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Molecular Analysis of *Mucuna pruriens* (L.) DC. and *Mucuna cochinchinensis* (Lour.) A. Chev. Using RAPD Markers Helps to Understand Genetic Variations

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Abstract: *Fifteen species of Mucuna species are reported so far from India which are being used in the name of Kapikacchu by the ayurvedic practitioners since ancient times. Kapikacchu is an ingredient of several commercial preparations that are being used as an aphrodisiac in male sexual disorders. The samples of Mucuna pruriens (L.) DC. and Mucuna cochinchinensis (Lour.) A. Chev. were authenticated, analyzed and compared to illustrate the phylogenetic relationships between them. In this study, a set of 25 plants RAPD universal primers (RPI 1 – RPI 25) were used for genomic DNA amplification. The amplicons were compared and the phylogenetic tree was drawn with the help of bioinformatic analysis tool UPGMA (Unweighted Pair Group Method with Arithmetic Mean) by applying Jaccard's distance method. The Jaccard's similarity coefficient values suggested good variability in these two Mucuna sps. 107 RAPD fragments were obtained using twenty five primers. The RAPD PCR (Random Amplification of Polymorphic DNA- Polymerase chain reaction) amplification resulted in different successive polymorphic banding patterns with primers in both the plants that indicate significant genetic variations between the species. The creation of some baseline data is an important approach to study the genetic markers of Mucuna species as the information on the genetic diversity of Mucuna species is inadequate.*

Keywords*: Mucuna pruriens; Velvet bean; Mucuna cochinchinensis; Lyon bean; RAPD profiling; Genetic divergence; Molecular polymorphism*

I. Introduction

The genus *Mucuna* belongs to the family Fabaceae (Leguminoceae) and includes about 150 species of annual and perennial legumes of tropical distribution. India is one of the natural centers of origin of the *Mucuna* in the world (Eilittä *et al*., 2002). To date fifteen species of *Mucuna* species are reported from India. In addition to their traditional medicinal use many species of the genus offer an excellent source as cover crop and green manure (Janardhanan and Lakshmanan, 1985; Mohan and Janardhanan, 1993). Almost all the species are reported to contain L-3,4-dihydroxy phenylalanine (L-Dopa), a non-protein amino acid that acts as precursor for the neurotransmitter dopamine, used in the treatment of Parkinson's disease (Manyam, 1995). In addition, *Mucuna* is also traditionally used in various other applications like, dye (Standley and Steyermark, 1946), treatment of pain and numbness of joints, and irregular menstruation (Ding *et al*., 1991).

Mucuna pruriens Bak. commonly known as Kivach, Alkusi, Cowhage, Kaunch, Velvet bean is an economically important medicinal plant found in bushes and hedges and dry deciduous, low forests throughout the plains of India (Sastry and Kavathekar 1990; Singh *et al*., 1996). It is reported to be native of China and Eastern India (Wilmot Dear, 1987). M. pruriens is a wild plant and it's every part is full of medicinal value. It's most important parts are seeds and roots which are good source of giving vital energies. Seeds are excellent source of L-DOPA (lavodopa 3,4- dihydroxyphenyl alanine) which is precursor of dopamine a neurotransmitter (Daxenbichler *et al*.,1971) used in the treatment of Parkinson's disease (British pharmacopoeia, 1973; Longhi *et al*., 2011). The seed of *M. cochienchinensis* contains carbohydrate 55.8%, protein 27.5% and fat 3.6%. The fruits yield 0.96% L-dopa (Anonymous, 2006)**.**

Mucuna cochinchinensis (Lour.) A. Chev., locally known as Lyon bean is an annual twining herb with white or pale purple flowers and glabrescent pods. It is widely distributed in the tropics and subtropics and cultivated mostly in Bengal and Bihar region of India for its edible pods and seeds. The fleshy and tender fruits of the plant are valued as vegetable (Anonymous, 2005).

Randomly Amplified Polymorphic DNA (RAPD) is convenient technique to analyse the genetic variation (Bhat *et al*., 1999). It has been extensively used for molecular fingerprinting and population diversity analysis (Albert *et al*., 1997, Saini *et al*., 2010). Availability of reliable polymorphic markers, often limits the accurate estimation of genetic variation among individuals or different populations.

In the present study, we have applied a DNA fingerprinting technique, random amplified polymorphic DNA (RAPD), to differentiate the three accessions of *Mucuna* as well as for genetic characterization of the selected accession.

II. Material And Methods

Collection of Plant Materials:

The leaves of *Mucuna pruriens* (L.) DC. and *Mucuna cochinchinensis* (Lour.) A. Chev. were collected from the Medicinal plants garden of National Research Ayurvedic Institute of Basic Ayurvedic Sciences, Pune. The plant materials were authenticated by Mrs. A. G. Mhase, Herbarium Assistant, and the specimens were preserved in the herbarium for reference.

DNA Extraction:

For RAPD analysis, healthy and non-infected leaves were collected from mother plant. The leaves samples were grounded in liquid nitrogen to a fine powder and were kept at -20°C until use**.** The total genomic DNA extraction from the leaves of both the plants was done by using 3B Black Biotech Biotools kit with approximately 100-120 mg of powdered sample of each. The DNA of both plants was then quantified using biophotometer (eppendorf) as well as standardized against known concentrations of DNA marker ladder (Promega, USA) on 1% agarose gels. DNA was then kept at 4°C until further use.

RAPD-PCR:

The protocol for polymerase chain reaction (PCR) was optimized by varying the concentration of template, *Taq* DNA polymerase and $MgCl_2$. Polymerase chain reaction (PCR) amplifications were performed in reaction mixtures containing 10 ng template DNA, 0.2 µM primer, 100 µM dNTP´s, 10 mM PCR buffer, 1.5 mM $MgCl_2$, and 1 unit *Taq*-polymerase in a total volume of 20 µl.

Amplifications were carried out in a thermo-cycler (Peltier P25+, Cyber lab) with the following program: initial denaturation step of 3 min at 94°C followed by 35 cycles of 45 sec at 94°C, 30 sec at 36°C, 1 min at 72°C, and a final extension step of 5 min at 72°C and 4ºC for the end hold. PCR products were resolved by electrophoresis in 1.5% agarose gels along with DNA Marker ladder (100-1000bp), the run was carried out at 75 Volts for approximately 60 minutes. Gels were stained with ethidium bromide, visualized under UV-light, and photographed GelDoc system (BioRad, USA). To ensure reproducibility of banding patterns, each amplification reaction was repeated twice. All the PCR reaction components and the Primers set were purchased from 3B Blackbio Biotech Biotools. The primers used for amplification of the genomic DNA are detailed in Table 1. Along with the 25 different reaction mixtures for 25 different primers, a negative control (NC - No DNA template) and a positive control (PC - No primer) were placed in thermal cycler to rule out the possibility of contamination. Reaction mixture wherein template DNA replaced by distilled water was used as negative control.

Table 1: Plant RAPD primers used for the study

No.	Primer code	Accession Numbers
1	RPl 1	AM765819
2	RPl 2	AM750044
3	RPl 3	AM773310
4	RPl 4	AM773769
5	RPl 5	AM773770
6	RPl 6	AM773771
7	RPl 7	AM773312
8	RPl 8	AM773773
9	RPl 9	AM773315
10	RPl 10	AM750045
11	RPl 11	AM911709
12	RPl 12	AM773316
13	RPl 13	AM750046
14	RPl 14	AM773774
15	RPl 15	AM773775
16	RPl 16	AM773776
17	RPl 17	AM911710
18	RPl 18	AM765830
19	RPl 19	AM773777
20	RPl 20	AM773317
21	RPl 21	AM765820
22	RPl 22	AM911711
23	RPl 23	AM911712
24	RPl 24	AM765821
25	RPl 25	AM750054

Statistical analysis:

Gelquest® and Clustervis® software were used to construct dendro-grams using the Unweighted Pair Group Method with Arithmetical Averages (UPGMA) by comparing the bands that were observed common in both plants to specific primers.

III. Results

The DNA obtained was 21.32 ng/µl (*M. pruriens*) and 20.5ng/µl (*M. cochinchinensis*).

As evident from RAPD profiles, the two accessions selected of *Mucuna* could be distinguished at the molecular level. The comparative analysis of all accessions was established on the basis of RAPD profiles generated by using decanucleotide primers. Figure 1 shows the characteristic RAPD profile of *M. pruriens*, whereas Figure 2 clearly depicts the genetic differentiation of *M. cochinchinensis*. Only the primers which displayed prominent, reproducible and distinguishable bands were considered for analysis. The 14 tested primers yielded totally 107 scorable bands with an average of three bands per primer which showed polymorphism. 60% polymorphism was observed in *M. cochinchinensis* and 92% in M. pruriens. All primers showed amplification for *M. pruriens* whereas primer number 4, 18 and 24 did not show any bands in the RAPD profile of *M. cochinchinensis.*

Figure 1: RAPD ***Mucuna* pruriens (L.) DC.**

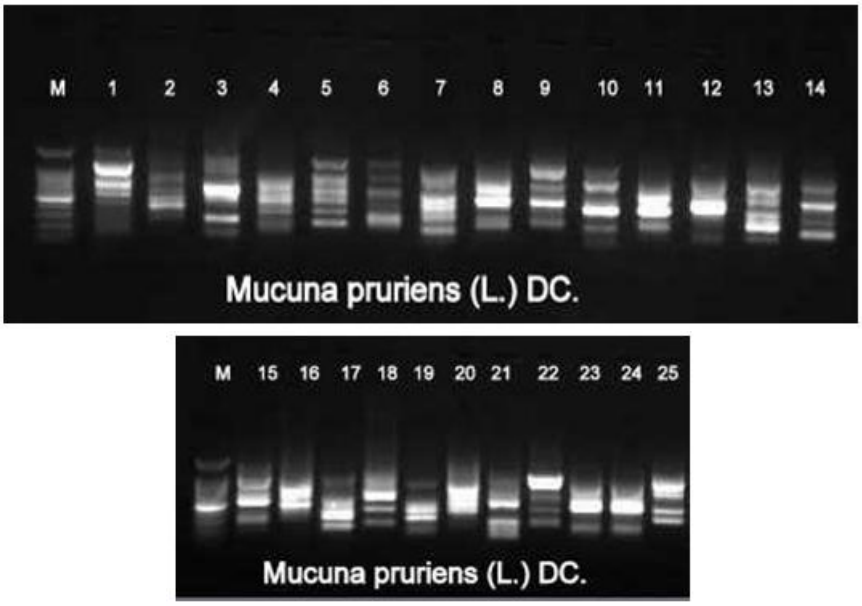
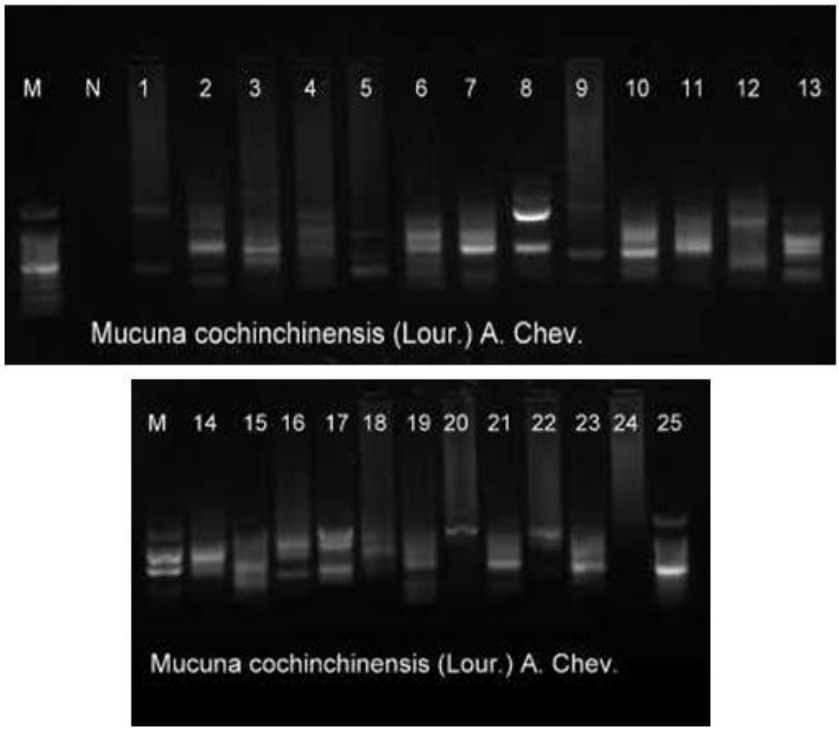
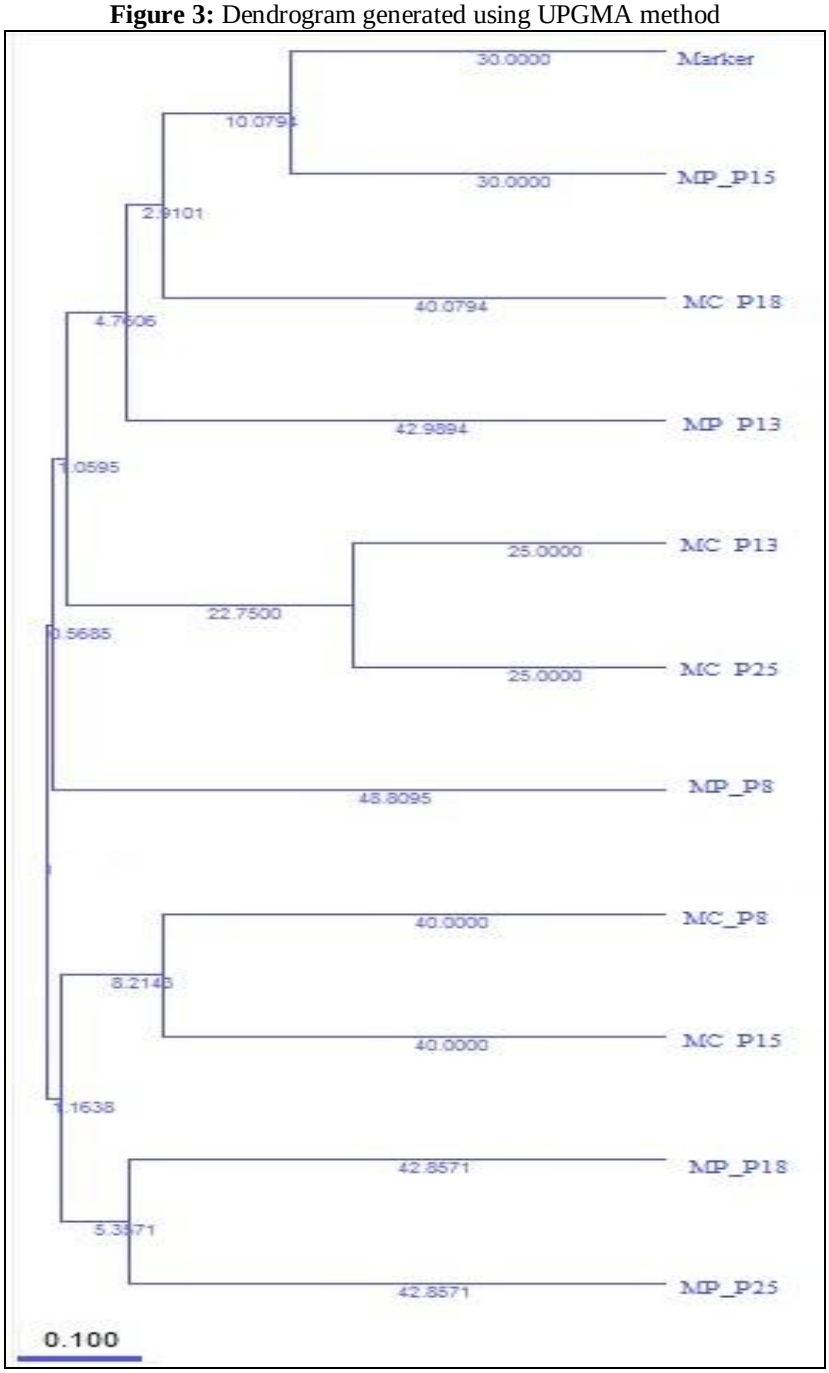


Figure 2: *Mucuna* cochinchinensis (Lour.) A. Chev.



The data scored on RAPD polymorphism and band sharing was aimed at analyzing the genetic similarity matrix obtained through multivariate analysis using Nei and Li's coefficient. The similarity coefficients were used to generate a tree for cluster analysis using the UPGMA method as evident from the phenogram (Figure 3).

Figure 3: Dendrogram generated using UPGMA method



IV. Discussion

RAPD analysis in the present study reveled very high level of Polymorphism (92%) for different *Mucuna* species. Padmesh *et al*., (2006) observed that 11 *Mucuna* accessions produced 90.1% polymorphism. Leelambika *et al*., (2010), observed 98% polymorphism in 18 accessions of *Mucuna*. Generated data showed RAPD markers are suitable tools to scan anonymous DNA of species with high secondary metabolite content such as *Mucuna*. The limited reliability of RAPD molecular markers was compensated by increasing the number of RAPD fragments. In this study, the large similarity values revealed by RAPD markers while making the phylogenetic tree provide greater confidence for assessment of genetic relationship among the species. RAPD profiling of these two plants have not yet reported.

Hadrys *et al*., (1992) used RAPD-PCR technique for studying genotype fusion at various levels, including those of individuals (parentage tests) and populations (identification of hybrid populations, species and subspecies). This profiling method was used for isolating soma-clone of altered genotypes in *Mentha arvensis* by Khanuja *et al*., 1998. The UPGMA tree showed that the two plants were related and have the common ancestral background. Though these two have different uses in Ayurvedic sciences they still can be tracked back. Based on the study the large range of similarity and dissimilarity values for the plants provides the greater confidence for assessment of genetic diversity and relationships.

V. Conclusion

The information on genetic diversity of *Mucuna* species evaluated using RAPD technique and the results provided a short and snappy view on the total genetic variability for *Mucuna* species. RAPD fingerprinting analysis is widely used in analyzing population and genetic relationships between and among species, establishing taxonomic status and evolutionary studies. Thus, this approach will be helpful in ranking the species according to their genetic inter-relatedness. This can provide a better platform for identification and authentication of *Mucuna* species. Therefore this study may contribute to provide germplasm conservationists with data in order to facilitate conservation efforts. In summary, the analysis of amplification spectra of 107 bands obtained by 25 primers clearly demonstrated the differentiation of selected accessions and established the effectiveness of this molecular tool for establishing the distinct identity of selected accessions of *Mucuna*.

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