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ORIGINAL ARTICLE

ASSESSMENT OF GENETIC DIVERSITY OF INDIGENOUS RIDGE GOURD [*LUFFA ACUTANGULA* (ROXB.) L.] GENOTYPES OF NORTH EAST INDIA AS REVEALED BY SDS-PAGE

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Abstract : Protein content was estimated in seeds of fourty genotypes of ridge gourd. Total seed storage proteins of ridge gourd genotypes analyzed by SDS-PAGE and showed substantial variation in banding pattern of total protein, which varied from 8-24 numbers of bands. The similarity index was calculated on the basis of presence (+) or absence (-) of bands and the dendrogram constructed grouped the 40 genotypes in two major clusters which were further sub divided in four sub clusters. The clustering pattern reflected that the genotype CHFRG35 was most distantly related to CHFRG36 and identified as potential genotypes for crossing programme and to develop new improved cultivars of desired traits. Assessment of genetic diversity of ridge gourd genotypes from various regions of India could be utilised for augmenting the database that would contribute towards systematic breeding programmes and minimizing genetic erosion.

Key words : Ridge gourd, *Luffa acutangula*, Genetic divsersity, SDS-PAGE, Seed protein profiling.

1. Introduction

Luffa acutangula (Roxb. L.) [2n=2x=26], commonly known as ridge gourd is an important vegetable of India available during summer to rainy season when there is scarcity of vegetables. Green fruits are cooked as vegetables. Knowledge of genetic diversity among existing cultivars of any crop is essential for long-term success in breeding programme and to maximize the exploitation of the germplasm resources. Seed protein electrophoresis is based on the concept that each genotype/cultivar is distinct and relatively homogenous at the genetic level. In recent years, SDS-PAGE is commonly employed as a supplementary approach for species identification and useful tool for back-tracking the evolution of various groups of plants. Seed proteins have the advantage of being scorable from unviable organs or tissues and the electrophoretic protocol for bulk protein is generally simpler than that for isozymes [Cook (1984)]. Sodium dodecyl sul-phate polyacrylamide gel electrophoresis (SDS-PAGE) is an simple and cost-effective, technique that provides the best resolution in the identification of germplasm by protein patterns and is extensively used for describing the seed protein diversity of crop germplasm [Fufa *et al.* (2005)]. Furthermore, seed proteins are used as genetic markers in the study of genetic variation because they are the primary products of structural genes, any change in the coding sequence of a gene generally reflects the corresponding change in the primary structure of protein [Srivalli *et al.* (1999)]. Based on available literatures, scanty information is available on the genetic variability of ridge gourd collection from NE region of India and Arunachal Pradesh in particular. Therefore, the present investigation was undertaken with an objective to evaluate the extent of divergence existing in fourty ridge gourd genotypes through morphological and seed protein profile as protein marker are highly polymorphic and stable. The information generated from the study would provide scientific basis for vegetable breeders to assist their needs and plan appropriate crop improvement programme.

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2. Materials and Methods

Plant materials

Collection of fourty ridge gourd genotypes from NE regions and other part of the India were grown at the experimental farm of department of Vegetable Science, College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh during April-September, 2013. The different genotypes along with their sources and morphological characteristics are mentioned in (Table 1).

Determination of protein

Seeds of all fourty genotypes of ridge gourd subjected for determination of protein content by the method described by Lowry *et al*. (1951). The amount

Table 1 : Morphological characters and source of collection of ridge gourd genotypes.

Genotype	Fresh fruit skin colour	Flesh colour	Smoothness of surface	Fruiting behaviour	Source of collection
CHFRG 1	Green	White	Intermediate	Solitary	Arunachal Pradesh, India
CHFRG 2	Green	Pale yellow	Intermediate	Solitary	Uttar Pradesh, India
CHFRG 3	Green	Pale yellow	Superficial	Solitary	Uttar Pradesh, India
CHFRG 5	Green	White	Intermediate	Solitary	Maharastra, India
CHFRG 6	Green	Pale yellow	Intermediate	Solitary	Uttar Pradesh, India
CHFRG 7	Green	Pale yellow	Intermediate	Solitary	Arunachal Pradesh, India
CHFRG 8	Light green	White	Intermediate	Solitary	Uttar Pradesh, India
CHFRG 9	Green	Pale yellow	Intermediate	Solitary	Uttar Pradesh, India
CHFRG 10	Light green	White	Superficial	Solitary	Uttar Pradesh, India
CHFRG 11	Green	White	Intermediate	Solitary	Uttarakhand, India
CHFRG 12	Dark green	Pale yellow	Intermediate	Solitary	New Delhi, India
CHFRG 13	Dark green	Pale yellow	Intermediate	Solitary	Uttar Pradesh, India
CHFRG 14	Green	Pale yellow	Intermediate	Solitary	Uttar Pradesh, India
CHFRG 15	Dark green	Pale yellow	Intermediate	Solitary	Uttar Pradesh, India
CHFRG 16	Green	Pale yellow	Prominent	Solitary	Uttar Pradesh, India
CHFRG 17	Dark green	White	Intermediate	Solitary	Uttar Pradesh, India
CHFRG 18	Green	White	Intermediate	Solitary	Karnataka, India
CHFRG 19	Green	White	Superficial	Solitary	Uttar Pradesh, India
CHFRG 21	Light green	White	Superficial	Solitary	Uttar Pradesh, India
CHFRG 22	Green	White	Intermediate	Solitary	Uttar Pradesh,India
CHFRG 23	Green	White	Intermediate	Solitary	Uttar Pradesh, India
CHFRG 24	Light green	Pale yellow	Prominent	Solitary	Uttar Pradesh, India
CHFRG 25	Green	Pale yellow	Intermediate	Solitary	Arunachal Pradesh, India
CHFRG 26	Light green	Pale yellow	Intermediate	Solitary	Rajasthan, India
CHFRG 27	Green	Pale yellow	Intermediate	Solitary	Arunachal Pradesh, India
CHFRG 28	Green	White	Intermediate	Solitary	Arunachal Pradesh, India
CHFRG 29	Green	Pale yellow	Intermediate	Solitary	Assam, India
CHFRG 30	Dark green	White	Intermediate	Solitary	Arunachal Pradesh, India
CHFRG 31	Green	White	Intermediate	Solitary	Uttar Pradesh, India
CHFRG 32	Green	White	Intermediate	Solitary	Uttar Pradesh, India
CHFRG 33	Green	White	Intermediate	Solitary	Uttar Pradesh, India
CHFRG 34	Dark green	Pale yellow	Prominent	Solitary	Uttar Pradesh, India
CHFRG 35	Green	White	Intermediate	Solitary	Assam, India
CHFRG 36	Light green	White	Intermediate	Solitary	Uttar Pradesh, India
CHFRG 37	Green	White	Intermediate	Solitary	Karnataka, India
CHFRG 38	Green	White	Prominent	Solitary	Manipur, India
CHFRG 39	Green	White	Intermediate	Solitary	Uttar Pradesh, India
CHFRG 40	Green	Pale yellow	Superficial	Solitary	Uttar Pradesh, India
CHFRG 43	Green	White	Intermediate	Solitary	Bihar, India
CHFRG 46	Green	White	Prominent	Solitary	Assam, India

of protein per gram of the sample was estimated by plotting a standard curve of absorbance at 660 nm versus 1µg of BSA.

Total seed protein extraction for SDS-PAGE

The seed storage protein profiling was done by SDS-PAGE as per the procedure by Laemmli (1970) with minor modifications. The seeds of fourty ridge gourd genotypes evaluated in the experimental field were collected. For each sample, 0.1 g seed was crushed using pestle and mortar and then mixed with 25µL of sample 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). Further, the sample was thoroughly homogenized, denatured in water bath at 100°C for 5 min and centrifuged at 10,000 rpm for 5 min. The supernatant collected was used as loading sample.

SDS-PAGE

The extracted soluble seed proteins were separated by SDS-PAGE in vertical gel slabs of 1mm thickness (5% stacking and 10% resolving gels) with a discontinuous buffer system. The samples were electrophoresed at 25 mA initially and then increased to 50 mA until the tracking dye reached the border line of stacking gel and separating gel. The gel was allowed for silver staining which involved treating with 0.02% sodium thiosulphate solution for 5 min and then washing twice thoroughly with double distilled water for 1 minute. Further, the gels were subjected to staining solution and shaken on gel rocker for 20 min in dark, thoroughly washed and then transferred to developing solution. The reaction was finally stopped with 12% acetic solution. Gel was washed intermittently but carefully with distilled water till the background blue colour of the gel vanished and the visibility of protein bands became clear for scoring. The electrophoretic profile was prepared based of protein mobility and band density expressed as Rm values.

Statistical data analysis of protein profiling

Scoring of the gels was done only for clear and reproducible bands as present (+) or absent (-). The similarity index [Nei and Li (1979)] between the genotypes was calculated based upon the presence or absence of bands by the following formula:

$$F = (2\ Z/X+Y) \times 100$$

where,

F = Similarity

Z = Number of similar bands between the genotypes and

X+Y = Total number of bands in the two genotypes.

Dissimilarity is usually defined as 1 minus similarity *i.e.* (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).

3. Results and Discussion

In the present investigation, seed protein profiling through SDS-PAGE in fourty genotypes of ridge gourd exhibited distinct pattern of polypeptide distribution (Fig. 1). The dendrogram based on UPGMA showed 93 bands in total as distinguished by silver staining and the Rm values varied from 0.01-0.97. A considerable variation ranging from 8-24 in number of storage protein band was observed. The highest number (24) of protein bands were seen in genotype CHFRG11 while CHFRG12 showed lowest number (8) of bands among the fourty genotypes. Band number 1 (R_m= 0.01) and band number 4 (R_m= 0.05) were present in genotypes CHFRG 46 only, similarly band number 79 (R_m= 0.83) and 92 (R_m= 0.96) was present only in genotype CHFRG7. Whereas, band number 10 (R_m=0.11), 41 (R_m=0.44), 78 (R_m=0.82), 80 (R_m=0.84), 93 (R_m= 0.97) were found only in CHFRG 33, CHFRG 6, CHFRG 23, CHFRG 32 and CHFRG 38 respectively. Band number 2 (R_m= 0.02) was observed in CHFRG 8 and CHFRG 9 exclusively. Similarly, band number 6 (R_m= 0.07) showed its presence only in CHFRG 32 and CHFRG 33, band number 7(R_m= 0.08) in CHFRG 32 and CHFRG 46, band number 11 (R_m= 0.12) in CHFRG 33 and CHFRG 46. The band number 14 was seen (R_m= 0.15) in CHFRG11 and CHFRG 24, band number 34 (R_m= 0.35) in CHFRG 5 and CHFRG 29, band number 40 (R_m= 0.42) in CHFRG 6 and CHFRG19. Further, genotypes CHFRG 8 and CHFRG 29 showed band number 46 (R_m= 0.49) and band number 58 (R_m= 0.61) were present in CHFRG 15 and CHFRG 25. The band number 73 (R_m= 0.76) showed its presence only in CHFRG15 and CHFRG 28, band number 82 (R_m= 0.86) in CHFRG 8 and CHFRG 24, 90 (R_m= 0.94) in CHFRG2 and CHFRG 8 and band number 91 (R_m= 0.95) was seen in CHFRG 26 and CHFRG 39 solely. Band number 25

Table 2 : Genetic distance estimates among 40 genotypes of ridge gourd SDS-PAGE analysis.

Genotype	1	2	3	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	43	46
CHFRG 1	0																																							
CHFRG 2	59	0																																						
CHFRG 3	72	87	0																																					
CHFRG5	67	88	67	0																																				
CHFRG 6	79	72	42	73	0																																			
CHFRG 7	89	62	85	86	65	0																																		
CHFRG 8	79	65	83	78	65	85	0																																	
CHFRG 9	92	68	94	94	68	73	55	0																																
CHFRG 10	94	70	84	85	68	73	79	65	0																															
CHFRG 11	78	73	71	77	52	71	66	58	41	0																														
CHFRG 12	69	91	84	86	92	81	100	100	100	100	0																													
CHFRG 13	73	92	58	87	95	89	86	84	94	89	58	0																												
CHFRG 14	91	100	79	100	100	89	93	84	88	89	58	37	0																											
CHFRG 15	84	93	87	88	94	84	94	92	82	84	81	84	84	0																										
CHFRG 16	100	100	87	100	100	83	94	77	88	89	80	74	57	84	0																									
CHFRG 17	100	100	86	94	100	70	100	84	94	95	67	72	53	92	46	0																								
CHFRG 18	82	92	72	94	100	89	93	92	81	89	69	73	64	84	66	53	0																							
CHFRG 19	92	100	94	75	94	95	87	71	82	90	72	84	75	77	52	66	50	0																						
CHFRG-21	100	92	87	88	100	83	100	93	88	95	80	74	74	84	25	64	74	68	0																					
CHFRG-22	91	92	79	94	100	89	86	84	81	83	100	82	73	84	48	72	55	84	66	0																				
CHFRG-23	81	73	60	68	78	91	78	75	85	72	93	73	80	94	80	100	94	84	80	80	0																			
CHFRG-24	68	70	63	59	68	77	73	89	85	78	93	94	94	100	100	94	100	100	100	100	58	0																		
CHFRG-25	70	83	75	71	70	78	80	78	77	66	87	100	100	89	89	88	88	83	100	94	60	39	0																	
CHFRG-26	56	59	70	60	82	80	76	87	73	67	84	93	100	87	100	100	93	100	100	93	59	50	58	0																
CHFRG-27	62	72	63	71	50	64	63	80	72	59	92	93	100	86	93	100	85	93	93	93	82	55	63	62	0															
CHFRG-28	100	94	88	66	76	85	70	74	78	70	92	100	100	94	93	93	93	73	79	78	59	67	58	75	68	0														
CHFRG-29	73	88	89	73	83	76	83	94	85	86	93	87	94	100	74	86	80	88	80	80	89	74	75	77	70	89	0													
CHFRG-30	94	82	73	74	67	72	62	58	70	63	86	80	80	94	88	94	87	88	88	74	57	54	66	72	71	49	90	0												
CHFRG-31	91	83	78	100	78	82	78	67	74	65	89	81	72	92	91	70	62	83	100	81	86	94	75	85	60	85	79	73	0											
CHFRG-32	83	78	70	86	75	79	80	85	68	66	81	77	77	84	83	76	77	95	89	77	61	68	74	65	58	75	81	62	64	0										
CHFRG-33	85	79	75	77	75	85	69	80	83	75	100	85	100	100	100	100	100	100	100	100	76	72	90	75	80	88	88	71	100	74	0									
CHFRG-34	86	94	82	83	76	80	94	87	73	65	100	93	100	73	86	93	93	80	93	93	83	62	64	88	62	82	83	89	84	64	94	0								
CHFRG-35	85	93	75	89	75	79	94	87	72	70	100	70	77	79	78	76	93	93	86	85	76	60	68	88	67	88	88	89	84	64	94	36	0							
CHFRG-36	87	94	73	90	78	77	95	88	75	73	93	67	74	82	68	66	80	82	75	87	79	65	66	89	71	89	90	85	87	67	95	38	12	0						
CHFRG-37	80	94	89	85	67	77	89	82	65	68	86	74	80	90	62	73	87	82	68	87	79	70	66	83	77	83	95	79	87	72	50	43	30	22	0					
CHFRG-38	72	87	77	78	77	85	83	88	63	61	68	93	79	87	66	78	86	80	80	79	79	63	70	70	69	88	89	78	86	65	88	64	63	56	50	0				
CHFRG-39	80	94	83	68	83	91	83	88	64	72	77	94	80	88	60	79	80	75	87	66	78	79	75	71	70	77	89	79	86	86	94	65	64	57	46	20	0			
CHFRG-40	80	81	89	73	78	81	83	69	64	72	85	94	94	94	74	79	87	81	87	87	84	64	60	83	76	71	84	73	79	81	94	81	82	73	68	66	62	0		
CHFRG-43	76	71	88	82	81	79	81	72	59	74	82	76	68	100	85	75	92	93	93	92	94	71	78	87	66	80	75	82	67	84	87	84	73	76	70	75	75	38	0	
CHFRG-46	86	94	77	73	77	100	77	88	95	71	100	86	93	87	94	100	100	94	94	93	66	73	80	88	88	76	89	78	93	90	57	88	40	50	50	50	50	89	100	0

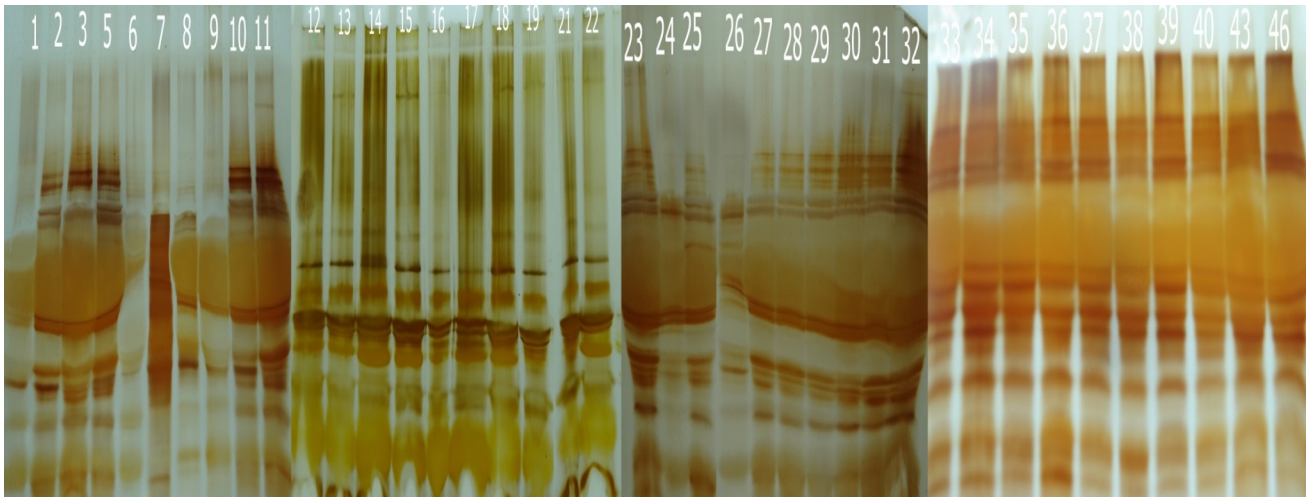

Fig. 1 : Seed protein profiles in fourty ridge gourd genotypes.

Table 3 : Major cluster produced by SDS-PAGE analysis.

Cluster	Sub cluster	Genotypes
I	IA	CHFRG 34, CHFRG 35, CHFRG 36, CHFRG37, CHFRG 38, CHFRG 39 and CHFRG 46
	IB	CHFRG 1, CHFRG 2, CHFRG 3, CHFRG 5, CHFRG 6, CHFRG 7, CHFRG 8, CHFRG 9, CHFRG 10, CHFRG11, CHFRG23, CHFRG24, CHFRG25, CHFRG26, CHFRG27, CHFRG28, CHFRG29, CHFRG30, CHFRG31, CHFRG32, CHFRG33, CHFRG40 and CHFRG43
II	IIA	CHFRG12, CHFRG13, CHFRG14, CHFRG16, CHFRG17, CHFRG18, CHFRG19, CHFRG21 and CHFRG22
	IIB	CHFRG-15

(R_m= 0.26) was observed in maximum (21) number of genotypes. Genotype CHFRG 17 with total 10 bands did not possess band 10 - 44. Similarly, CHFRG12 with minimum 8 bands showed absence of band number 10-36 and 66-93. Likewise, presence of band number from 1-28 was not observed in CHFRG 31, which possessed total of 10 bands and CHFRG 46 did not show any band after band number 43 with total of 17 bands. The tree cluster analysis grouped the forty genotypes into 2 major clusters which were further sub-divided into two sub clusters each (Fig. 2). The first sub cluster (IA) contained the genotypes CHFRG 34, CHFRG 35, CHFRG 36, CHFRG 37, CHFRG 38 and CHFRG 39 and CHFRG 46. Second sub cluster was found to contain 23 genotypes *viz*., CHFRG1, CHFRG 2, CHFRG 3, CHFRG 5-11, CHFRG 23-33, CHFRG 40 and CHFRG 43. The third sub cluster contains 9 genotypes viz, CHFRG12-14, CHFRG16-19, CHFRG 21 and CHFRG 22. Only one genotype *i.e.* CHFRG 15 was observed in fourth sub cluster. The seed protein analysis through SDS-PAGE exhibited substantial variation in the genotypes under study which advocates large possibility in search of outperforming genotypes for different traits and novel genes identification in ridge gourd landraces from different regions of India. Elucidation of genetic diversity in germplasm is the foundation that aids in efficient sampling and utilization of germplasm by identifying and/or eliminating duplications in the gene stock and helps in the establishment of core collection [Yatung *et al.* (2014)]. This facilitates in proper gene bank management and planning experiments for crop improvement. The genetic divergence in the germplasm is average diversity of all loci [Nei and Li (1979)]. Assessing the genetic relatedness and affinities among crop genotypes assist to acquire information on the specific traits possessed by the genotypes; developing improved cultivars etc. besides meeting diversified objectives of breeders [Dubey *et al.* (2016)]. Genotypes lacking information on characterization, evaluation and biochemical analysis limits their utilization. In the recent years, various electrophoretic technique based on storage protein patterns are routinely used for the identification and the characterization of crops. Researchers indicate that polymorphism of seed storage protein profiles find wide application in studies of taxonomic classification,

mutant screening, genetic variability analysis, cultivar identification etc. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is considered to be an economical, simple and extensively used biochemical technique for analysis of genetic structure of germplasm. The seed storage protein profiling based on SDS-PAGE is considered as reliable, cost effective means for germplasm characterization with the advantage of being independent of environmental influences too. In the foregoing study, the 40 ridge gourd genotypes characterised using SDS-PAGE exhibited considerable variation in number of polypeptide bands varying from 8 to 24. The genotype CHFRG11 exhibited highest number (24) of protein bands while CHFRG12 showed the lowest number (8) of bands. Cluster analysis using SDS-PAGE banding patterns generated dendrogram represented clear separation of 40 genotypes of ridge gourd (Fig. 2). The genetic distance estimates among 40 genotypes of ridge gourd using SDS-PAGE analysis exhibited a maximum degree of genetic distance exhibiting value of 100 between many combinations, which indicate their close affinity. Further, the genotype CHFRG35 with CHFRG36 exhibited minimum genetic distance as evident by its lowest value *i.e.* 12. Several studies on characterisation of germplasm as revealed by SDS-PAGE are report. It was also observed that some genotypes collected from different locations to be closely related while some genotypes from the same source had dissimilar genetic makeup. This indicates germplasm exchange/ intermixing between regions and the information generated in context to intra and inter regional diversity could be efficiently exploited for ridge gourd improvement programmes. The tree cluster analysis through SDS-PAGE generated more sub groupings and thus presented better depiction of variability in comparison to morphological data. Although, the study could reveal considerable variation among few genotypes of ridge gourd using seed protein polymorphism but needs to be extended to more number of genotypes in order to reach towards a concrete conclusion [Dubey *et al*. (2015)]. The present findings are in concordance with results of Singh *et al.* (2009) that SDS-PAGE exhibiting different banding pattern of polypeptides could be utilised for cultivar identification. Similar studies [Anu and Peter (2003)] also reported that proteins and enzymes are significant biochemical genetic markers for assessing variability

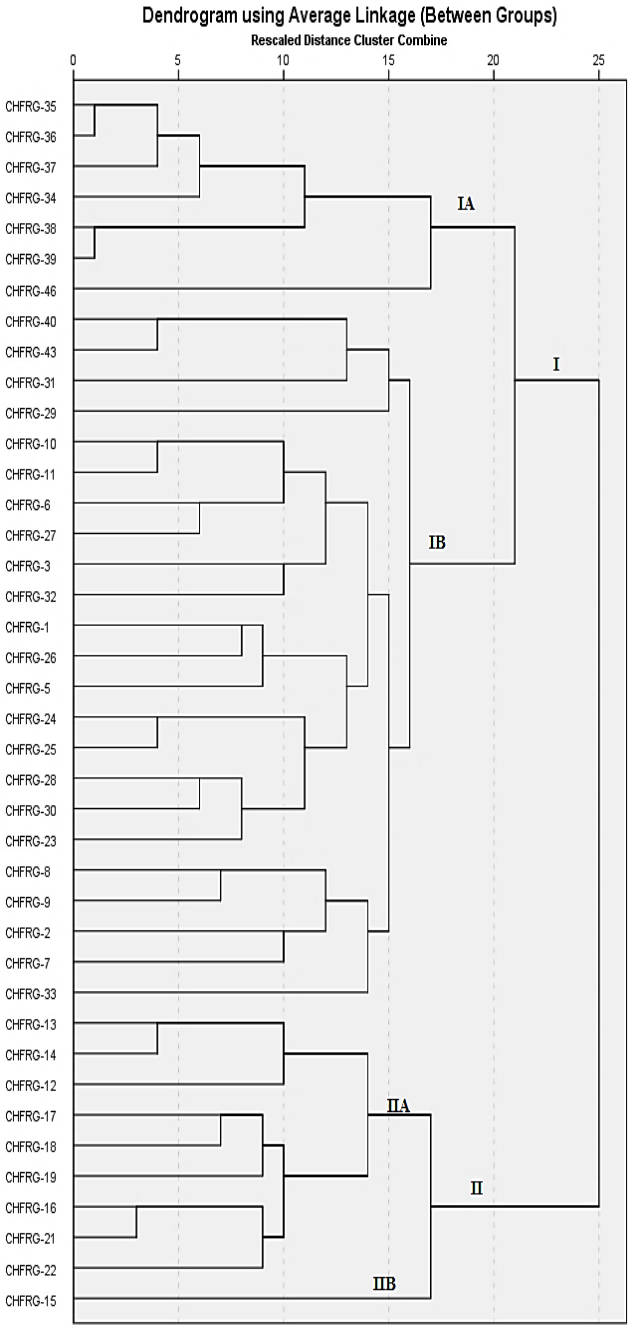

Fig. 2 : UPGMA of fourty ridge gourd genotypes based on total seed protein profiles obtained by SDS-PAGE.

and taxonomy in plant kingdom. Thus, it is quite apparent that storage protein profiling is a proven, stable technique to differentiate cultivars/genotypes, which appears to be morphologically similar or dissimilar. Further, extensive studies based on molecular markers are essential for valid taxonomic classification of ridge gourd and generating unambiguous phylo-genetic relationships. Reports substantiate that seed protein polymorphism was found to be an indicative character for establishing genetic relationship in eight taxa of chilli peppers obtained by disc electrophoresis [Panda *et al*.

(1986), Dubey and Ram (2008)]. Hence, data generated by SDS-PAGE based on seed protein would always be considered a predominant, supporting tool for evaluation of germplasm characterisation, species/ cultivar identification etc. compared to morphological markers.

4. Conclusion

The assessment of genetic diversity among forty ridge gourd genotypes revealed by SDS–PAGE analysis generated relatively good amount of polymorphism in protein banding pattern with more sub groupings and thus presented enhanced level of diversity as compared to morphological markers. The number of proteins bands varied from 8-24 in the genotypes under study through SDS-PAGE. The genotypes CHFRG35 and CHFRG36 exhibited minimum genetic distance (12.00) and were most distantly related. Further, it is also observed that some genotypes from different geographical locations were closely related and genotypes from the same source had dissimilar genetic makeup. The diverse genotypes identified could be exploited as choice material for breeders and to develop improved cultivars. Although, seed storage protein profile in ridge gourd genotypes revealed considerable variability but more extensive studies generating high resolution of discrimination would help researchers to establish a concrete picture of phylogenetic relationship. The genotypes necessitates to be subjected for characterization through advanced molecular techniques in order to outreach duplication in collections, identify superior genotypes for qualitative as well as qualitative characters, assist in tagging of gene for specific traits linked through genetic markers etc. This would significantly support efficient utilisation, management and conservation of genetic resources and prove a boon for researchers, farmers as well as mankind.

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