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SEED PROTEIN PROFILING OF COWPEA FOR GENETIC DIVERGENCE USING SDS-PAGE

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Abstract

The genetic variation of seed storage protein was assayed by SDS-PAGE for 20 cowpea accessions. On the basis of SDS-PAGE, 36 reproducible bands were used for analysis and genetic diversity was estimated based on the number of different protein peptides. Total seed proteins were separated through electrophoresis polyacrylamide gels using standard protocols. 36 (100%) of the protein bands detected were polymorphic. Similarity coefficients of among the accessions ranged from 0.25 to 1. Maximum similarity coefficient value occurred between IC-51154 *vs* EC-97306, IC-51154 *vs* EC-472272, EC-97306 *vs* EC-472272 and EC-911718 *vs* EC-472272 were 100% and the minimum similarity coefficient value occurred between IC-15665 *vs* IC-402106 was 25%. A dendrogram was constructed using similarity matrix data obtained from among accessions revealing relation on seed storage protein basis using Jaccard similarity coefficient of NTSYS-pc software. Dendrogram classified the 20 cowpea accessions into 6 different clusters *i.e.* cluster A, B, C, D, E and F comprising of 2, 5, 5, 2, 4 and 2 cowpea accessions, respectively. Based on clustering, accessions IC-15665 and IC-402106 had the highest genetic distance and can be applied as parents in hybridization in plant breeding to get highest hybrid vigor. It is concluded that seed storage protein profiling could be helpful markers in the studies of genetic diversity. This study will be helpful for the future breeding program of cowpea accessions.

Key words: Cowpea, genetic diversity, cluster analysis, SDS-PAGE, seed storage protein.

Introduction

Cowpea is an important leguminous vegetable crop, grown both in kharif and spring summer season crop in most parts of India. It is not only an important pulse crop but also used as an excellent fodder, green manure and soil improving cover crop because of its high protein content (23-29%). It has multifarious uses like as fodder, cover crop and green manure and provides high quality protein in the form of vegetable and pulse to human nutrition. It is early, multi seasonal and multipurpose crop in the tropical and subtropical countries (Singh *et al.*, 1997). Cowpea is consumed in many forms: the young leaves, green pods, and green seeds are used as vegetables; dry seeds are used in various food preparations; and the haulms are fed to livestock as nutritious supplement to cereal fodder.

Assessments of genetic variability based on biochemical markers (such as protein) of different crops have been studied by different researchers. Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) is a quick and accurate proteins based polymorphism method among different plant species, as it is free from any environmental effect (Dhawale *et al.*, 2015; Das and Mukherjee, 1995). SDS-PAGE is used as a promising tool for distinguishing cultivars of particular crop species due to its validity and simplicity for describing genetic structure of crop germplasm (Nisar *et al.*, 2011), but its implication has been limited mainly to cereals due to less polymorphism in most of the legumes (Ghafoor *et al*., 2002). Seed storage proteins have been used as genetic markers obtained by electrophoresis to resolve the taxonomic and evolutionary problems of several crop plants (Ladizinsky & Hymowitz 1979; Das and Mukarjee 1995). The SDS-PAGE is a practical and reliable method for species identification because seed storage proteins are largely independent of environmental fluctuation (Gepts, 1989). During the present investigation 20 accessions of cowpea were evaluated to fingerprint the diversity and differentiation in total seed storage proteins.

The objectives of present investigation was to find out the genetic diversity in cowpea accessions by using the total seed storage proteins through SDS- PAGE profiling.

Material and Methods

Plant material: Twenty cowpea accessions were obtained from Scientific & Applied Research Centre (SARC), Meerut (table-1). The genetic material was grown in pot for germination and growth at Scientific and Applied Research Centre, Meerut with standard agronomical practices. The seeds were collected, dried and rinsed with distill water to remove the dust particles and any other impurities and then dried again for the experiment.

Protein extraction: 4-5 seeds of each cowpea accessions under investigation were grounded to fine powder using mortar and pestle. 15 mg powdered sample was taken for extraction of protein, mixed with 500µl of extraction buffer (0.05M Tris HCL buffer, pH 8.0, containing 0.2% SDS, 5M Urea and 1% β-Mercaptoethanol). The contents were mixed thoroughly using vortex mixer and the samples were centrifuged for 5 minutes at 5000 rpm, the supernatant of the sample was stored in deep freezer until further use. 20µ1 of the supernatant of the sample was mixed with 5 µ1 of Bromophenol Blue dye (0.05% w/v) and placed on PAGE at 100 volts for 30 minutes and 150 volts for further 2 hours and were than stained and de- stained for observation.

SDS-PAGE profiling: Proteins profiling of samples was performed using SDS-polyacrylamide gels as described by Laemmli (1970). The electrophoretic procedure was carried out using slab type SDS-PAGE with 12.5% polyacrylamide gel. A 12.5% resolving gel (3.0M Tris-HCL, pH 9, 0.4% SDS and 4.5% stacking gel (0.4M Tris-HCL pH 7.0, 0.4 % SDS) was prepared and polymerized chemically by addition of 17 mL of N, N', N', N' tetramethylenediamine (Wako) and 10% Ammonium persulphate. Electrode buffer solution was poured into the bottom pool of the apparatus. Gel plates were placed in the apparatus carefully so as to prevent bubbles formation at the bottom of gel plates. Then electrode buffer (0.025M Tris, 1.29M Glycine, 0.125% SDS) was added to the top pool of the apparatus. Four ml of the extracted protein was loaded with the micropipette into each wells of the gel.

Electrophoresis: Electrophoresis was carried out at 100V for 3 hours till Bromophenol blue marker reached bottom of the gel. After electrophoresis the gels were stained in staining solution (methanol 44%, acetic acid 6%, commassie breliant blue 0.225%) for one hour and then transferred to destaining solution (methanol 20%, acetic acid 5%) and kept on shaker over night. The resulting gels were photographed to visualize the protein band patterns.

Data analysis: Gels were scored for the presence (1) and absence (0) of every protein band to prepare a binary data matrix. These binary data were used to analyze using NTSYS-pc (Numerical Taxonomy System, Version 2.2, Rohlf 2002). The SIMQUAL sub-programme was used to calculate the Jaccard's coefficient using following formula (Jaccard, 1908).

Jaccard's coefficient= $N_{AB}/ (N_{AB}+N_A+N_B)$

Where, N_A and N_B represents no. of bands in sample A and sample B, respectively. N_{AB} is the number of bands shared in the samples. Similarity matrices as computed by the programme were used to construct the UPGMA (un-weighted pair group method with arithmetic average) (Sneath and Sokal, 1973) dendrograms to elucidate the diversity among the accessions studied.

Results and Discussion

Total seed proteins were separated through

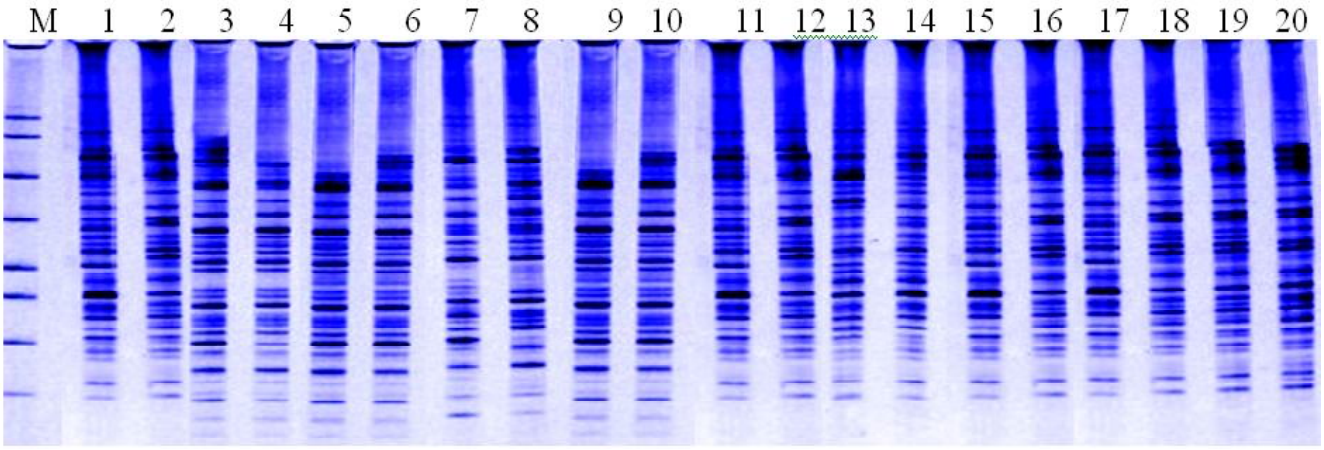

Fig. 1: Banding pattern of SDS-PAGE showing diversity among cowpea accessions

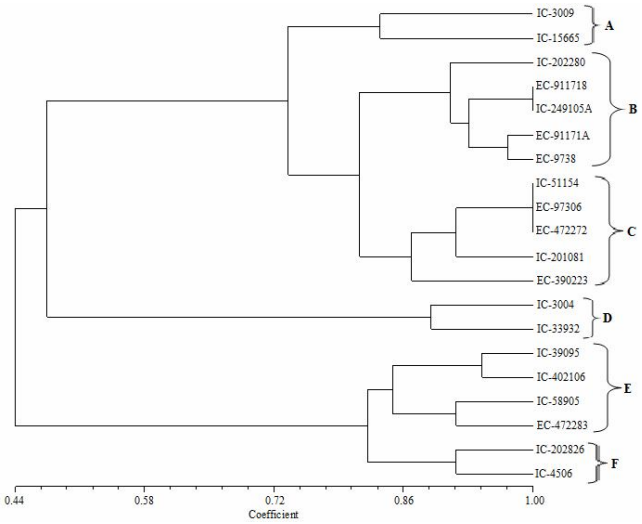

Fig. 2: UPGMA based cluster analysis of 20 cowpea using SDS-PAGE

electrophoresis polyacrylamide gels using standard protocols. On the basis of the relative mobility of seed proteins on the gel, 36 bands were detected in this study, which were used for examining the genetic diversity (Fig. 1). All 36 (100%) of the seed storage protein bands detected were polymorphic. On the basis of SDS-PAGE, 36 polymorphic bands were used for analysis and genetic diversity was estimated based on the number of different protein peptides.

Total seed storage protein extracted from 20 cowpea accessions were separated using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Results of SDS-PAGE profile are presented in fig. 1. A dendrogram was constructed using similarity matrix data obtained from 20 cowpea accessions revealing relation on seed storage protein pattern basis using Jaccard similarity coefficient of NTSYS-pc software. Genetic similarity coefficients of 20 cowpea accessions based on SDS-PAGE ranged from 0.25 to 1.00 (table-3). Maximum similarity coefficient value occurred between IC-51154 *vs* EC-97306, IC-51154 *vs* EC-472272, EC-97306 *vs* EC-472272 and EC-911718 *vs* EC-472272 were 100% and the minimum similarity coefficient value occurred between IC-15665 *vs* IC-402106 was 25%. A dendrogram was constructed by clustering of 20 cowpea accessions are shown in fig. 2. The resulting dendrogram classified the 20 cowpea accessions into 6 different clusters *i.e.* cluster A, B, C, D, E and F comprising of 2, 5, 5, 2, 4 and 2 cowpea accessions, respectively (table-2).

Cluster A consisted of only two cowpea accessions namely IC-3009 and IC-15665, which showed the similarity coefficient value of 0.83. Cluster B grouped into two sub-clusters *viz*; sub-cluster B1 and sub-cluster B2. Sub-cluster B1 comprised of 3 accessions namely IC-202280, EC-911718 and IC-249105A, in which the maximum similarity coefficient occurred between EC-911718 *vs* IC-249105A with a value of 1.0 and the minimum similarity coefficient occurred between IC-202280 *vs* EC-911178, IC-202280 *vs* IC-249105A with a value of 0.917. Sub-cluster B2 comprised of only two accessions namely EC-91171A and EC-9738, which showed the similarity coefficient value of 0.972. Cluster C grouped into two sub-clusters *viz*; sub-cluster C1 and sub-cluster C2. Sub-cluster C1 comprised of 3 accessions namely IC-51154, EC-97306 and EC-472272, in which the similarity coefficient occurred between IC-51154 *vs* EC-97306, IC-51154 *vs* EC-472272 and EC-97306 *vs* EC-472272 with a similar value of 1. Sub-cluster C2 comprised of only two accessions namely IC-201081 and EC-390223, which showed the similarity coefficient value of 0.889. Cluster D consisted of only two accessions of cowpea namely IC-3004 and IC-33932, which showed the similarity coefficient value of 0.889. Cluster E grouped into two sub-clusters *viz*; sub-cluster E1 and sub-cluster E2. Sub-cluster E1 consisted of only two accessions of

Table 1: List of Cowpea accessions

S.No.	Accessions	S.No.	Accessions
1	IC-3009	**11**	IC-51154
2	IC-15665	**12**	IC-202280
3	IC-39095	**13**	EC-390223
4	IC-402106	**14**	IC-201081
5	IC-58905	**15**	EC-97306
6	EC-472283	**16**	EC-911718
7	IC-3004	**17**	EC-472272
8	IC-33932	**18**	IC-249105A
9	IC-202826	**19**	EC-91171A
10	IC-4506	**20**	EC-9738

Table 2: Distribution of 20 cowpea accessions into 6 different clusters

S. No.	Cluster No.	No. of cowpea accessions	Accessions
1.	A	02	IC-3009, IC-15665
2.	B	05	IC-202280, EC-911718, IC-249105A, EC-91171A, EC-9738
3.	C	05	IC-51154, EC-97306, EC-472272, IC-201081, EC-390223
4.	D	02	IC-3004, IC-33932
5.	E	04	IC-39095, IC-402106, IC-58905, EC-472283
6.	F	02	IC-202826, IC-4506

Table 3: Genetic similarity coefficient of 20 cowpea accessions derived from SDS-PAGE

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1.000																			
2	0.833	1.000																		
3	0.361	0.306	1.000																	
4	0.306	0.250	0.944	1.000																
5	0.361	0.361	0.833	0.833	1.000															
6	0.389	0.333	0.861	0.861	0.917	1.000														
7	0.333	0.500	0.528	0.472	0.417	0.444	1.000													
8	0.444	0.611	0.472	0.417	0.361	0.389	0.889	1.000												
9	0.333	0.333	0.806	0.806	0.861	0.778	0.444	0.444	1.000											
10	0.361	0.306	0.833	0.833	0.778	0.861	0.472	0.472	0.917	1.000										
11	0.722	0.722	0.417	0.361	0.528	0.500	0.389	0.444	0.500	0.472	1.000									
12	0.750	0.861	0.333	0.278	0.444	0.417	0.472	0.528	0.417	0.389	0.861	1.000								
13	0.639	0.694	0.444	0.444	0.500	0.472	0.361	0.417	0.472	0.444	0.861	0.778	1.000							
14	0.694	0.750	0.444	0.389	0.500	0.472	0.417	0.528	0.528	0.500	0.917	0.833	0.889	1.000						
15	0.722	0.722	0.417	0.361	0.528	0.500	0.389	0.444	0.500	0.472	1.000	0.861	0.861	0.917	1.000					
16	0.722	0.778	0.417	0.361	0.472	0.500	0.500	0.556	0.444	0.472	0.833	0.917	0.750	0.806	0.833	1.000				
17	0.722	0.722	0.417	0.361	0.528	0.500	0.389	0.444	0.500	0.472	1.000	0.861	0.861	0.917	1.000	0.833	1.000			
18	0.722	0.778	0.417	0.361	0.472	0.500	0.500	0.556	0.444	0.472	0.833	0.917	0.750	0.806	0.833	1.000	0.833	1.000		
19	0.722	0.778	0.417	0.361	0.528	0.500	0.500	0.556	0.500	0.472	0.833	0.917	0.750	0.806	0.833	0.944	0.833	0.944	1.000	
20	0.694	0.750	0.389	0.389	0.556	0.528	0.472	0.528	0.528	0.500	0.806	0.889	0.722	0.778	0.806	0.917	0.806	0.917	0.972	1.000

cowpea namely IC-39095 and IC-402106, which showed the similarity coefficient value of 0.944. Sub-cluster E2 consisted of only two accessions of cowpea namely IC-58905 and EC-472283, which showed the similarity coefficient value of 0.917. Cluster F consisted of only two accessions of cowpea namely IC-202826 and IC-4506, which showed the similarity coefficient value of 0.917. SDS-PAGE has a potential to establish distinctiveness among the accessions. Similar attempts for establishing distinctiveness by SDS-PAGE was reported by various workers including Malik *et al*., 2009 in Soybean; Buckseth and Singh, 2016 in Pea; Hameed *et al.*, 2009 and Gupta *et al.*, 2016 in chickpea genotypes.

SDS PAGE has been proved as one of the most important parameters for studying total protein as well as storage proteins in crops. Storage proteins are not affected by environmental fluctuations, their profiling using SDS-PAGE technology is particularly considered as a reliable tool for economic characterization of germplasm but the consistency and accuracy of the protein based studies depend completely on the protein isolation (Javid *et al.*, 2004; Iqbal *et al.*, 2005). It is concluded that the accessions IC-15665 and IC-402106 were most diverse among all cowpea accessions and may be further utilized as potent accessions in cowpea breeding programme for the varietal development.

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