop improvement programme.

Results

Protein distribution patterns in thirty genotypes of chilli were studied. The electrophoretic seed protein profiles of the same have been outlined in the form of electrophorograms based on UPGMA resulting in distinct clusters (Fig 3). A total of 92 protein bands were identified by silver staining. The genotypes showed considerable variation in protein band number ranging from 7-19. Among the genotypes CHFC14 and CHFC15 showed maximum number (19) of protein bands while the minimum number (7) of bands was present in genotypes CHFC3, CHFC4 and CHFC21 (supplementary Table1). Band number 1 was present in genotype CHFC1 and CHFC2 only. CHFC1 and CHFC2 were also found to be unique in bearing the band number 9,10, 13, 39, 70 and 74 which were absent in all other genotypes. Band number 11, 12, 14 and 16 were exclusively present in genotype CHFC25. Band number 15, 21 and 27 were present in CHFC24, band number 19 was present in CHFC19 solely. Band number 42, 52, 61, 63 and 77 was exclusively present in CHFC30. Genotypes CHFC14 and CHFC15 with highest number of bands (19 each) do not contain band number 1-23. Band number 53 was present in maximum (15) number of genotypes. Rm values ranged from 0.145 - 0.992 (supplementary Table 1). The 30 genotypes could be grouped into three big clusters. Genotypes CHFC11-20 formed cluster (I), genotypes CHFC21 - 30 formed cluster (II) and genotypes CHFC1-10 formed cluster (III) (Fig 3). Of these CHFC11, CHFC14, CHFC15, CHFC17 and CHFC18 were collected from East Siang, Arunachal Pradesh whereas genotypes CHFC12 from Lower Subansiri, Arunachal Pradesh and CHFC13, CHFC19 and CHFC20 from East khasi hills, Meghalaya while CHFC16 from Bisunpur, Manipur. Details of the other cluster groups are given in (Table 1). A dendrogram drawn based on the percentage similarity showed that there were 9 sub clusters (Table 3). Genotypes CHFC14-18 formed the first sub cluster. Of these CHFC14, 15 and 18 were collected from East Siang, Arunachal Pradesh whereas CHFC16 was collected from Bisunpur, Manipur. The second sub cluster was formed by genotypes CHFC11-13. Of these CHFC11was from East Siang whereas CHFC12 was from Lower Subansiri, Arunachal Pradesh while CHFC13 was from East khasi hills, Meghalaya. Genotypes CHFC19-20 formed third sub cluster and originating from East khasi hills, Meghalaya. Fourth sub cluster was formed by genotypes CHFC21-24. Of these genotypes CHFC21-22 were from East Siang, Arunachal Pradesh whereas CHFC23 was from Jaunpur, Uttar Pradesh and CHFC24 was East Khasi hills, Meghalaya. Fifth sub cluster was formed by genotypes CHFC25 - 27. Of these genotype CHFC25 was from East district, Sikkim whereas CHFC26 and CHFC27 were from East Siang, Arunachal Pradesh. The sixth cluster was formed by genotypes CHFC28-30. Of these genotypes CHFC28-29 were collected from East Siang, Arunachal Pradesh while CHFC30 was collected from Pune, Maharastra. Cluster seven was formed by the genotypes CHFC1-2 which were collected from East Sing, Arunachal Pradesh. Cluster eighth was formed by the genotypes CHFC6-10 of which CHFC6, 8, 9 and CHFC10 were collected from same area East Siang, Arunachal Pradesh while CHFC7 was from East district, Sikkim. The genotypes CHFC3 - 5 formed cluster ninth. Of these genotype CHFC3 was collected from Bisunpur, Manipur while CHFC4 - 5 were collected from East Siang, Arunachal Pradesh. Average similarity was highest (99. 510%) between genotypes CHFC14 and CHFC15. Genotype CHFC14 and CHFC15 were collected from East Siang, Arunachal Pradesh while genotype CHFC9 was found to have minimum similarity with genotype CHFC18 (53.098 %). The genotypes under study collected from different places and can be distinguished on the basis of seed protein used in the present study. This suggests that there is a potential for identification of new genes in chilli from landraces of NEH region of India.

Discussion

Knowledge of genetic diversity has been successfully used for efficient germplasm management and utilization, genetic finger printing and selection (Engles et al., 2002). Genetic diversity of a genotype is average diversity of all loci (Nei and Li, 1979). Different kind of electrophoretic methods based on storage protein patterns have been used for the identification and the characterization of crop (Karihaloo et al., 2002). Some investigators proposed that seed protein profiles may be useful as an indicator of taxonomic relationships within some species (Duran et al., 2005) but ones said that this method was insufficient for the discrimination at the cultivar level (Panella et al., 1993). In present study our findings indicated that SDS-PAGE of seed proteins supplied additional banding pattern for the discrimination of the chilli genotypes. The results were in agreement with the findings of Kumar et al. (2010). Recently, several studies suggested that the application of numerical analysis, coupled with the utilization of a standardized identification system instead of simple quantative comparison of protein patterns provides an effective approach to the investigation of taxonomic relationships among crop species (Karihaloo et al., 2002; Lioli et al., 2005). Here in the present investigation, SPSS for windows package (version 14) was used to analyse the data because of the difficulties in the visual interpretation of SDS-PAGE of seed protein profiles. The numerical analysis of SDS-PAGE of seed protein profiles showed that each cluster had slight discriminative protein banding profile. For example, the cluster I includes10

Table 2. Genetic distance estimates among 30 genotypes of chilli using SDS-PAGE analysis.

Genotypes	CHF C1	CHF C2	CHF C3	CHF C4	CHF C5	CHF C6	CHF C7	CHF C8	CHF C9	CHF C10	CHF C11	CHF C12	CHF C13	CHF C14	CHF C15	CHF C16	CHF C17	CHF C18	CHF C19	CHF C20	CHF C21	CHF C22	CHF C23	CHF C24	CHF C25	CHF C26	CHF C27	CHF C28	CHF C29	CHF C30
CHFC1	0.000																													
CHFC2	0.627	0.000																												
CHFC3	18.339	18.150	0.000																											
CHFC4	17.766	17.577	0.640	0.000																										
CHFC5	23.629	23.440	8.923	9.196	0.000																									
CHFC6	23.774	23.584	14.567	15.207	13.316	0.000																								
CHFC7	24.311	24.122	14.674	15.314	13.106	1.628	0.000																							
CHFC8	17.567	17.378	10.456	11.096	14.394	7.880	8.458	0.000																						
CHFC9	22.556	22.366	14.881	15.521	14.023	1.219	2.411	7.845	0.000																					
CHFC10	23.363	23.174	15.747	16.387	13.728	1.626	2.823	7.712	1.808	0.000																				
CHFC11	35.205	35.391	39.870	39.230	33.753	41.098	41.021	40.274	41.198	40.348	0.000																			
CHFC12	34.639	34.825	38.941	38.301	32.824	40.629	40.093	39.805	40.729	39.879	9.091	0.000																		
CHFC13	34.435	34.621	40.072	39.432	33.955	41.759	41.223	40.935	41.859	41.009	11.030	4.965	0.000																	
CHFC14	38.532	38.718	44.820	44.180	38.703	46.103	45.971	45.279	46.203	45.353	13.799	9.318	7.509	0.000																
CHFC15	38.406	38.592	44.344	43.704	38.227	45.628	45.496	44.804	45.728	44.878	13.324	8.843	7.022	0.490	0.000															
CHFC16	38.760	38.946	44.840	44.200	38.723	46.084	45.992	45.260	46.184	45.334	14.128	9.745	7.295	2.931	2.654	0.000														
CHFC17	33.394	33.580	37.854	37.214	31.737	39.082	39.006	38.185	39.183	38.332	10.759	8.383	8.701	10.021	9.906	9.825	0.000													
CHFC18	38.656	38.842	45.573	44.933	39.456	46.801	46.725	45.904	46.902	46.051	13.307	10.596	8.862	4.958	5.041	4.480	10.547	0.000												
CHFC19	29.084	29.270	34.773	34.133	28.656	36.461	35.924	35.637	36.561	35.710	13.348	13.278	13.291	15.575	15.445	15.252	12.286	15.041	0.000											
CHFC20	33.200	33.386	38.998	38.358	32.881	40.686	40.150	39.862	40.786	39.936	11.152	8.122	7.984	10.681	10.191	9.948	8.694	10.386	8.127	0.000										
CHFC21	24.692	24.882	31.169	30.529	34.205	36.173	36.924	31.499	35.881	36.354	29.441	31.343	31.573	34.202	34.467	34.911	30.345	32.326	27.536	30.639	0.000									
CHFC22	27.244	27.433	34.254	33.614	36.377	38.513	38.661	34.584	38.613	38.434	29.267	33.108	32.894	35.563	35.828	36.232	32.109	33.850	28.853	31.956	7.551	0.000								
CHFC23	27.733	27.922	34.743	34.103	36.866	39.002	39.150	35.073	39.102	38.923	29.756	33.597	33.383	36.052	36.317	36.721	32.598	34.339	29.256	32.359	7.928	0.557	0.000							
CHFC24	23.690	23.879	31.055	30.415	33.295	35.326	35.495	31.439	35.426	35.322	28.987	32.459	32.288	35.395	35.268	35.596	31.486	33.358	28.090	31.102	9.098	7.462	7.525	0.000						
CHFC25	24.407	24.593	30.830	30.190	34.135	36.038	36.567	31.784	36.106	36.057	32.769	32.197	31.980	34.566	34.832	35.340	31.270	33.695	28.243	30.990	16.437	18.304	17.815	18.827	0.000					
CHFC26	25.484	25.674	32.850	32.210	34.669	36.546	37.172	33.693	36.646	36.553	32.503	32.683	32.469	35.138	35.403	35.807	31.704	34.066	28.553	31.373	17.609	19.055	18.566	19.153	4.505	0.000				
CHFC27	29.163	29.349	36.525	35.885	38.467	40.332	40.899	37.368	40.421	40.351	36.325	36.468	36.254	38.923	39.188	39.592	35.469	36.943	32.055	35.157	19.506	21.178	20.689	20.990	7.782	4.255	0.000			
CHFC28	23.861	24.050	31.226	30.586	29.802	34.021	33.369	31.639	34.122	32.827	26.903	26.631	26.417	29.086	29.351	29.755	25.632	27.106	22.218	25.320	13.402	14.912	14.516	15.275	14.361	13.672	10.200	0.000		
CHFC29	26.262	26.451	33.627	32.987	31.714	35.964	35.312	33.958	36.064	35.214	29.281	29.009	28.795	31.464	31.729	32.133	28.010	29.484	24.596	27.698	16.538	18.043	18.106	17.647	17.340	16.614	13.154	3.771	0.000	
CHFC30	20.337	20.527	27.703	27.063	28.955	30.912	31.537	28.086	31.013	30.909	29.306	27.895	27.681	30.350	30.615	31.019	26.973	29.857	24.466	26.584	13.240	15.489	15.552	13.079	14.050	13.090	15.956	9.744	12.116	0.000

Fig 1. (A) India map showing geographical distribution of the chilli genotypes which was collected from Arunachal Pradesh (a and b), Sikkim (c), Meghalaya (d), Manipur (e), Uttar Pradesh (f) and Maharashtra (e). (B) A representative picture showing variation in corolla colour, (C) fruiting behavior and (D) fruit shape and colour.

of reference chilli genotypes (CHFC11 - 20), sharing many protein bands. The members of sub cluster Ia (Table 3) which were from East Siang, Arunachal Pradesh having highest intra-cluster similarities (99.51%). This finding confirmed that the genotype CHFC14 and CHFC15 may be very close at genetic level even though there was much difference at

Table 3. Major cluster produced by SDS-PAGE analysis in 30 chilli genotypes.

Cluster	Sub cluster	Genotypes
I	I a	CHFC-14, CHFC-15, CHFC-16 and CHFC-18
	I b	CHFC-12, CHFC-13, CHFC-11 and CHFC-17
	I c	CHFC-19 and CHFC-20
II	II a	CHFC-22, CHFC-24, CHFC-21 and CHFC-23
	II b	CHFC-26, CHFC-27and CHFC-25
	II c	CHFC-28, CHFC-29 and CHFC-30
III	III a	CHFC-1 and CHFC-2
	III b	CHFC-6, CHFC-7, CHFC-9, CHFC-10 and CHFC-8
	III c	CHFC-3, CHFC-4 and CHFC-5

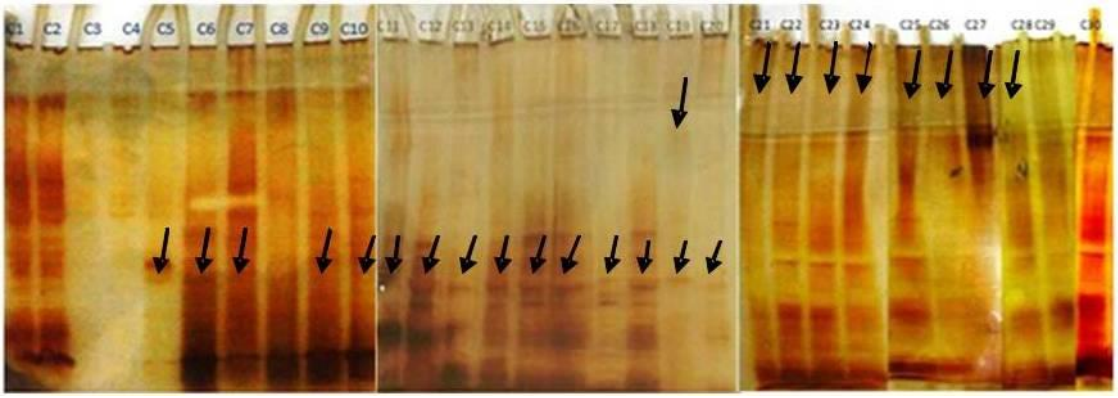

Fig 2. Seed storage protein profiles in thirty chilli genotypes. Arrows represents the specific bands.

morphological traits. Similar findings were also reported by Kumar and Tata (2010) and Berber et al. (2011). Additionally, our findings showed that the genotypes CHFC9 and CHFC18 belongs to different sub cluster and maximum genetic distance (53.098 %) though they were collected from East Siang, Arunachal Pradesh which were having almost similar phenotypic traits. Similar report with present finding was also reported by Kumar et al. (2010) with conclusion that seed storage protein profiles could be useful markers in cultivar identification in chilli. It was also observed that genotypes from different regions were observed to be closely related and genotypes from the same region had different genetic back ground. Intra regional diversity could be as a valuable source as inter regional diversity for chilli improvement. The landraces evaluated in present study were shown to have useful horticultural characteristics which exhibit a higher genetic potentiality. The genotype CHFC9 was most distantly related to CHFC18. Similar report with present findings was also reported by Akbar et al. (2010) in their study on phylogeny and genetic diversity studies in *Capsicum* using seed storage proteins. Hence, it was recommended that these two genotypes could be utilised for crossing programme to create more genetic diversity or segregants of desired characteristics through chilli breeding programmes. Thus, SDS-PAGE marker data provided more sub groupings and revealed higher amount of diversity as compared to morphological data. It is evident from the present study that genetic relationship estimated from protein banding pattern enhanced the resolution of diversity and thus provided a better picture of variability as compared to morphological markers. In conformity of present work, Singh et al. (2009) concluded that SDS-PAGE of soluble seed proteins showed different banding patterns, which might be used for varietal identification. Although SDS-PAGE analysis could show discrete variation among few genotypes of chilli under study, this protein marker should be applied in future to more number of genotypes to arrive at a reasonable conclusion. Similar finding was also reported by Anu and Peter (2003) by demonstrating that proteins and enzymes are important parameters in biochemical taxonomy. Therefore, seed protein electrophoresis could be proved to be a successful technique in certain cases to distinguish morphologically indistinguishable genotypes. Extension of this method may be useful for chilli taxonomy studies and establishment of the phylogenetic relationship without any ambiguity. Similarly, Panda et al. (1986) also confirmed that the seed protein profile of eight taxa of chilli peppers obtained by disc electrophoresis was found to be a diagnostic character in the study of phylogenetic relationships. Hence, SDS-PAGE based on seed protein is a predominant tool to study molecular systematic for identification of genotypes and to discriminate between morphologically similar genotypes in a limited way.

Materials and Methods

Plant materials

Thirty chilli (*Capsicum annuum* L) genotypes were collected from different part of the North eastern and other part of India and cultivated at research farm of department of Vegetable Science, College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh, India during March-October, 2011. The list of genotypes along with their sources and morphological traits is given in (Table 1).

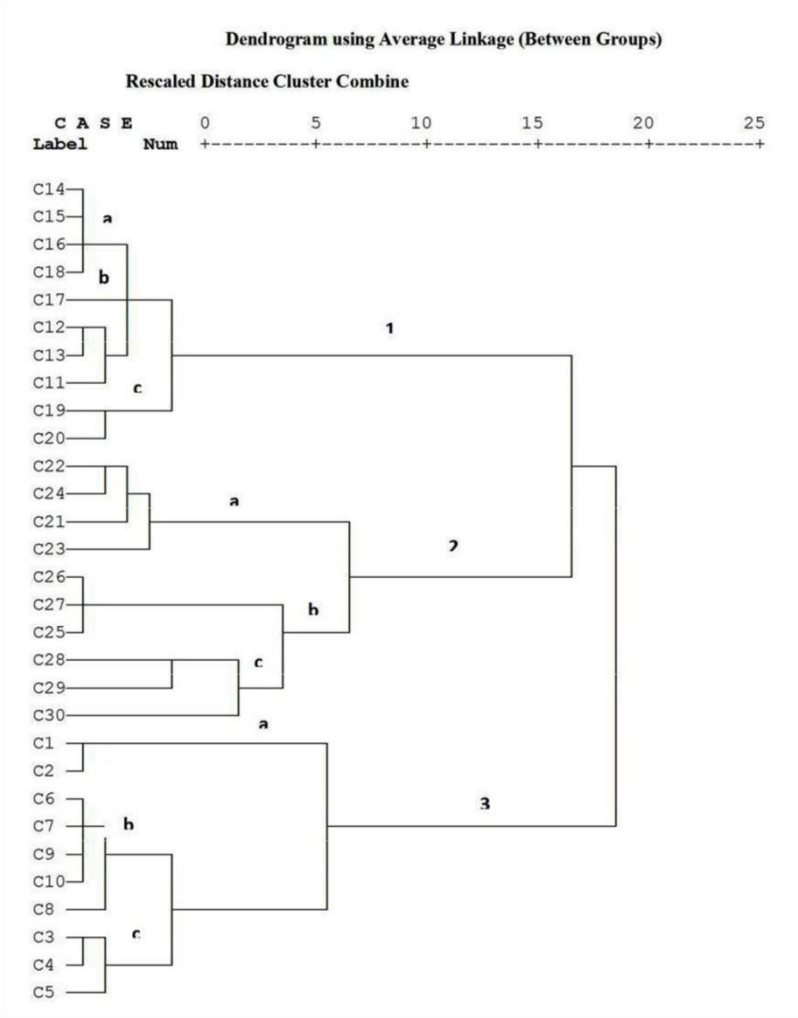

Fig 3. UPGMA of thirty chilli genotypes based on total seed protein profiles obtained by SDS-PAGE.

Determination of protein

Estimation of protein was done as per procedure described by Lowry et al. (1951) in seeds of thirty genotypes of chilli collected for the study. A standard curve of absorbance at 660 nm versus 1µg of BSA was drawn and from this standard curve, the amount of protein in the sample tube was determined as protein per gram of the sample.

Extraction of total seed proteins for SDS-PAGE

The variability of seed storage proteins was analyzed by SDS-PAGE as per procedure described by Laemmli (1970). Seeds of thirty chilli genotypes evaluated in the field were collected and 0.1 g seed was taken in pestle and mortar and added 25µL of buffer (0.06 M Tris -HCl, 2.5% Glycerol, 0.5% SDS, 1.25% β-mercaptoethanol, 0.1% TCA, 10 mM urea, 1 mM EDTA). The sample was homogenized and heated in a boiling water bath for 5 min at 100°C for denaturation of proteins. The protocol followed in the present study was in accordance with Kumar and Tata (2010) with some modifications.

SDS-PAGE

The soluble seed proteins were subjected to SDS-PAGE in gel slabs of 1mm thickness (5% stacking and 10% resolving gels). Electrophoresis was performed with a discontinuous buffer system in a vertical electrophoresis unit. The gel was run at 25 mA until the tracking dye approached the bottom of the gel. The system was disconnected and the gel was taken out from the slab. The gel was then subjected to silver staining as per procedure described by Mortz et al. (2001) through sensitizing with 0.02% sodium thiosulphate solution for 5 minutes and then washing twice with double distilled water for 1 minute. It was then transferred to staining solution and kept on gel rocker for 20 minutes in dark. The gel was then washed twice with distilled water for 45 seconds, transferred to developing solution and finally the reaction was stopped with 12% acetic solution. Gel was washed thoroughly but gently with double distilled water until protein bands became clearly visible for bands scoring**.** The electrophorograms were prepared on the basis of protein mobility and the density expressed in Rm values.

Statistical data analysis of protein profiling

The gels were scored as presence (+) or absence (-) of protein bands. Depending upon the presence or absence of bands, similarity index (Nei and Li, 1979) between the genotypes was calculated by the following formula:

$$SI = \frac{2Z}{X+Y} \times 100$$

Where, Z = Number of similar bands between the genotypes and X+Y = Total number of bands in the two genotypes

compared. Dissimilarity is usually defined as 1 minus similarity (1-F) (Virk et al., 1995) and distance matrix of dissimilarity was produced for a set of individuals. Dendrogram was generated using an unweighted pair group method with arithmetic mean analysis (UPGMA) by use of statistical software SPSS for windows package (Version 14).

Conclusion

SDS-PAGE of seed storage proteins can be economically used to assess genetic diversity in chilli germplasm. Based on the resolved protein profiles CHFC9 was most distantly related to CHFC18. The genotypes from different regions were observed to be closely related and genotypes from the same region had different genetic back ground. Intra regional diversity could be as a valuable source as inter regional diversity for chilli improvement. Genotypes with similar banding patterns should be further characterized by 2-D electrophoresis. Advanced molecular techniques could be employed to identify duplicate genotypes in the germplasm collection for efficient management and to tag important gene available in the germplasm through linkage to DNA markers.

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