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Assessment of Genetic Diversity in *Mucuna pruriens* cultivars using SDS-PAGE and RAPD Markers

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Abstract: **SDS-PAGE protein profiling and RAPD techniques have been found to be highly useful and accurate for the determination of both inter-specific and intraspecific genetic variations in plants. In present investigation five accessions of *Mucuna pruriens* were taken for protein profiling using SDS-PAGE marker and protein profiling and UPGMA cluster analysis and selected three cultivars the samples were analyzed for genetic variation by RAPD analysis using three random primers for each seed samples. On the basis of RAPD cluster analysis *Mucuna pruriens* accessions were also grouped into two clusters. Major cluster I consisted of two accessions sharing 30.0% similarity. The Similarity value for all the accession ranged from 15.0% to 82.0%. The findings of the molecular markers were substantiating each other.**

Keywords: Mucuna pruriens, **protein profiling, SDS-PAGE marker.**

1. MATERIAL AND METHODS

(i). SDS-PAGE

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 Electrophoretic apparatus was set and run as per protocol (Chawla, 2003) . 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).

(ii). RAPD Analysis

Seeds from selected accessions of *Mucuna pruriens*, were taken, soaked in sterile water and removed seed coats of soaked seeds, and genomic DNA was extracted following the standard method of Krishna and Jowali (1997), with required modification.

Genomic DNA samples were checked for purity by running it in an agarose gel (0.8%). The quantities of DNA were determined by measuring ratio of absorbance (A) at 260 & 280 nm (A260/A280). 1 µl of a λ DNA marker (10 µg μl^{-1}) was also loaded for visual quantification. Genetic diversity among selected isolates was also assessed by RAPD primer using the 11 RAPD-Primer set purchased from Bangalore Genei, India. The presence or absence of individual, distinct and reproducible bands was scored as "1" for presence and "0" for absence. Binary data was pooled and used to construct a composite dendrogram. The software NTSYSpc version 2.02i (Rohlf, 1995) was used to calculate the Jaccard distance coefficient (Jaccard, 1908) and construct the dendrogram using the Unweighted Pair-Group Method with Arithmetic

Average (UPGMA). Zymogram is the diagrammatic representation of the enzyme or protein band location in the strip of a gel. It is extrapolated on the basis of relative mobility of each band in the gel. Scoring of bands was done by Rm value and + sign was given for presence of band. A schematic diagram was made for analysis of banding pattern**.**

Table 1. RAPD primers used for diversity analysis

RAPD Primers	Primer sequence (5'-3')	Total number of bands	No. of Polymorphic Bands	Percent of Polymorphic Bands
OPB-B1	GTTTCGCTCC	8	3	37.5
OPB-B2	CCTTGACGCA	6	5	83.3
OPB-B3	CCACAGCAGT	5	3	60.0

2. RESULTS AND DISCUSSION

(i). SDS-PAGE

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.

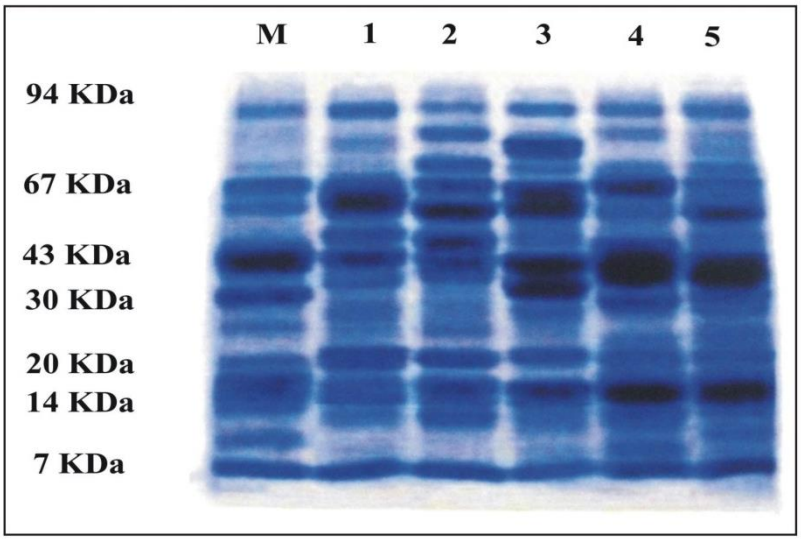

Fig. 1(a): Protein Fingerprinting of selected accessions of *Mucuna pruriens.*

: 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

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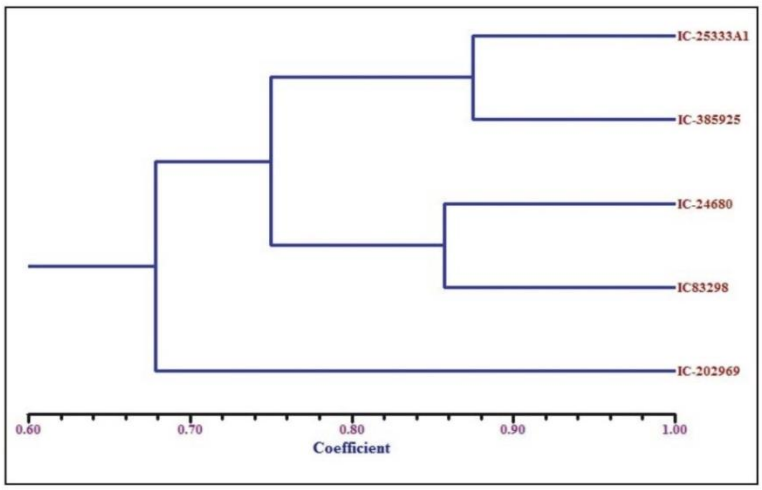

Fig.1 (b): UPGMA dendrogram resulting from Protein Fingerprinting showing diversity among selected accessions of *Mucuna pruriens*.

(ii). RAPD Analysis

RAPD analysis of three accessions of *Mucuna pruriens* out of five whose SDS-PAGE was carried which and high showed high divergence were subjected to RAPD analysis out using 3 decamer random RAPD primers (OPB-B1, OPB-B2, OPB-B3). RAPD analysis in *Mucuna pruriens* primer OPB-B2 produced 6 amplicons. RAPD data were used to make pair wise comparison of the accessions based on shared and unique amplification products to generate a similarity matrix with Exeter Software, Setauket, NY software package (NTSYS-Pc 2.02e). Similarity value for all the accession ranged from 15.0% to 82.0%.

Accessions IC385925 and IC24680 depicted genetic closeness (similarity coefficient 30%) and IC83298 showed genetic divergence from the members of cluster I (similarity coefficient 15%) and same results were found at protein level (Fig. 2 a & b). Kalidass and Mohan (2010) also used RAPD makers for assessing genetic diversity among the cultivars of *Mucuna pruriens*. Dhawan *et al*. (2011) successfully also carried out comparative genetic analysis of trichome-less and normal pod genotypes of *Mucuna pruriens* using RAPD-PCR analysis. Capo-Chichi *et al*. (2003) reported genetic variability within the cultivated species of *Mucuna* by using AFLP (amplified fragment length polymorphism) and classified different taxa on the basis of level of variation.

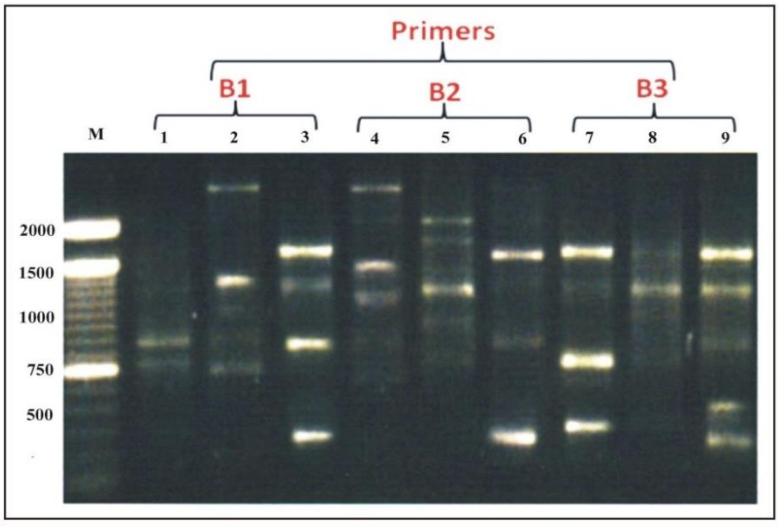

Fig. 2 (a) RAPD profile of *Mucuna pruriens* accessions using three different primers. Lane 1,4,7- IC-385925, Lane2,5,8- IC-24680 and Lane3,6,9- IC-83298. M- molecular Marker (range: 250-2000bp)

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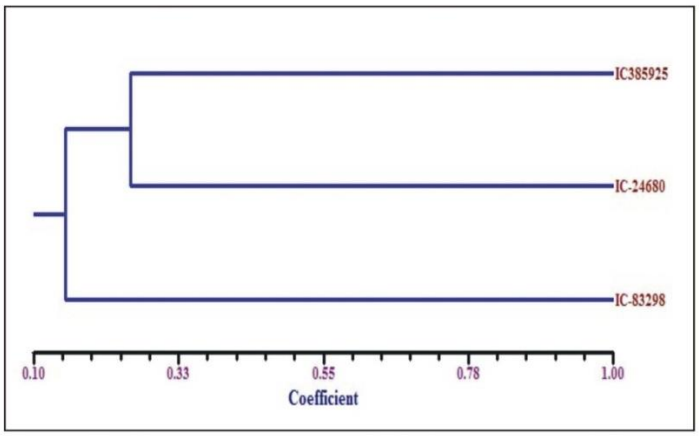

Fig. 2 (b) Composite UPGMA dendrogram of *Mucuna pruriens* on the basis of RAPD analysis based on Nei and Li (1979) genetic distances.

3. CONCLUSION

In present investigation Protein Profiling by SDS-PAGE and RAPD analysis technique to estimate genetic diversity and phylogenetic relationship among *Mucuna pruriens* was found highly useful. Accessions of *Mucuna* genotypes were highly diverse in nature and protein profiling, RAPD analysis and UPGMA cluster analysis results showed that these technique s are highly applicable to study genetic diversity at molecular level.

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