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Study of Genetic Diversity in Selected Accessions of *Mucunapruriens* SDS-PAGE Markers

Abstract

SDS-PAGE is frequently used protein profiling method to assess genetic diversity. In present investigation five accessions of *Mucuna pruriens* were taken for protein profiling using SDS-PAGE marker and protein profiling and UPGMA cluster analysis results showed that among the five accessions of *Mucuna pruriens* accessions IC-24680 and IC-83298 depicted more distance than other members of cluster II and accession IC-202969 showed highest divergence at protein level. Finally, protein profiling and UPGMA cluster analysis was carried out in selected accessions of, Mucuna pruriens**.**

Keywords: Protein Profiling, Genetic Diversity, Phylogenetic

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Introduction

Mucuna pruriens is also an important tropical legume commonly known as 'Velvet bean', or Cow Itch. *Mucuna pruriens* (Velvet bean) an annual climbing legume, originally came from southern China and eastern India where it was at one time widely cultivated as a green vegetable crop (Burkill, 1966, Duke 1983 and Wilmot-Dear 1984). The genus *Mucuna pruriens* covers perhaps 100 species of annual and perennial legumes, including the annual velvet bean. Velvet beans are thought to have originated in Asia (India) and to have been introduced into the western hemisphere via Mauritius.

SDS-PAGE is a powerful analytical technique for the separation, characterization and estimation of relative molecular mass of proteins. Electrophoresis is the movement of net charged molecules in an electric field. When proteins are separated on Polyacrylamide, the procedure is called Polyacrylamide gel electrophoresis (PAGE). When Sodium Dodecyl Sulphate is used with the process, the procedure is abbreviated as SDS-PAGE. SDS coats proteins with a uniform negative charge and all coated proteins in electric field migrate at same speed. Proteins of different size experience different degree of sieving because of specific pore size in a gel, which affects their mobility, and it is inversely proportional to their size.

Objective of Study

The objective of present investigation was to carry out Protein Profiling by SDS-PAGE technique to estimate genetic diversity and phylogenetic relationship among *Mucuna pruriens* genotypes to identify the better genotypes for plant breeding purpose.

Review of Literature

Harini and Sathyanarayana (2009) studied Polyacrylamide gel electrophoretic analysis of the brown and non-brown somatic embryos of *Mucuna pruriens* and revealed quantitative differences inprotein contents between the two.

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 *(Pisumsativum, Vignaradiata*, *Cicerarientum* and *Vignamungo).* 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 Gonçalveset.et al. (2016) in *C. ensiformis* used SDS-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. One gram seeds of each accessions were ground to fine powder in a pestle and mortar and 10 mg was weighed in 1.5 ml micro centrifuge tube. Subsequently 400 µl protein extraction buffer (Tris-HCl 0.05 M, pH – 8.0, 0.02% SDS, 30.3% urea, 1% 2-mercaptoethanol) was added to each micro centrifuge tube and kept overnight at 40°C, and centrifuged at 13,000 rpm for 10 min. The supernatant contains the dissolved extracted protein ready for experiment purposes and could be kept for longer time at 4°C.

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 buffer and 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).

Result and Discussion

The total seed protein extracts of *Mucuna pruriens* 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. In *Mucuna pruriens* a total of 14 polypeptide bands were recorded. The size of these polypeptide bands ranged from 7.0 to 94.0 kDa. Out of these polypeptide bands 11 were common among all five genotypes and 3 bands were polymorphic (Fig. 1).

Accessions of *Mucuna pruriens* were highly diverse in nature and protein profiling and UPGMA cluster analysis results showed that among the five accessions of *Mucuna pruriens* accessions IC-24680 and IC-83298 depicted more distance than other members of cluster II and accession IC-202969 showed highest divergence at protein level (Fig 2) . Gupta and Kak (2007) using SDS-PAGE electrophoresis technique also observed genetic diversity in some genotypes of *Mucuna pruriens*. Similar technique was also used for differentiating the proteins in raw and roasted seeds of *Canavalia cathartica* from southwest coast of India by Bhargva*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

In present investigation Protein Profiling by SDS-PAGE technique to estimate genetic diversity and phylogenetic relationship among *Mucunapruriens* was found highly useful. Accessions of *Mucuna genotypes* were highly diverse in nature and protein profiling and UPGMA cluster analysis results showed that among the five accessions of *Mucunagenotypes* accessions IC-24680 and IC-83298 depicted more distance than other members of cluster II and accession IC-202969 showed highest divergence at protein level.

Fig. 1(a): Protein Fingerprinting of selected accessions of *Mucuna pruriens.*
: M- Molecular Marker (Range: 7.0-94 KDa)
Lane 1: IC-25333A1, Lane 2: IC- 385925, Lane 3: IC-24680, Lane 4: IC- 202969, Lane5: IC-83298

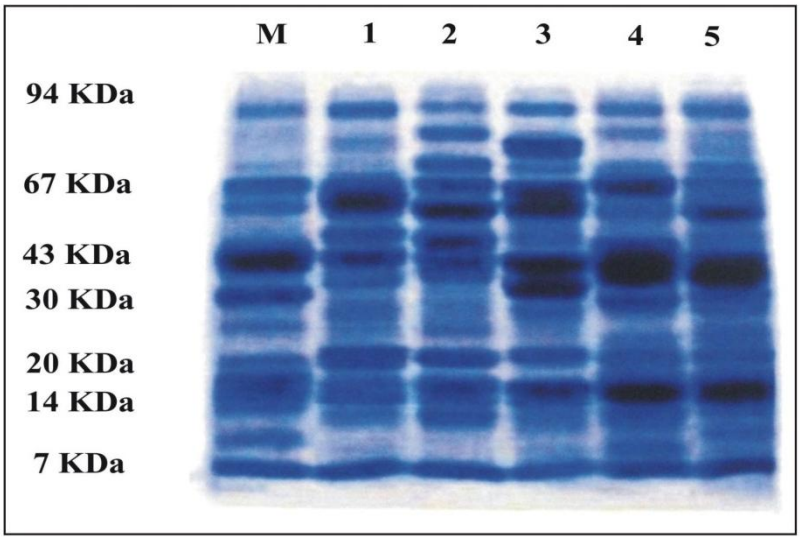
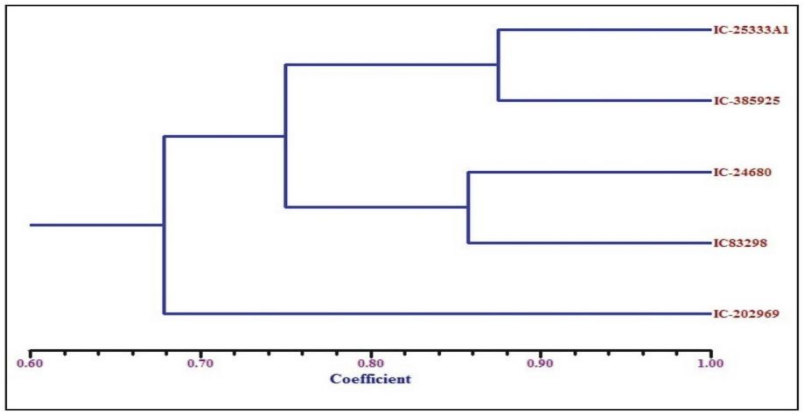


Fig.1(b):UPGMA dendrogram resulting from Protein Fingerprinting showing diversity among selected accessions of *Mucuna pruriens*.



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