P: ISSN NO.: 2321-290X RNI : UPBIL/2013/55327 VOL-6* ISSUE-9* May- 2019
E: ISSN NO.: 2349-980X **Shrinkhla Ek Shodhparak Vaicharik Patrika**

SDS-PAGE Marker as Tool of Identification in *Canavalia gladiata* (Sword Bean)

Anil Kumar
Associate Professor,
Deptt. of Botany,
R.B.S. College,
Agra, U.P., India

Abstract

A Seed protein analysis using SDS-PAGE is particularly considered as a reliable technique because seed storage proteins are highly independent of environmental fluctuations. The high stability of seed protein profile and its additive nature makes it a promising tool for distinguishing genotypes of particular plant species. Therefore, in the present studies SDS-PAGE technique was employed for analysis of seed protein diversity in *Canavalia gladiata* with different accessions. SDS-PAGE technique has been successfully applied in many different plant species to estimate genetic diversity and phylogenetic relationship among genotypes. Protein profiling and UPGMA cluster analysis of *Canavalia gladiata* for five accessions was carried out.

Keywords: SDS-PAGE, Genetic Diversity, Protein Profiling and UPGMA Cluster Analysis.

Introduction

Canavalia bean, commonly known as the 'Sword bean' is a major tropical and subtropical legume crop along with *Dolichos lablab* bean and velvet bean and represented about 48 species contributing a major part to agriculture(Smartt, 1976) which are yet to be exploited fully as food source.

The plant height may vary from 4.0 to 10 m. The degree of twining, the size of the seed, pods, the number and the colour of the seed and pods show considerable variation. The seed pods are usually broad and curved with strongly developed ridges. They are about 15-30 cm long and 3.0- 5.0-5.5 cm broad, containing on average 8 to 16 seeds. Seeds are 2.5 -3.5 cm long, white or red in color with a dark brown hilum extending the entire length of the seed. Germination is epigeal (Purseglove, 1968). The seed has a tough thick coat which makes it unpopular among the other beans.

Canavalia bean is a multipurpose crop and almost all the parts of the plant are of great economic importance but pods and seeds are main source of food and forage. Seeds of sword bean are main source of proteins and also rich in carbohydrates, various vitamins and nutrients. In Sri Lanka the immature pods are made in to curry directly or sometimes after boiling the pods with water, which could be to remove the effects of L-DOPA. Other than its nutritive value Sword bean has role in agriculture as green manure and sometimes this is grown as a cover crop and play important role in environmental protection in agro ecosystems.In the present investigation SDS-PAGE technique was employed for analysis of seed protein diversity in selected genotypes of *Canavalia gladiata*. SDS-PAGE technique had successfully been applied in many different plant species to estimate genetic diversity and phylogenetic relationship among genotypes. Finally, protein profiling and UPGMA cluster analysis was carried outin selected accessions of *Canavalia gladiata*.

Aim of the Study

The objective of present investigation was to use SDS-PAGE technique estimate genetic diversity and phylogenetic relationship among *Canavalia gladiata* genotypes to identify the better genotypes for plant breeding purpose.

Review of Literature

Gept P. (1989) identified some seed storage proteins as molecular marker in various crop plants and emphasized on the identification obtained by the elecrophoretic analysis of seed protein as a tool for varietal identification. Bianchi-Hall *et al*. (1992) worked out the diversity of seed storage protein patterns in wild pea nut (Fabaceae). The

objectives of the study were to evaluate variability within *Arachis* sp. accessions and to classify taxa based on protein composition.. The study showed that great diversity exists for protein profiles and seed storage proteins have potential for aiding species classification and for serving as markers for interspecific hybridization studies.Da Silva *etal*. (2005) employed SDS-PAGE study in identification of lectin proteins of *Canavalia brasiliensis*. Proteins from callus culture established from cotyledons of mature seeds used and by this study some differences were found between sluble protein content and bands (intensity and number) for callus growth curve. Irfan Emre*et al*. (2006) studied role of seed proteins in taxonomy of some *Lathyrus* sp. grown in Turkey using SDS-PAGE analysis. Oko (2012) investigated the chemical nutrient composition and the phytochemical content of the leaves of *Mucuna poggei*, using standard methods. Pang *et al*. (2012) carried out SDS-PAGE analysis of germinated seed storage proteins of Horse Gram and found that it can be economically used to assess genetic variation and relation in germplasm and further stated that the specific bands of germinated seed storage protein profiles may be used as markers for identification of the mutants/genotypes. Ranjan *et al*. (2012) extracted the seed proteins offour different leguminous plants (*Pisum sativum, Vigna radiata, Cicer arientum* and *Vigna mungo).* They determined the yield of protein as well as the resolution of protein bands separated on Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). Rayane Natshe Gonçalves*et.al*. (2016) in *C. ensiformis* usedSDS-PAGE analysis to study diversity in proteases. These enzymes cleaved hemoglobin, bovine serum albumin, casein, and gelatin at different levels.

Material and Methods

The method suggested previously **(**Damania*et al*. 1983**)** was used as method for extraction of proteins. The protein concentration was determined in each sample by Bradford (1976) method with Bovine Serum Albumin as standard. The concentration of protein for each sample was determined by measuring absorbance at 595 nm in a UV-VIS spectrophotometer (SPECORD 40). The final concentration of protein in each sample was reduced to 20-25µg/ml by using following formula.

Y=0.0013 X

Where,

Y= Absorbance at 595 nm

X=Amount of protein present (µg/ml)

The Electrophoretic apparatus was set and run as per protocol (Chawla, 2003) .One volume of above extracted protein was mixed with one volume of 2x treatment bufferand mixed subsequently placed in boiling water bath for 90 seconds. Cool it at room temperature and store at 4ºC. 40µg of extracted protein was loaded in each well**.** 10 - 20 µl of 2x sample treatment buffer was added in each eppendorf tube consisting of extracted protein samples. The tubes were kept in a boiling water bath (100ºC) for 90 seconds. The samples were centrifuged at 8,000 - 10,000 rpm for 15 min at 4ºC. The 40µg of each extracted protein samples of different cultivars were loaded into separate wells of gel. Electric supply of 120 volts was regularized to Electrophoretic kit till the Bromo Phenol Blue dye reached the bottom. 10 µl protein molecular weight marker was loaded in one well along with the samples for determination of molecular weight of different seed proteins.

After the completion of electrophoresis, gel was carefully removed from the sand witched plates and washed with distilled water and placed in the staining solution (CBBS) for three to four hours. After that gel was placed in destaining (3% NaCl solution) solution untill the bands become visible against clear background (Bassam*et al*. 1991).

Results and Discussion

The total seed protein extracts of all three genera (*Dolichos lablab, Mucuna pruriens* and *Canavalia gladiata*) subjected to SDS-PAGE analysis and revealed significant variation in polypeptide banding pattern. Bands with same mobility were considered as identical fragments, regardless of their staining intensity. The total bands observed with apparent molecular weight range of 7KDa -100KDa could be distinguished.

A total of 25 polypeptide bands were recorded for each accession of *Canavalia gladiata*. The size of these polypeptide bands ranged from 17.0 to 67.0 kDa. Out of these polypeptide bands 20 were common among all five genotypes and 5 bands were polymorphic.For protein profiling and Unweighted Pair Group Method Analysis (UPGMA) of *Canavalia gladiata;* five accessions of each were carried out. On the basis of protein profiling and UPGMA cluster analysis in five accessions of *Canavalia gladiata*. were grouped into two clusters. Major cluster I consisted of single accession (IC-44599), sharing 48.0% similarity with cluster II (Fig. 1b). Cluster II was further divided into two, clade I (IC-44618B, IC-44590 and IC-44592) with 100.0% similarity and in clade II single accession (IC-45248) shared 76.5% similarity with clade I accessions. Accessions of *Canavalia gladiata* were less diverse in nature and protein profiling and UPGMA cluster analysis revealed that similarity between the members of cluster I was less (48%) and single accession (IC-45248) of cluster II showed maximum divergence among all five accessions. SDS-PAGE was also used for differentiating the proteins in raw and roasted seeds of *Canavalia cathartica* from southwest coast of India by Seena*et al*. (2005).Blagrove and Gillespie (1978) in winged bean, and Hameed*et al*.(2009) in Kabuli Chickpea genotypes reported significant polymorphism through SDS-PAGE analysis to reveal genetic diversity. Thus, SDS-PAGE analysis provides strong basis for the discrimination of genotypes on the basis of specific polypeptide fragments.

Conclusion

Protein profiling and UPGMA cluster analysis of *Canavalia gladiata* five accessions of each were carried out. Selected accessions of *Canavalia gladiata* were less diverse in nature and protein profiling and UPGMA cluster analysis revealed that similarity between the members of cluster I was less (48%) and single accession (IC-45248) of cluster II showed maximum divergence among all five

accessions..Therefore, SDS-PAGE analysis provided strong basis for the discrimination of genotypes on the basis of specific polypeptide fragments.

Fig. 1(a): Protein Fingerprinting of selected accessions of *Canavalia gladiata* : M- Molecular Marker (Range: 7.0-94 KDa) Lane 1: IC- 44599, Lane 2: IC-44618B, Lane 3: IC-44590, Lane 4: IC- 44592, Lane5: IC-45248

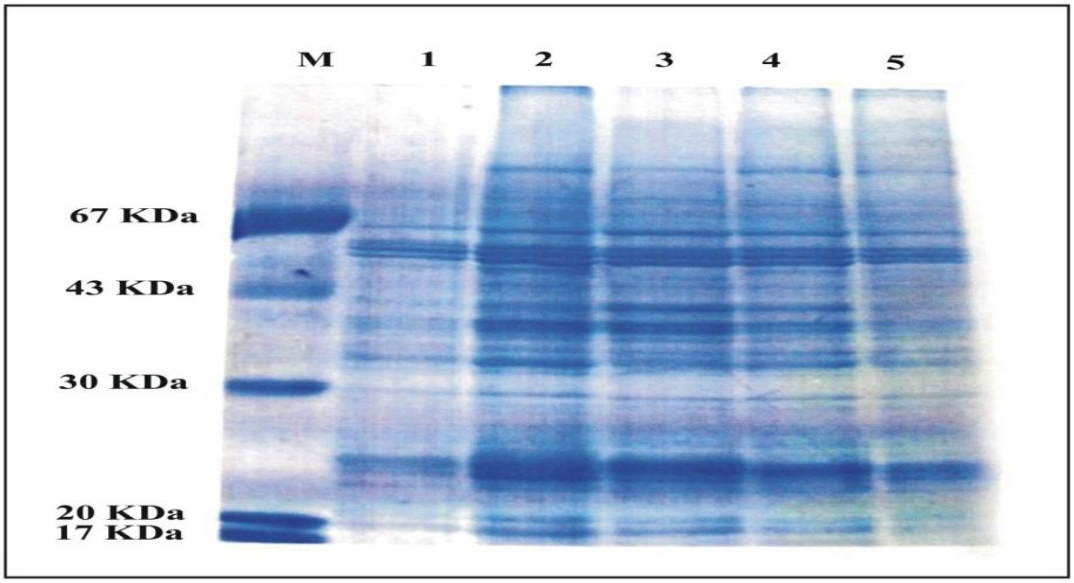
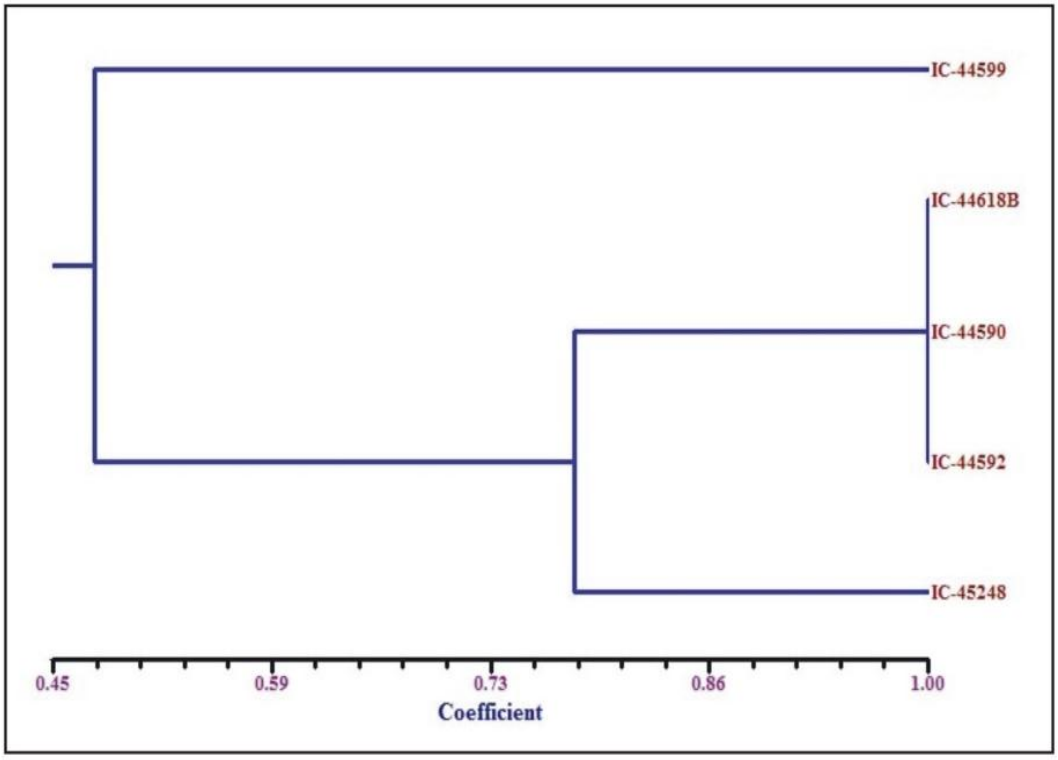


Fig.1(b): UPGMA dendrogram resulting from Protein Fingerprinting showing diversity among selected accessions of *Canavalia gladiata*



Refrences

Burkil I H. 1966. A dictionary of the economic products of Malaya Peninsula: Singapore: Government Printer.

Blagrove RJ and Gillespie JM. 1978. Variability of the seed globulins of winged bean, Psophocarpus tetragonolobus (L) DC. Aust J. Plant Physiol. 5: 371–375.

Bhagya B, Sridhar K R. and Seena S. 2006. Biochemical and protein quality evaluation of tender pods of wild legume Canavalia cathartica of coastal sand dunes. Livestock Research for Rural Development18: 1-20.

Begchi GD, Tripathy T. 1986. Scanning electron microscopic studies on some medicinally

important seeds of Papilionaceae, Geophytology.16 (1): 122-128.

Bradford MM. 1976.A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding.Anal Biochem.72: 248–254.

Duke JA. 1983. Lablab purpureus (L.) Sweet. In: Handbook of legumes of world economic importance. New York, USA; Plenum Press.pp 102-106.

Damania A B, Porceddu E and Jackson M T. 1983.A rapid method for the evaluation of variation in germplasm collection of cereals using Polyacrylamide gel electrophoresis. Euphytica.32: 887-883.

Da Silva Fabia M B, Moreira Renato A, Horta Ana C G, Silva Andre Luis C. 2005. The Lectin Content of Cotyledonary Callus from Canavalia brasiliensis (Mart. ex. Benth). Digital Repository Infrastructure Vision for European Research (DRIVER)

Gupta Veena and Kak Anjali 2007.Genetic diversity analysis in Mucuna pruriens germplasm using SDS-Poly-acryl Amide gel electrophoresis (PAGE). International Journal of Plant Sciences.2 (1): 124 – 127.

Gept P. 1989.Genetic diversity of seed storage proteins in plants. Plant Population Genetics, Breeding and Genetic Resources; 64-82.

Hameed A, Shah T M, Atta B M, Iqbal N, Haq M A and Ali H. 2009. Comparative seed storage protein profiling of kabuli chickpea genotypes, Pak. J. Bot.41 (2): 703.

Oko AO. 2012. Nutritional and phyto-chemical compositions of leaves of Mucuna poggei. Journal of Biology and Life Science.3 (1): 232 – 242.

Pang, Pek, Geok, Afandi Asrul, Shefiqur Rahman and Kumar Saha Ranajit 2012. Sodium Dodecyl SulphatePolyacrylAminde Gel Electrophoresis pattern of Horse Gram seed storage protein during germination. International Research Journal of Biological Sciences.1 (4): 39 – 50.

Pang, Pek, Geok, Afandi Asrul, Shefiqur Rahman and Kumar Saha Ranajit 2012. Sodium Dodecyl SulphatePolyacrylAminde Gel Electrophoresis pattern of Horse Gram seed storage protein during germination. International Research Journal of Biological Sciences.1 (4): 39 – 50.

Rayane Natshe Gonçalves, Suellen Duarte Gozzini Barbosa, and Raquel Elisa da Silva-López (2016). Proteases from Canavalia ensiformis: Active and Thermostable Enzymes with Potential of Application in Biotechnology. Biotechnology Research International Volume 2016, Article ID 3427098, 11 pages.

Ranjan Sanjeev, Matcha Rajesh, Madhuri B and NarendraBabu PVR. 2012. Comparative evaluation of protein extraction methods from few leguminous seeds. International Journal of Advanced Biotechnology and Research.3 (2): 558 – 563.

Wilmot-Dear C M. 1984. A revision of Mucuna (Leguminosae-Phaseoleae) in Chinaand Japan. Kew Bull.39: 23-65.