Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2768

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (2):** 491-496 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Genetic Diversity in Cowpea [*Vigna unguiculata* (L.) Walp.] Accessions using Protein Profiling

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Received: 29.03.2017 | Revised: 11.04.2017 | Accepted: 12.04.2017

ABSTRACT

An investigation was carried out on 52 accessions of cowpea to determine the variation in their storage seed protein subunit bands profile obtained through Sodium Dodecyl Sulphate - Polyacrylamide Gel Electrophoresis (SDS-PAGE). The proteins were extracted from cotyledons to represent both water and salt soluble proteins. Results revealed that protein subunit bands ranged between 97.4 kD to less than 18.4 kD molecular weight(MW). The bands of the accessions could be placed in five distinct regions. Those of region I and II were monomorphic and intensely stained and identified to range between 97.4 kD to 43.0 kD. The bands of region III and IV were relatively more polymorphic than those of region V. These bands were in the MW range of 43.0 kD to 10.0 kD. The binary data generated from the polymorphic bands over the accessions were used to compute Jaccard's similarity coefficients. The similarity matrix thus prepared was used to construct a dendrogram which, in turn, distributed the accessions in seven clusters. One of the accessions, was most diverse from the rest. Cluster I was comprised of three accessions with mottled seed coat color whereas in a sub cluster of cluster 7 two mutant derivatives of same parent were included. Cluster 2 showed a sub cluster of three accessions with very small seed weight and volume as well. Rest of the clusters did not reveal any characteristic similarity among their accessions.

Key words: *Cowpea, Genetic diversity, SDS-PAGE, seed storage proteins.*

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is a food legume crop and being protein rich (20-25%) plays an important nutritional role in human diet of developing countries of the tropics and subtropics. In the state of Rajasthan of India, which is largely represented by arid and semi-arid agro-climatic zones, cowpea is of great importance because of its short duration, dependable yield potential, quick growing habit and as it proves to be one of the climate resilient crops. It can be grown as cover crop to conserve soil and as a green manure crop for improving soil fertility besides its use as nutritious fodder to the livestock. Its multifarious uses poses for need of a wide spectrum of varietal development.

Cite this article: Sharma, D.B. and Krishna, K.R., Genetic Diversity in Cowpea [*Vigna unguiculata* (L.) Walp.] Accessions using Protein Profiling, *Int. J. Pure App. Biosci.* **5(2):** 491-496 (2017). doi: http://dx.doi.org/10.18782/2320-7051.2768

Therefore, documentation of usable diversity between accessions of the germplasm is a pre-requisite for specific crop improvement programme. In this direction, the characterization of diversity in a germplasm based on seed storage protein profiles reveled by SDS-PAGE can be a useful supplement to the diversity assessed using morphogenetic traits for selection of parents in a hybridization programme. Many plant proteins and their subunits exhibit extensive polymorphism in relation to size and charge and are encoded at several loci within the genome[3]. Therefore, the study of polymorphic proteins by electrophoresis is one of the most convenient methods for describing variability in plant genetic resources and for cultivar identification[5].

MATERIAL AND METHODS:

The seeds of a total of 52 accessions of cowpea (*Vigna unguiculata*) were obtained from Department of Plant Breeding & Genetics, S.K.N. college of Agriculture, Jobner.In this study these accessions were given numeric designations as 1-52. The seed coat and embryo were removed from dried seeds and cotyledons were grind to fine powder and flour so obtained was used for protein extraction immediately. Proteins were extracted by grinding either in 2ml of water, 2ml of 0.5M NaCl or in 1 ml of water followed by subsequent grinding in 1ml of 1M NaCl, respectively[11]. The extracted protein samples so obtained were used for Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis (SDS-PAGE). SDS–PAGE was carried out as described by Laemmeli[6]. Extracted protein samples (1ml) were transferred into Eppendorf tubes and centrifuged for 3 minutes at 10,000 rpm. One half milliliter (0.5ml) supernatant was transferred into a fresh Eppendorf tube and denatured with 0.5ml cracking buffer (0.2M Tris-Hcl buffer pH 6.8, 10% SDS, 20% glycerol, 10 mM β-mercaptoethanol, 0.05% bromophenol blue) at 80^0C in a water bath for 15 minutes. Bromophenol blue (BPB) added to the cracking buffer served as tracking dye to monitor the movements of protein bands in the gel. The protein samples (40µl) were loaded into the wells of the polyacrylamide gel slab prepared for electrophoresis. The molecular weights of the dissociated polypeptides were determined by using standard marker proteins obtained from Merk provided by Genei, Bangaluru (Range 18.4 to 97.4KD). Gels were scored for the presence (1) and absence (0) of every protein band. The binary data so obtained were analyzed using NTSYS-pc (Numerical Taxonomy System, Version 2.1, Rohlf2000). The SIMQUAL sub-program was used to calculate the Jaccard's coefficient using following formula[4]. Jaccard's coefficient= NAB/ (NAB+NA+NB). Statistical stability of the branches in the cluster was estimated by bootstrap analysis with 2000 replicates, using Winboot software program[14]. The molecular weight of the unknown protein subunits was extrapolated from plotting its Rf value and that of marker protein bands by developing a standard curve on a semi-log graph.

RESULTS AND DISCUSSION

Legume seeds, in general, predominantly contain albumins (20-35%) and globulins (43-55 %). Of these, albumins are water soluble and globulins are salt soluble and together account for 63-90% of the total seed proteins[10]. Therefore, in the present investigation only albumins and globulins were analyzed.

In the present investigation, the proteins from the cotyledons were isolated as described by Tripathy *et.al.*,[11]. Since albumins are water soluble and globulins are salt soluble, therefore, an experiment was first conducted to their simultaneous extraction. Lane 1 in Figure 1 was loaded with water extracted sample and lane 2 with 0.5 M NaCl extracted sample. In lane 3 the sample ground in 1.0 ml chilled distilled water followed by 1.0 ml of 1 M NaCl was loaded to represent both, water and salt soluble proteins.

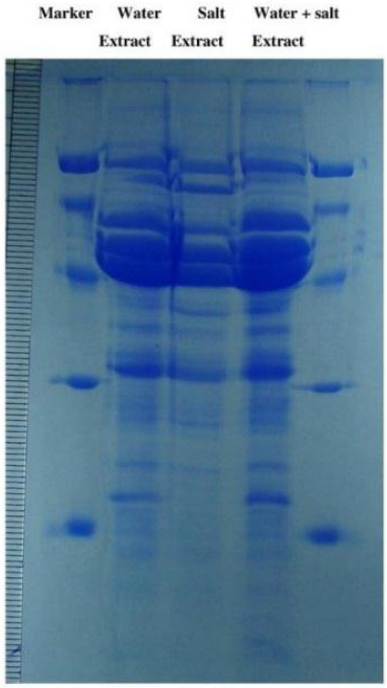

Fig.1.Electrophoregram demonstrating appearance of protein bands of seed storage protein of cowpea accession-5 extracted in water and / or salt (0.5 M Na Cl).

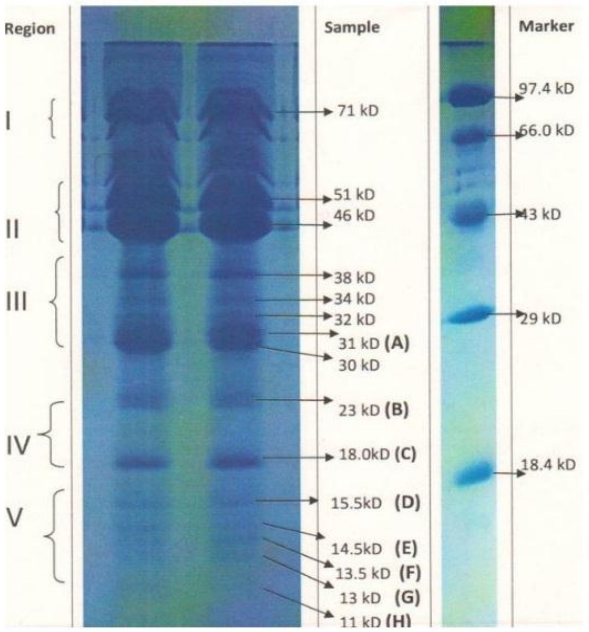

Fig.2. Identification of regions of protein band with their approximate molecular weight

A perusal of all three lanes in Figure 1 reveal that the protein bands of lane 3 represents those which were present in lane 1 or 2 and are relatively darker. Therefore, in all subsequent experiments proteins from were extracted to represent both the fractions, *i.e.* water and salt soluble ones. These results are consistent with those of Tripathy *et. al.*,[11].

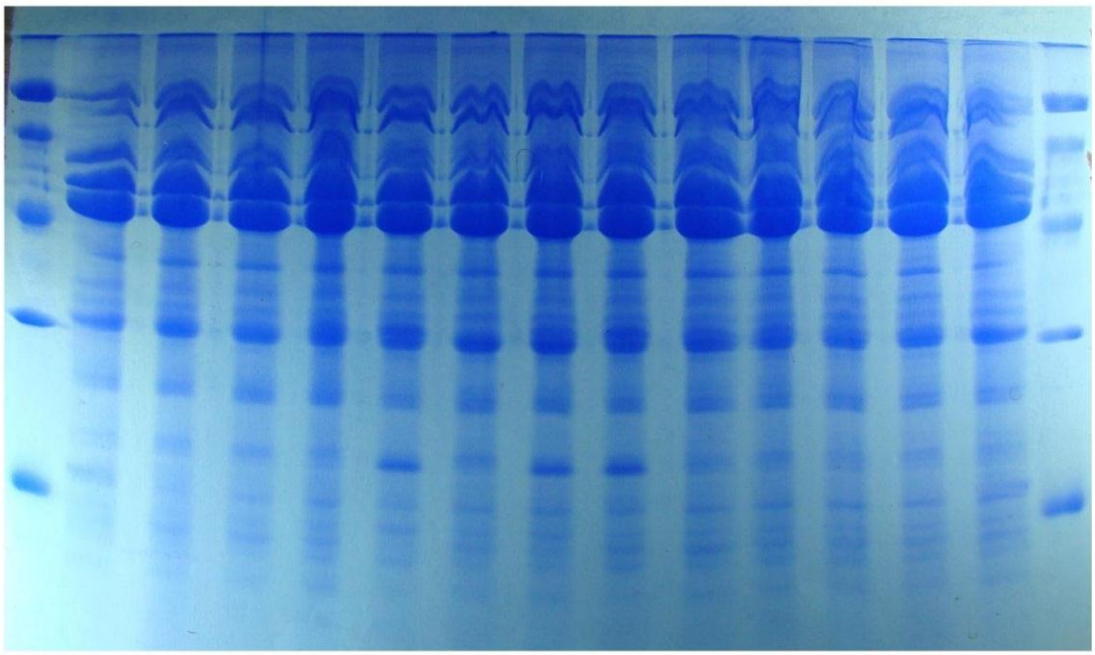

Fig 3. A view of gel revealing protein subunit bands of accessions 40-52. .First and last lanes represent marker proteins.

The electrophoregrams of 4 gels (13 x 4 = 52) each revealing banding pattern of 13 samples were prepared (a representative gel has been shown in Fig. 3). Reproducibility of protein bands of the cowpea accessions on the gel was assured by repeating the experiment twice. A typical banding pattern of cowpea sample has been shown in Figure 2. Each of the distinct bands was identified by comparing its MW with the marker protein lane. The marker was comprised of 5 distinct proteins of 97.4, 66.0, 43.0, 29.0 and 18.4 kD MW.

A perusal of proteins bands revealed that the bands could be identified to form 5 regions. The bands of higher MW, i.e. region I and II were invariably monomorphic. However the bands of region III and IV (designated A-D) were relatively more polymorphic than those of region 5(designated E-F). Thus a total of 8 bands were recognized for scoring purpose. The polymorphic potential of band A was found to be maximum (71.2 %) followed by band C (51.93%) and band D (34.62 %) and that for band H being least (9.62%). The above three polymorphic bands were between the MW range of 43- 18.4 kD (Table not shown).

A dendrogram was constructed using Jaccard's similarity coefficients obtained for protein band binary data observed on the 52 accessions of cowpea employing NTSYSpc programme (Fig 4). The cluster analysis on the accessions revealed 7 distinct clusters. The clustering was more apparent after performing the bootstrap analysis using Winboot programme at 2000 cycles. The salient finding of the clustering could be summarized. One of the accessions designated *Accession No.25* was entirely separated from all other accessions and scored values of lower order with all the genotypes (average similarity coefficient being 0.16 over rest of the accessions and hence, maximum diverse from the rest). The *Accession Nos.27and 28* were in same cluster with very high confidence limit and are, in fact, mutants of the same parent. The *Accession No.1, 6 and 7* had same seed coat color pattern, i.e. mottled pattern of black and white coat color, whereas, *Accession Nos.23, 22 and 15* shared characteristically low 100 seed weight and seed volume thus could be placed in one cluster.

The basic objective of present study was to explore possibility of using the SDS-PAGE approach to identify diverse genotypes of cowpea. On the basis of present results it may be concluded that using SDS-PAGE on cotyledonary proteins (albumins + globulins) in cowpea it is possible to observe a usable protein band polymorphism to study the diversity of the accessions and it is possible to cluster the genotypes with similar band pattern. Some of the genotypes may be identified on their specific banding pattern and information could help make decisions regarding the choice for selecting as parents for improvement of cowpea productivity through hybridization.

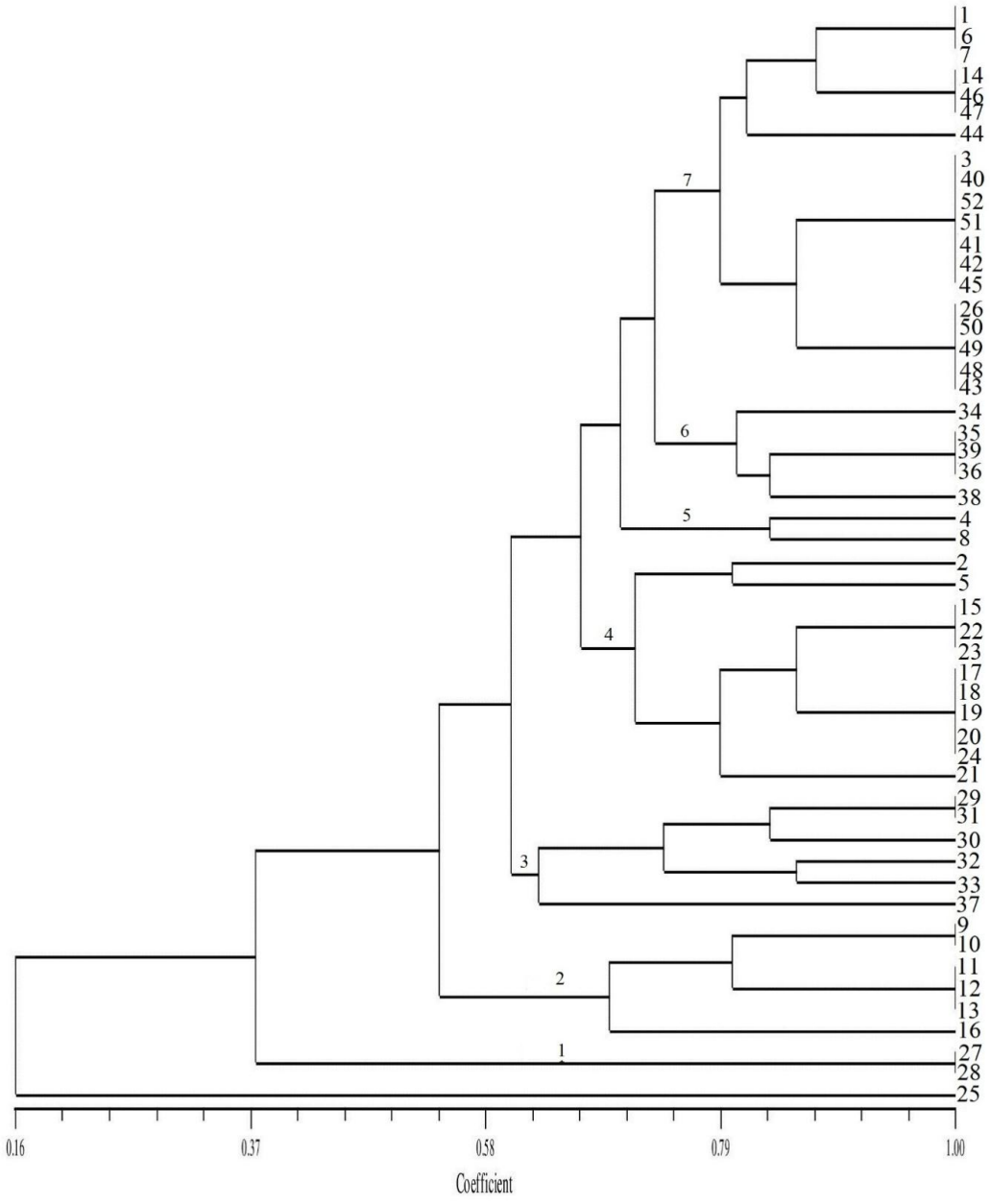

Fig.4. Dendrogram of the 52 cowpea accession revealed by UPGMA cluster analysis of SDS Page based genetic similarity estimates.

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