ResearchArticle

Seed Protein Profiling, An Efficient Method in Diversity Analysis of Dolichos Bean (*Lablab purpureus* L. Sweet.) from Northeast India

MM Shulee Ariina*, SD Warade, SP Kanaujia, Yabi Gadi, Athikho Alice Kayia and M. Chandrakumar Singh

Department of Vegetable Science, College of Horticulture and Forestry, CAU, Pasighat, Arunachal Pradesh, India -791102

Abstract

For the present study, 22 diverse genotypes of dolichos beans were evaluated for genetic diversity using the total seed proteins through SDS-PAGE and morphological characteristics. All the germplasm clearly revealed remarkable polymorphism from their protein banding patterns. On the basis of banding pattern, the 47 number of protein bands were observed in all the genotypes. Total protein band which ranged from 20 to 35 Relative mobility (Rm) values 40.74 to 95.83. The result showed that genotype CHF-10 showed maximum numbers (35) of protein bands while the minimum numbers (20) of bands were present in genotypes CHF-12, CHF-18, CHF-19 and CHF-20. Seed protein banding pattern through SDS-PAGE showed that the genotypes CHF-18 and CHF-3 were more distantly related to each other at genotypic level. Hence, these genotypes could be utilized for breeding programme.

Keywords:dolichos, genetic diversity, Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

***Correspondence**
Author: MM Shulee Ariina
Email:ariinalee2@gmail.com

Introduction

Dolichos bean is widely distributed in many tropical and subtropical countries where it has become naturalised [1]. Northeast India is blessed with rich biodiversity including Dolichos bean where it is grown as local cultivar or landraces. Landraces are variable plant populations adapted to local agro climatic conditions, which are locally named, selected and maintained by the traditional farmers to meet their social, economical, cultural and ecological needs [2].

The botanical name of Dolichos bean is *Lablab purpureus* L. (Sweet) with chromosome 2n=2x=22, 24. It is member of the family Leguminoseae, sub-family Faboideae, tribe phaseoleae and sub-tribe phaseolinae. Although the plant is a perennial herbaceous plant but often it is grown as an annual crop [3] with bushy, erect or climbing races. It plays an important role in protein nourishment as it contains 20-30% protein on dry matter basis [4]. Hence, it is some of the best sources of plant protein for millions of people in tropical and sub-tropical countries, particularly the poor and developing countries.

Among the biochemical techniques, Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is an economical, simple and extensively used biochemical technique for describing the seed protein diversity of crop germplasm [5].

Although this crop has originated in India and large number of indigenous strains is available, very little work has been done for genetic improvement of yield and quality. A great range of variation exists for the plant and pod characters among the accessions grown all over the country [6]. The efforts of improving the crop by utilizing indigenous and exotic germplasm have been useful in breaking the yield barriers resulting in compact plant type, reduced duration and photo-insensitive types. Hence, comprehensive germplasm collection and evaluation, identification of suitable genotypes and investigation of its value are essential followed by selection among diverse types and analysis of segregating generations. Keeping in view the above consideration, the present study was undertaken to evaluate the variability in 22 germplasm of North-eastern region of India, through morphometric and seed protein analysis to provide a scientific basis for future selection and crop improvement programme.

Materials and Method

Plant materials

For the present study, 22 diverse genotypes of dolichos bean [*Lablab purpureus* L. (Sweet)] were collected from different location of north eastern region, India. The list of genotypes along with their source of origin and morphological traits are given in (**Table 1**).The experimental materials for the present study were evaluated at

Vegetable Research Farm, College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh, India.

Table 1 Dolichos genotypes with their sources of collection and morphological characters

S. No.	Genotype	Source	GPS Coordinates	Growth Habit	Standard Petal Colour	Pod Colour	Pod Curvature
1	CHF-1	Mao, Manipur	25°31'N,94°9'E	Semi determinate	White	Green	Slightly curved
2	CHF-2	Phek, Nagaland	25°34'N,94°17'E	Semi determinate	Light yellow	Cream	Moderately curved
3	CHF-3	Senapati, Manipur	25°16'N,94°1'E	Semi determinate	Light yellow	Cream	Slightly curved
4	CHF-4	Namchi, Sikkim	27°10'N,88°22'E	Indeterminate	Pink	Green with purple suture	Straight
5	CHF-5	East Siang, Arunachal Pradesh	28°6'N,95°50'E	Semi determinate	White	Green	Slightly curved
6	CHF-6	East Siang, Arunachal Pradesh	28°6'N, 95°50'E	Semi determinate	Light yellow	Cream	Moderately curved
7	CHF-7	Makhan, Manipur	25°26'N, 94°6'E	Semi determinate	White	Green	Slightly curved
8	CHF-8	Collection from IIVR, Varanasi	25°28'N, 82°96'E	Indeterminate	White	Green	Straight
9	CHF-9	Collection from IIVR, Varanasi	25°28'N, 82°96'E	Indeterminate	Purple	Purple	Straight
10	CHF-10	West Khasi Hills, Meghalaya	25°33'N, 91°37'E	Indeterminate	White	Green	Slightly curved
11	CHF-11	West Khasi Hills, Meghalaya	25°33'N, 91°37'E	Semi determinate	White	Green	Straight
12	CHF-12	Champhai, Mizoram	23°27'N, 91°19'E	Semi determinate	White	Green	Slightly curved
13	CHF-13	Bishramganj, Tripura	23°36'N, 91°21'E	Determinate	White	Green	Moderately curved
14	CHF-14	Pasighat, Arunacahal Pradesh	28°6'N, 95°15'E	Indeterminate	White	Green	Slightly curved
15	CHF-15	Agartala, Tripura	23°50'N, 91°17'E	Semi determinate	Light yellow	Cream	Slightly curved
16	CHF-16	Chandel, Manipur	24°27'N, 94°1'E	Indeterminate	White	Green	Moderately curved
17	CHF-17	Tamenglong, Manipur	24°59'N, 93°30'E	Indeterminate	Pink	Green with purple suture	Slightly curved
18	CHF-18	Upper Siang, Arunachal Pradesh	28°32'N, 95°6'E	Indeterminate	White	Green	Slightly curved
19	CHF-19	Ukhrul, Manipur	25°3'N, 94°20'E	Indeterminate	White	Green	Slightly curved
20	CHF-20	Senapati, Manipur	25°16'N, 94°1'E	Semi determinate	White	Green	Moderately curved
21	CHF-21	Kokrajhar, Assam	26°24'N, 90°16'E	Determinate	White	Green	Straight
22	CHF-22	Keinou, Manipur	24°40'N, 93°47'E	Indeterminate	White	Green	Straight

Estimation of Protein

Estimation of protein was done as per Lowry's method [7]. Seeds of 22 germplasm lines were collected and subjected to gel electrophoresis. 1 g of seed sample were macerated in mortar and pestle with 5 mL 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) and transferred to centrifuge tubes. The materials were then centrifuged at 8000 rpm for 20 min. The supernatants were collected and the procedure was repeated 4-5 times. The supernatants were mixed and volume made up to 50 mL with phosphate buffer. 1 mL of 20% TCA was added to 1 mL of the extract and the mixture was kept for 30 min. The mixture was then centrifuged at 8000 rpm for 20 min. The resultant pellets were washed twice with acetone and again centrifuged. The supernatant was then discarded. The pellet was collected and dissolved in 5 mL of 0.1N NaOH till it had

dissolved. 1 mL of the aliquot was taken in which 5 mL of freshly prepared alkaline copper sulphate reagent were added and mixed properly. After 10 min, 0.5 mL of Folin's reagent was added and mixed instantaneously and allowed to develop colour for 30 min. Absorbance at 660 nm was recorded after setting the instrument with reagent blank which contained 1 mL of 0.1 N NaOH instead of the sample aliquot.

In another set of tubes, suitable aliquots of BSA solution (in the range of 0-100 µl) were taken and volume made up to 1 mL with 0.1 N NaOH and allowed to develop colour as described above. A standard curve of absorbance at 660 nm versus 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 gel electrophoresis

Polyacrylamide gel electrophoresis in presence of denaturing agent (SDS) was carried out as per procedure described by Laemmli (1970) [8] with some modifications. Acrylamide Solution: 29.2 g acrylamide and 0.8 g bisacrylamide were dissolved in water and the final volume was made up to 100 mL and stored at 4^0C. Separating Gel Buffer: 1.5M Tris-HCl of pH 8.8 was prepared and stored at 4^0C. Stacking Gel Buffer: 1M Tris-HCl of pH 6.8 was prepared and stored at 4^0C. Sodium Dodecyl Sulphate solution: 2% aqueous solution of SDS was prepared. Ammonium per sulphate solution: 10% aqueous solution of ammonium persulphate was prepared. Bromophenol blue solution: 0.1% aqueous solution of bromophenol blue was prepared. Electrophoresis buffer: 3.0 g of Tris base and 14.4 g of glycine was dissolved in water and the final volume was made up to 1liter. The final pH was adjusted to 8.3 with glycine solution. The gel was then subjected to silver staining and the protein bands on the gels were visualized by silver staining [9].

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 vertical electrophoresis unit. Cathode and anode terminals were connected to the electrophoretic power supply and the SDS-PAGE was started by applying a voltage of 80V which was increased to 100V when the dye font has moved in to the separating gel. The gel was run until the bromophenol blue reaches the bottom of separating gel. The power supply was switched off when the tracking dye approach the bottom of the gel. The system was disconnected and the gel was taken out from the slab. The gel was then immersed in silver staining solution and kept on a gel rocker for 4 hours.

Statistical data analysis for protein profiling

The gels were scored as presence (+) or absence (-) of protein polypeptide bands. Depending upon the presence or absence of polypeptide bands, similarity index (SI) [10] between the genotypes was calculated by the following formula:

$$SI= \left(\frac{2Z}{X+Y}\right) X\ 100$$

Where, Z= Number of similar bands between the genotypes andX+Y =Total number of bands in the two genotypes compared [11].

Dendrogram was generated using an unweighted pair group method with arithmetic mean analysis (UPGMA) by using statistical software SPSS for windows package (Version 16).

Result and Discussion

The morphological variations under this study are summarized in (Table 1). The electrophoretic seed protein profiles of the same have been outlined in the form of electrophorograms based on UPGMA resulting in distinct clusters (**Figure 1**) and were studied and summarized in (**Table 2**). Cluster analysis of banding pattern of 22 genotypes based on similarity and UPGMA resulted in distinct clusters (**Figure 2**). A total of 47 protein bands as per Rm values were identified by silver staining. The genotypes showed considerable variation in protein band number ranging from 20-35. Among the genotypes CHF-10(West Khasi Hills, Meghalaya) showed maximum numbers (35) of protein bands while the minimum numbers (20) of bands were present in genotypes CHF-12(Champhai, Mizoram), CHF-18(Upper Siang, Arunachal Pradesh), CHF-19(Ukhrul, Manipur) and CHF-20(Senapati, Manipur). Band number 4 (Rm=0.241) was only present in genotype CHF-10. Similarly, band number 18 (Rm=0.489) and band number (Rm=0.729) was found to be present only in genotype CHF-1(Mao, Manipur).

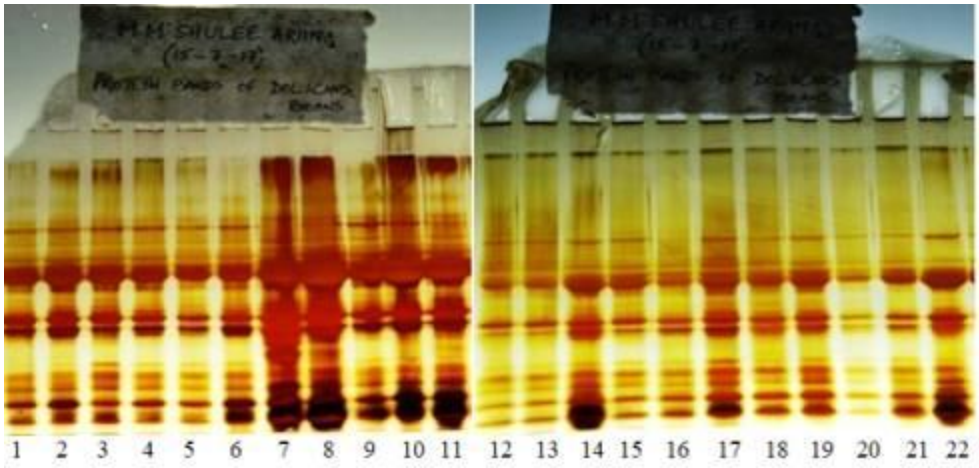


Figure 1 Seed protein banding pattern of 22 genotypes of Dolichos bean

Table 2 Major cluster produced by SDS-PAGE analysis in 22 genotypes of Dolichos

Cluster	Number of genotypes	Genotypes
Cluster I	13	CHF-13, CHF-14, CHF-16, CHF-5, CHF-7, CHF-12, CHF-19, CHF-1,CHF-16, CHF-17, CHF-4, CHF-18,CHF-15
Cluster II	5	CHF-11, CHF-21, CHF-3, CHF-2,CHF-20
Cluster III	1	CHF-22
Cluster IV	1	CHF-10
Cluster V	2	CHF-8, CHF-9

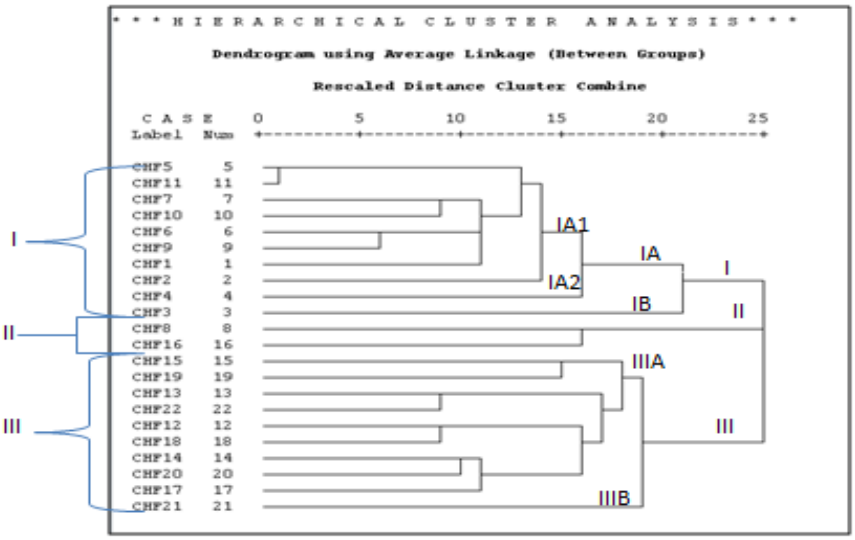


Figure 2 UPGMA of 22 Dolichos genotypes based on total seed protein profiles obtained by SDS-PAGE

Band number 2 (Rm=0.211), band number 3 (Rm=0.226), band number 8 (Rm=0.316), band number 22 (Rm=0.571) and band number 30 (Rm=0.692) were found to be present in both CHF-10 and CHF-11 (both from West Khasi Hills, Meghalaya) only, whereas it was absent in all other genotypes.Band number 10 (Rm=0.338), band number 11 (Rm=0.353), band number 12 (Rm=0.375), band number 21 (Rm=0.564) and band number 25 (Rm=0.617) was found to be present in all the genotypes.

Clustering Analysis

Based on the dendrogram, all the genotypes could be grouped into 3 major clusters. Cluster I was further sub-divided into 2 sub clusters, sub cluster IA was again sub-divided into 2 sub-sub clusters. Cluster I was found to contain 10 genotypes while cluster II was found to contain 2 genotypes (CHF-8 and CHF-16) and cluster III contain 10 genotypes again. The first sub-sub cluster (IA1) was found to be incorporating 8 genotypes *viz.*, CHF-5, CHF-11, CHF-7, CHF10, CHF-6, CHF-9, CHF-1, CHF-2. Second sub-sub cluster (IA2) was found to be incorporating 1 genotype CHF-4. Third sub-sub cluster (IIIA1) was found to be incorporating 2 genotypes *viz.*,CHF-15 and CHF-19.

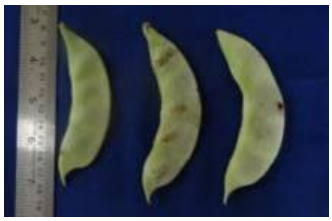
Fourth sub-sub cluster (IIIA2) was found to contain 7 genotypes *viz.*, CHF-13, CHF-22, CHF-12, CHF-18, CHF-14, CHF-20 and CHF-17.The similarity index coefficient matrix utilizing SDS-PAGE analysis for the 22 genotypes of Dolichos bean under study has been presented in **Table 3**. Percentage similarity index generated by SDS-PAGE analysis in the germplasm under study ranged from 40.74 to 95.83. Values of percentage similarity index coefficient matrix suggested least genetic distance of genotypes was observed between CHF-18(Upper Siang, Arunachal Pradesh) with CHF-3(Senapati, Manipur) at 40.74% while maximum similarity index was observed between the genotypes CHF-13(Bishramganj, Tripura) with CHF-12(Champhai, Mizoram) at 95.83%.

Table 3 Percentage similarity index estimates among 22 genotypes of Dolichos bean using SDS-PAGE analysis

Genotypes	CHF-1	CHF-2	CHF-3	CHF-4	CHF-5	CHF-6	CHF-7	CHF-8	CHF-9	CHF-10	CHF-11	CHF-12	CHF-13	CHF-14	CHF-15	CHF-16	CHF-17	CHF-18	CHF-19	CHF-20	CHF-21	CHF-22
CHF-1	0.0																					
CHF-2	82.53	0.0																				
CHF-3	83.87	92.06	0.0																			
CHF-4	86.66	85.24	93.33	0.0																		
CHF-5	81.35	86.66	91.52	91.22	0.0																	
CHF-6	75.4	74.19	81.24	84.74	82.75	0.0																
CHF-7	75	80.64	78.12	77.41	78.68	75.75	0.0															
CHF-8	82.53	84.37	82.53	81.96	76.66	77.41	89.23	0.0														
CHF-9	74.57	76.66	71.18	80.7	71.42	68.96	75.4	83.33	0.0													
CHF-10	74.62	79.41	71.64	73.84	75	69.69	72.46	79.41	81.25	0.0												
CHF-11	66.66	71.42	72.46	62.68	57.57	67.64	67.6	77.14	75.75	86.48	0.0											
CHF-12	65.45	53.57	61.81	56.6	57.69	66.66	42.1	78.57	53.84	60	54.83	0.0										
CHF-13	69.09	57.14	50.9	60.37	61.53	66.66	59.64	71.42	57.69	63.33	54.83	95.83	0.0									
CHF-14	71.42	56.14	50	55.55	60.37	65.45	62.06	70.17	56.6	62.29	53.96	83.33	89.79	0.0								
CHF-15	71.42	55.17	52.63	58.18	59.25	67.85	64.4	72.41	62.96	61.29	59.37	88	92	86.27	0.0							
CHF-16	71.69	59.25	56.6	62.74	64	73.07	69.09	70.37	64	65.51	63.33	82.6	91.3	85.1	91.66	0.0						
CHF-17	71.42	56.14	50	59.25	64.15	72.58	58.62	80.7	64.4	62.29	57.14	81.96	85.71	80	90.19	93.61	0.0					
CHF-18	69.23	52.83	40.74	56	44.06	70.58	62.96	83.01	69.38	63.15	54.23	71.11	80	78.26	80.85	83.72	86.95	0.0				
CHF-19	66.66	58.18	44.44	53.84	58.82	60.37	50	83.63	66.66	64.4	55.73	76.59	80.85	79.16	81.63	85.44	83.33	86.36	0.0			
CHF-20	66.66	53.84	47.05	57.14	58.33	68	49.05	84.61	70.83	67.85	55.17	77.27	81.25	75.55	82.69	81.81	78.02	87.26	93.02	0.0		
CHF-21	69.23	56.6	46.15	56	65.3	43.13	51.85	75.61	61.66	66.66	57.14	75.55	80	78.26	85.1	93.61	86.95	85.71	90.9	87.8	0.0	
CHF-22	72.72	53.57	43.63	52.83	61.53	44.12	49.12	71.42	57.69	60	54.83	70.83	70.83	73.46	80	86.95	81.63	80	89.36	86.36	93.33	0.0

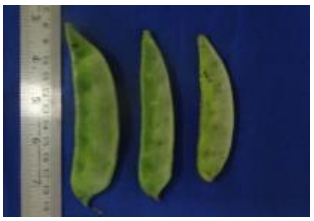
Seed protein variants have been observed to be the most widely used biochemical genetic markers during the last quarter century. Its success depends upon the polymorphism of seed and seedling proteins and the fact that these proteins represent primary gene products and are largely unaffected by the environmental interactions [12]. The Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was carried out to determine the protein banding patterns of 22 Indian bean genotypes. The genotypes showed considerable variation in band number of protein in present investigation which ranged from 20-35. Among the genotypes CHF-10 showed maximum numbers (35) of protein bands while the minimum numbers (20) of bands were present in genotype CHF-12, CHF-18, CHF-19 and CHF-20.


CHF-1


CHF-2

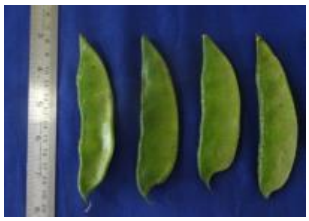

CHF-3

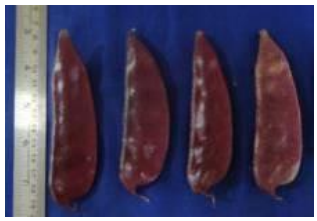

CHF-4


CHF-5


CHF-6


CHF-7

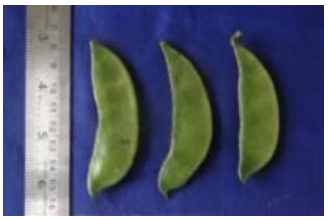

CHF-8


CHF-9


CHF-10

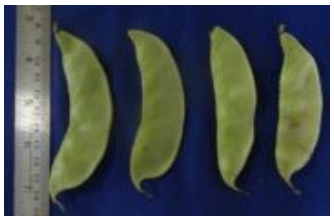

CHF-11


CHF-12


CHF-13

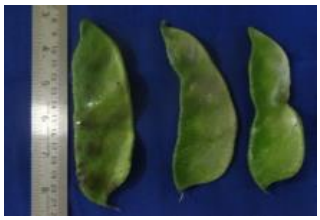

CHF-14


CHF-15


CHF-16


CHF-17


CHF-18


CHF-19


CHF-20


CHF-21

CHF-22

Figure 3 Glimpses of Dolichos bean genotypes

Cluster analysis utilizing SDS-PAGE banding patterns produced a dendrogram depicting clear separation of genotypes. The genotype CHF-22 and CHF-10 formed an individual cluster in dendrogram generated by Tocher's method analysis but the dendrogram based on SDS-PAGE banding pattern grouped genotype CHF-22 with CHF-13, CHF-12, CHF-18, CHF-14, CHF-20 and CHF-17 in fourth sub-sub cluster (IIIA2), while genotype CHF-10 with CHF-5, CHF-11, CHF-7, CHF-6, CHF-9, CHF-1 and CHF-2 in first sub-sub cluster (IA1). This shows that CHF-22 is more closely related to CHF-13, CHF-12, CHF-18, CHF-14, CHF-20 and CHF-17. Also, CHF-10 is more closely related to CHF-5, CHF-11, CHF-7, CHF-6, CHF-9, CHF-1 and CHF-2 as depicted in dendrogram generated by SDS-PAGE banding pattern.

The similarity index among 22 genotypes of dolichos bean using SDS-PAGE analysis showed a maximum degree of similarity index percentage(Table 3) exhibiting a value of 95.83 between genotypes CHF-13 with CHF-12. Further, the genotype CHF-18 with CHF-3 showed minimum percentage of similarity index between each other as evident by lowest value of similarity index i.e. 40.74. Genetic diversity analysis, based on seed protein profile using SDS-PAGE was also studied and similar findings were reported by Chakraborty (2017) [13], Mahamune *et al.* (2017) [14],Singh *et al.* (2017) [15] in pea andSarma *et al*. (2010) [16] in Indian bean (Dolichos Lablab).

Conclusion

Seed protein banding pattern through SDS-PAGE showed that the genotypes CHF-18 (Upper Siang, Arunachal Pradesh) and CHF-3 (Senapati, Manipur) were more distantly related to each other, hence, it can be recommended that these genotypes could be utilized for crossing programme to create more genetic diversity or segregants of desired characteristics through dolichos breeding programmes.

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