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Studies on seed protein profiling in wild brinjal (*Solanum gilo* Raddi) genotypes of Northeast India

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Abstract
North eastern region of India exhibits wide variability in fruit morphology, bearing habit and crop duration of brinjal. In the present study, fifteen wild brinjal (*Solanum gilo* Raddi) genotypes from different areas of North eastern hill were collected and estimation of protein was done in seeds of fifteen genotypes of wild brinjal. The analysis showed considerable variation in banding pattern of total protein which ranged from 18-38 numbers of band. The genotype CHFG-13 was most distantly related to CHFG-14. Hence, it was recommended that genotype CHFG-13 and CHFG-14 could be utilised for crossing programme to create more genetic diversity or segregants of desired characteristics through brinjal breeding programmes. Hence, overview of diversity of wild brinjal (*Solanum gilo* Raddi) 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: *Solanum gilo* Raddi, Genetic diversity, PAGE electrophoresis, SDS

Introduction
Solanum gilo Raddi is commonly known as bitter brinjal. It is an important indigenous leaf and fruit vegetable in tropical Africa; cultivated and consumed largely in Africa (Sunseri *et al*., 2010). Wide variations exists within and between the species including variation in characters like diameter of corolla, petiole length, leaf blade width, plant branching, fruit shape and colour (Chinedu *et al*., 2011) [4]. Their uses in indigenous medicine range from weight reduction to treatment of several ailments including asthma, allergic rhinitis, nasal catarrh, skin infections, rheumatic disease and swollen joint pains, gastro-oesophageal reflux disease, constipation, dyspepsia (Bello *et al*., 2005) [3]. Many reports have been studied on the genetic diversity but information of wild species of *Solanum* spp. is still lacking and divergence is usually related to adaptation to different geographical areas or climates or different ecological habitats. In the process of adaptation, populations may become genetically distinct (Webb *et al*., 1988).

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. The simplicity and cost effectiveness of SDS- PAGE technique has led to the vigorous used for analysing diversity of germplasm in the form of seed protein (Fuffa *et al*., 2005; Iqbal *et al*., 2005). Moreover, seed protein are primary product of structural genes which gave better precision for genetic diversity analysis (Srivalli *et al*., 1999). Diversity of brinjal by SDS-PAGE of seed proteins was conducted by Karihaloo *et al*., (2002). Dubey and Ram (2008) also used SDS-PAGE for assessment of genetic diversity in bottle gourd. NEH region comprising the states of Arunachal Pradesh, Assam, Nagaland, Meghalaya, Manipur, Tripura, Mizoram and Sikkim. Genetic resources of *Solanum gilo* Raddi landraces in North eastern region of India have not been well documented, however, a wide range of variability for several attributes *viz*., fruit size, color, bearing habit are observed. Therefore, a need exists for proper documentation and analysis of wild brinjal (*Solanum gilo*

Raddi) genotypes from different geographical locations in India. The present study generated data on the genetic diversity of wild brinjal (*Solanum gilo* Raddi) 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. Although wild brinjal (*Solanum gilo* Raddi) is cultivated in all the state of North eastern region of India but there is no improved variety that can be recommended to the farmers for its commercial cultivation in the region. For future selection and crop improvement programme, the morphology and seed protein analysis was undertaken among the highly variable fifteen wild brinjal (*Solanum gilo* Raddi) genotypes existing in the North eastern region of India.

Materials and Methods

Plant materials

Across north eastern region of India, highly diverse fifteen wild brinjal (*Solanum gilo* Raddi) genotypes were collected. During August (2015) to April (2016), the crop was raised at research farm of department of Vegetable Science, College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh, India The list of genotypes along with their sources and morphological traits is given in (Table 1).

Determination of protein

Estimation of protein was done as per procedure described by Lowry *et al*. (1951) in seeds of fifteen genotypes of wild brinjal collected for the study. A standard curve of absorbance at 660nm versus 1 µg 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 SDS-PAGE technique was employed to analyse the variability of seed storage proteins (Laemmli, 1970). Seeds of fifteen wild brinjal 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℃ 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 exposed to SDS-PAGE in gel slabs of 1 mm 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 ands 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. The similarity index (Nei and Li., 1979) between the genotypes were calculated on the basis of presence or absence of bands as follows:

$$SI = \frac{2Z}{X+Y} x100$$

Where, Z= Number of similar bands between the genotypes and X+Y = Total number of bands in the two genotypes compared. Dendrogram was generated using an unweighted pair group method with arithmetic mean analysis (UPGMA) by use of statistical software windows package (Version 16).

Result

Protein distribution patterns in fifteen genotypes of wild brinjal (*Solanum gilo* Raddi) were studied (Fig.1). The electrophorograms based on UPGMA resulting in distinct clusters have been generated on the basis of the electrophoretic seed protein profiles (Fig.2). A total of 71 protein bands were identified by silver staining. The genotypes showed considerable variation in protein band number ranging from 18-38. Among the genotypes CHFG-2 showed maximum number (38) of protein bands while the minimum number (18) of bands were present in genotypes CHFG-13 (supplementary Table 1). Band number 1 was present in genotype CHFG-13 only. CHFG-6, CHFG-1 and CHFG-14 were also found to be unique in bearing the number 5, 67 and 71 which were absent in all other genotypes. Band number 8 and 9 were exclusively present in genotype CHFG-2. Band number 11, 13, 14, 18, 20, 21, 22, 24, 25, 27, 31, 35, 37, 40, 41, 46, 51, 52, 58, 60, 64 were present in both the genotypes CHFG-6 and CHFG-7. Band number 23 was present in genotypes CHFG-1, CHFG-2, CHFG-3, CHFG-4, CHFG-5, CHFG-8, CHFG-9, CHFG-10, CHFG-11, CHFG-12, CHFG-13, CHFG-14, and CHFG-15. Band number 7 was found to be present in CHFG-1 and CHFG-2 only, whereas it was absent in all other genotypes. Similarly, band number 36 was found to be present in CHFG-4 and CHFG-10 only, whereas it was absent in all other genotypes. Band number 43, 44 and 47 were also found to be present only in two genotypes. Band number 29 was found to be present in CHFG-3, CHFG-4 and CHFG-6 only, whereas it was absent in all other genotypes. Similarly, band number 30 was found to be present in CHFG-1, CHFG-9 and CHFG-10 only, whereas it was absent in all other genotypes. Band number 42, 48, 49, 50, 61, 62, 66 and 69 were also found to be present only in three genotypes. Band number 21 was present in all the genotypes followed by band number 23 and band number 41 which were found to be present in 13 genotypes. Rm values ranged from 0.10 to 0.82 (supplementary table 1). The 15 genotypes could be grouped into two major clusters. Cluster I was found to contain 14 genotypes while cluster II was found to contain only 1 genotype (CHFG-13) (Fig.2). Of these CHFG-5, CHFG-8, CHFG-13 were collected from Arunachal Pradesh whereas genotypes CHFG-1, CHFG-2, CHFG-3, CHFG-4, CHFG-7, CHFG-11, CHFG-12 and CHFG-15 were collected from Mizoram. Details of the other cluster groups are given in (Table 1). A dendrogram drawn based on the percentage similarity showed that there were 4 sub clusters (Table 2). Genotypes CHFG-3, CHFG-4, CHFG-

5, CHFG-6, CHFG-7, CHFG-8 formed the first sub cluster. Of these, CHFG-3, CHFG-4, CHFG-7 were collected from Mizoram whereas CHFG-5 and CHFG-8 were collected from Arunachal Pradesh while CHFG-6 from East Sikkim. The second sub cluster was formed by genotypes CHFG-2, CHFG-9, CHFG-10, CHFG-12. Of these CHFG-2 and CHFG-12 were from Mizoram whereas CHFG-9 was from Nagaland while CHFG-10 from Manipur. Genotypes CHFG-14, CHFG-15 formed third sub cluster. Of these CHFG-14 was from Meghalaya while CHFG-15 from Mizoram. The genotypes CHFG-1, CHFG-11 formed fourth sub cluster. Of these CHFG-1 and CHFG-11 were collected from Mizoram. Average similarity was highest (76.0%) between genotypes CHFG6- and CHFG-7 while genotype CHFG-13 was found to have minimum similarity with genotype CHFG-14 (22.0%) (Table 3). The seed protein can differentiate the genetic variability existing among diverse germplasms from various locations. This suggests that there is a potential for identification of new genes in wild brinjal (*Solanum gilo* Raddi) from landraces of NEH region of India.

Discussion

For efficient germplasm management and utilization, genetic finger printing and selection, the extent of information on genetic diversity is successfully employed (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). The seed profiling has been used successfully as taxonomic relationship within some species (Duran *et al*., 2005) while other suggested the insufficiency of this method for discrimination at the cultivar level (Panella *et al*., 1993). The present findings showed additional banding pattern of SDS-PAGE from seed proteins is vital for discrimination of the brinjal 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 quantitative 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 16) was used to analyse the data because of the difficulties in the visual interpretation of SDS-PAGE of seed protein profiles. The cluster generated from seed protein SDS-PAGE showed minute discriminative protein banding profile. For example, the cluster I includes of reference wild brinjal genotypes (CHFG-3, CHFG-4, CHFG-5, CHFG-6, CHFG-7, CHFG-8), sharing many protein bands. The members of cluster IA (Table 3) which were from Sikkim and Mizoram having highest intra-cluster similarities (76.0%). This finding confirmed that the genotype CHFG-6 and CHFG-7 may be very close at genetic level even though there was much difference at morphological traits. Similar findings were also reported in chilli by Kumar and Tata (2010) and Barber and Yasar (2011). Additionally, our findings showed that the genotypes CHFG-13 and CHFG-14 belongs to sub cluster and maximum genetic distance (22.0%) 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 wild brinjal (*Solanum gilo* Raddi). It was also observed that genotypes from different regions were observed to be closely related and genotypes from the same region had different genetic background. Intra regional diversity could be as a valuable source as inter regional diversity for brinjal improvement. The landraces evaluated in present study were shown to have useful horticultural characteristics which exhibit a higher genetic potentiality. The genotype CHFG-13 was most distantly related to CHFG-14. Hence, it was recommended that these two genotypes could be utilised for crossing programme to create more genetic diversity or segregates of desired characteristics through brinjal 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 wild brinjal 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. Henceforth, morphologically indistinguishable genotypes can be effectively differentiated by seed protein electrophoresis technique. Extension of this method may be useful for brinjal taxonomy studies and establishment of the phylogenetic relationships. Therefore, seed protein SDS-PAGE is an efficient systematic molecular technique for genotypes identification and discrimination between morphologically similar genotypes in a limited way.

Conclusion

Seed proteins SDS-PAGE can be economically used to study genetic diversity in bitter brinjal germplasm. Based on the resolved protein profiles CHFG-13 was most distantly related to CHFG-14. Intra-regional as well as inter-regional diversity could be a valuable source for bitter brinjal improvement. Genotypes with similar banding patterns should be further characterized by 2-D electrophoresis. Advanced molecular techniques could be employed to identify duplicate genotypes for efficient management of bitter brinjal germplasm and to tag important gene available in the germplasm through linkage to DNA markers.

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Table 1: List of wild brinjal (*Solanum gilo*) genotypes with their source and morphological traits

Genotype	Source	Leaf blade width	Leaf blade lobing	Fruit colour	Fruit shape	Fruiting habit
CHFG-1	A landrace of Lawngtlai, Mizoram	Wide	Intermediate	Light green	About 1/2 way from base to tip	Cluster and solitary
CHFG-2	A land race of Khawzawl, Mizoram	Intermediate	Strong	White	About 1/2 way from base to tip	Cluster and solitary
CHFG-3	A landrace of Sialsuk, Mizoram	Intermediate	Strong	Purple	About 1/2 way from base to tip	Cluster and solitary
CHFG-4	A landrace of Keifang, Mizoram	Wide	Intermediate	Dark green	About 1/2 way from base to tip	Cluster and solitary
CHFG-5	A landrace of Balek, Arunachal Pradesh	Intermediate	Strong	Green	About 3/4 way from base to tip	Cluster and solitary
CHFG-6	A landrace of Yang yang, East Sikkim	Wide	Intermediate	Light green	About 1/2 way from base to tip	Cluster and solitary
CHFG-7	A landrace of Mizoram	Wide	Intermediate	Light green	About 1/2 way from base to tip	Cluster and solitary
CHFG-8	A landrace of Ledum, Arunachal Pradesh	Intermediate	Strong	Green	About 3/4 way from base to tip	Cluster and solitary
CHFG-9	A landrace of Nagaland	Wide	Strong	Dark green	About 1/2 way from base to tip	Cluster and solitary
CHFG-10	A landrace of Churachandpur, Manipur	Intermediate	Intermediate	Purple	About 3/4 way from base to tip	Cluster and solitary
CHFG-11	A landrace of Mizoram	Intermediate	Strong	White	About 1/2 way from base to tip	Cluster and solitary
CHFG-12	A landrace of Mizoram	Wide	Intermediate	Blackish green	About 1/2 way from base to tip	Cluster and solitary
CHFG-13	A landrace of Aalo, West Siang, Arunachal Pradesh	Wide	Intermediate	Light green	About 1/2 way from base to tip	Cluster and solitary
CHFG-14	A landrace of Shillong, Meghalaya	Intermediate	Intermediate	Green	About 3/4 way from base to tip	Cluster and solitary
CHFG-15	A landrace of East Lungdar, Mizoram	Intermediate	Intermediate	Green	About 1/2 way from base to tip	Cluster and solitary

Table 2: Major cluster produced by SDS-PAGE analysis in fifteen genotype of wild brinjal

Cluster	Sub cluster	Sub-sub cluster	Genotypes
I	I A	I A 1	CHFG-3, CHFG-4, CHFG-5, CHFG-6, CHFG-7, CHFG-8
		I A 2	CHFG-2, CHFG-9, CHFG-10, CHFG-12
	I B	I B 1	CHFG-14, CHFG-15
		I B 2	CHFG-1, CHFG-11
II	CHFG-13		

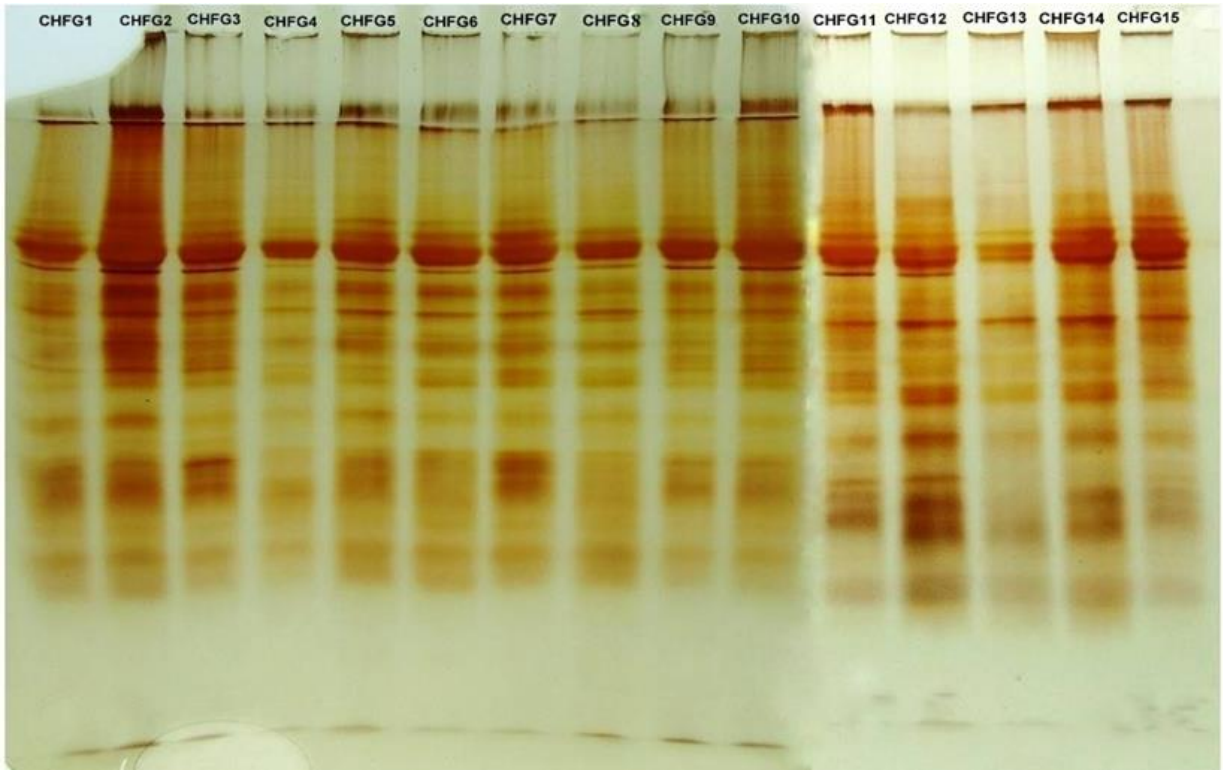

Fig 1: Seed protein banding pattern in fifteen wild brinjal genotypes

Table 3: Similarity index estimates among 15 genotypes of wild brinjal using SDS-PAGE analysis

Genotypes	CHFG-1	CHFG-2	CHFG-3	CHFG-4	CHFG-5	CHFG-6	CHFG-7	CHFG-8	CHFG-9	CHFG-10	CHFG-11	CHFG-12	CHFG-13	CHFG-14	CHFG-15
CHFG-1	1.00														
CHFG-2	0.50	1.00													
CHFG-3	0.44	0.56	1.00												
CHFG-4	0.30	0.49	0.53	1.00											
CHFG-5	0.32	0.53	0.62	0.62	1.00										
CHFG-6	0.45	0.46	0.60	0.52	0.65	1.00									
CHFG-7	0.41	0.51	0.68	0.58	0.75	0.76	1.00								
CHFG-8	0.48	0.38	0.56	0.46	0.57	0.54	0.57	1.00							
CHFG-9	0.38	0.64	0.53	0.40	0.53	0.55	0.50	0.50	1.00						
CHFG-10	0.37	0.63	0.52	0.58	0.41	0.47	0.46	0.38	0.65	1.00					
CHFG-11	0.48	0.45	0.45	0.38	0.44	0.35	0.42	0.45	0.39	0.38	1.00				
CHFG-12	0.31	0.51	0.49	0.43	0.52	0.43	0.46	0.34	0.43	0.53	0.46	1.00			
CHFG-13	0.25	0.39	0.31	0.39	0.37	0.35	0.34	0.33	0.40	0.47	0.38	0.43	1.00		
CHFG-14	0.39	0. 40	0.50	0.24	0.43	0.45	0.44	0.44	0.33	0.31	0.44	0.48	0.22	1.00	
CHFG-15	0.44	0.29	0.45	0.29	0.53	0.43	0.50	0.54	0.43	0.26	0.45	0.38	0.23	0.72	1.00

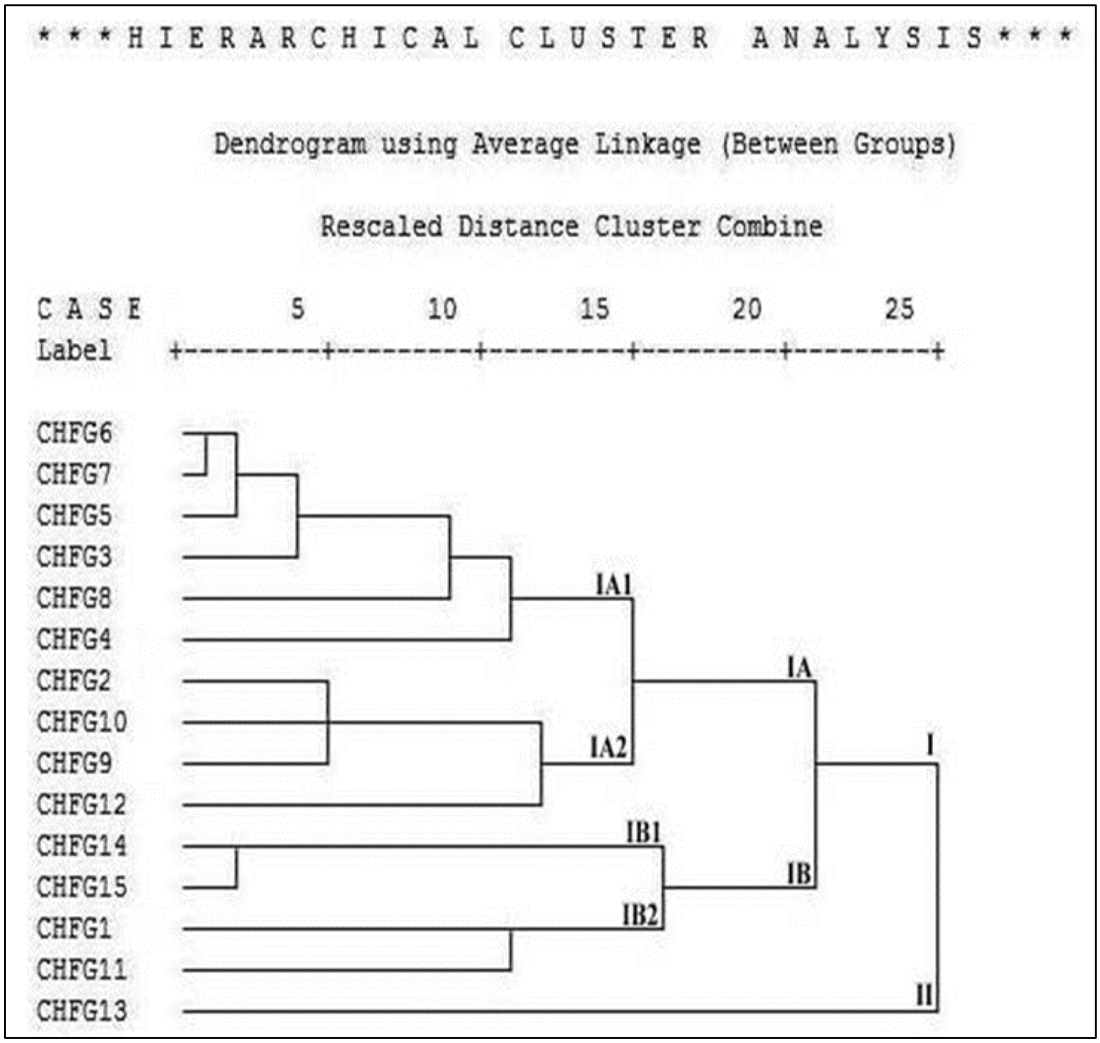

Fig 2: UPGMA of fifteen wild brinjal genotypes based on total seed protein profiles obtained by SDS-PAGE

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