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GENOME ANALYSIS OF DIFFERENT STRAINS OF *MUCUNA PRURIENS* L. USING MOLECULAR MARKERS

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ABSTRACT

Genomic diversity in four accessions of self-pollinated velvet bean- *Mucuna pruriens* (L.) DC. (comprising of varieties *pruriens* and *utilis*) were analyzed at the genomic DNA level using RAPD and ISSR primers. Both these analyses have indicated polymorphism among these strains. Absolute monomorphic patterns could not be obtained from any of the primers used in this study. The present study strongly indicated that the variety *pruriens* is genetically more diverse than the variety *utilis*. The UPGMA cluster analysis of both the RAPD and ISSR data also clearly revealed the close relatedness of all the 3 strains of the variety *utilis*. The only strain of variety *pruriens* is most distantly related to all the three strains of the other variety (var *utilis*).

Keywords: DNA; Genome; ISSR; *Mucuna*; RAPD

1. INTRODUCTION:

Mucuna pruriens (L.) DC., of Fabaceae, is a self-pollinated tropical plant **[1]** with massive nutritional and agronomic potentials. It is an important source of L-DOPA (3,4dihydroxy-L-phenylalanin) – the precursor of dopamine used in the treatment of Parkinson's disease. The plant is widely cultivated as a green manure **[2, 3]**. It has been used as source of food and a pasture crop **[2]**. The lack of information on taxonomy of *Mucuna* has hindered the effective utilization of *Mucuna*'s genetic resources. The wide geographical and climatic distribution of the crop is also likely

to induce genetic diversity in it, which needs to be ascertained before any cultivar development program. Since there is a confusion regarding the taxonomy of velvet bean, it is important to conduct research at the species level to assess the genetic diversity and phenetic relationships among accessions before any breeding is initiated. Study of morphological and chromosomal variability **[4]** is the classical way of assessing genetic diversity. For many species, especially minor crops, it is still the only approach used. An assessment of genetic diversity based only on morphological and agronomic traits might be biased because distinct morphotypes can result from a few mutations. However, with molecular marker techniques, powerful tools have been developed so that genetic resources can accurately be assessed and characterized. Most of these techniques are based on the analysis of nucleic acid molecules which are information-rich and provide a reliable estimation of relatedness, phylogeny, and inheritance of genetic characteristics **[5]**.

Genetic study at molecular level is important to determine variability or diversity, which is a pre-requisite for genome evolution. Moreover, it is also used in diverse areas like verifying the affinities and the limits between species, to detect forms of reproduction, to evaluate levels of migration and dispersion in populations and for the identification of threatened species. The basic data for these studies are called molecular markers **[6]**.

Molecular techniques using polymerase chain reaction (PCR)-based assays for DNA amplification have provided new insights into the systematic and evolutionary trends of different organisms. The most commonly used methods are PCR-hybridization, PCR-size polymorphism, random PCR-restriction fragment length polymorphism, and random amplified polymorphic DNA-PCR **[2]**.

The molecular markers like RAPD are now used worldwide for molecular studies. RAPD has been developed for genetic mapping, fingerprinting, and is widely used in inter- and intra-specific population polymorphism analyses of different organisms. It has proved to be a powerful tool for differentiating between different species or subspecies and for genetic analysis of phylogenetic relationships among strains or populations for a variety of microorganisms, plants, animals and mammals **[7]**.

Random amplified polymorphic DNA (RAPD) is a truly multiplex PCR-based

molecular marker system **[7, 8]** which uses short oligonucleotides primers of arbitrary sequence and low-stringency PCR to amplify discrete DNA fragments that can be used as molecular markers. RAPD analysis is rapid, inexpensive and easy to perform. It has been used extensively for genetic studies, for example, analysis of genetic variation in bacteria, fungi and plants **[9]** and construction of the first linkage maps for certain plant species **[10]**. Mapping with RAPD markers has had a strong impact on tree breeding **[11]**.

The utility of PCR-based RAPD or inter-simple sequence repeat (ISSR) variations, as phylogenetic markers for investigating evolutionary relationships among plants, has been established **[12-14]**. However, a number of disadvantages are also associated with RAPD markers. Most RAPD markers are dominant, and thus, are less suitable for linkage analysis than co-dominant markers like RFLP and AFLP markers **[2, 15]**. In addition, several studies have reported poor reproducibility for RAPD markers **[16]**. The most serious error that occurs in the RAPD assay is competition between different DNA fragments for amplification **[15, 17]**. This phenomenon in which a RAPD band successfully amplified in one genotype is undetected in another, results in incorrect genotype interpretations. For polymorphic bands, the frequency of errors due to competition has been found to be approximately 15% in genomes of both high and low complexity **[17]**.

In comparison with other polymorphism methods, RAPD offers many advantages like: a combination of simplicity with no radioactivity; amplification of anonymous fragments of the target DNA; the requirements of minimum amounts of target DNA; the acceptance of an unlimited number of primers; no requirements of previous knowledge of the sequences to be amplified and hybridization analysis; a rapid procedure for performing and screening a large number of loci simultaneously, in a much shorter time than screening for other genetic assays. Therefore, RAPD profiles remain stable and present good reproducibility under rigidly controlled conditions, even when using DNA from different extractions or the same sample under different conditions **[8]**.

In the present study, RAPD and ISSR assays of the genomic DNA has been carried out on four different strains of *Mucuna pruriens* to examine the level of genetic diversity as well as to study the phylogenetic relationship among them.

2. MATERIALS AND METHODS:

2.1. Materials:

Young and healthy leaves of field grown plants of four strains of *Mucuna pruriens* L. were used in the present investigation. Seeds of three strains of *Mucuna pruriens* var *utilis* [Strain Nos.: IC 471876 **(I)**, IC 241679 **(II)**, IC 392338 **(III)**] were obtained from NBPGR, New Delhi. Plants of one strain of *Mucuna pruriens* var *pruriens* **(IV)** were collected in wild from West Midnapur district of West Bengal, India.

2.2. Methods:

DNA from young leaves was extracted using modified Doyle and Doyle **[18, 19]** method as described by Lahiri **[20]**.

The concentration of the DNA solution was quantified by taking the absorbance at 260 nm (A_{260}) in a UV spectrophotometer using the following formula. The purity of DNA was obtained by the ratio of A_{260}/A_{280}.

DNA (μg/ml) = 50 x O.D. at 260 nm x Dilution factor.

2.3. RAPD and ISSR studies:

Procedure:

RAPD assays were carried out in 25 μl reaction mixture containing 1X *Taq* DNA polymerase buffer, 0.2 mM dNTP mix (Fermentas), 3 mM $MgCl_2$, 0.5 μM primer (Sigma-Aldrich), 1 U *Taq* DNA polymerase (Fermentas), and 50 ng template DNA. Reactions without DNA were used as negative control. PCR reactions were carried out in a Thermocycler (Eppendorf Master Cycler Gradient). For the RAPD assay, the Thermocycler was programmed for an initial denaturation step of 4 minutes at 94°C, followed by 40 cycles of 1 minute at 94°C, 1 minute at 36°C and 2 minutes at 72°C. The last cycle was followed by a final extension at 72°C for 10 minutes and a hold temperature of 4°C at the end **[20]**.

For ISSR assay, the Thermocycler was programmed like that of the RAPD assay. However, the primer annealing temperature was set at 55°C. Amplified products were resolved on a 1.2% agarose gel in 1X TBE buffer, at 50V for 3 hours and stained with ethidium bromide (0.5 μg/ml). Gels were digitally photographed under UV illuminator. Gene Ruler from Fermentas was used as marker. The sequences of all the primers used in RAPD and ISSR assays are presented in **Table 1**.

DNA amplifications with each primer were repeated thrice to ensure reproducibility. Clear, intense and reproducible bands were scored.

Each amplified band (using ISSR and RAPD primers) was treated as a unit character. Reproducible amplified DNA

fragments were scored and transformed into binary character matrices (1 for presence, 0 for absence). UPGMA (unweighted pair group method with arithmetic mean) cluster analysis was done with the help of Statistica 6.0 software.

3. RESULTS AND DISCUSSION:

The highest amount of total genomic DNA content was extracted from strain IV (268.50 µg/g of tissue) and the least amount from strain III (160.50 µg/g of tissue) **(Table 2)**. The values of the ratio of A_{260} /A_{280} revealed the extraction of almost pure DNA without any contamination **(Table 2)**.

Ten random RAPD primers were used for the estimation of inter and intra-varietal variation in *Mucuna pruriens*. RAPD assay generated 114 bands, of which 62 were polymorphic (54.3% polymorphism) **(Table 3)**. On an average, each primer generated 11.4 bands of which 6.2 were polymorphic. The number of products generated by the RAPD oligonucleotide primers varied from 8 (B08 and OPA 09) to 15 (OPP 11 and OPA 15). None of the primers produced 100% monomorphism across the accessions **(Table 3, Figure 1)**.

The four ISSR primers generated 47 bands of which 27 were polymorphic (57.4% polymorphism) **(Table 4, Figure 2)**. On an average, each ISSR primer generated 11.75 bands out of which 6.75 were polymorphic. The number of products produced by each primer varied from 8 (ISSR 1 and 3) to 17 (ISSR 4). Here too, none of the primers produced 100% monomorphism across the accessions **(Figure 2)**.

The UPGMA cluster analysis of both the RAPD and ISSR data clearly reveals that strain I and II of the variety *utilis* are very closely related to each other than the other two strains. Strain III of var *utilis* is more related to strain II and strain I of the same variety. The only strain of variety *pruriens* is most distantly related to all the three strains of the other variety (var *utilis*) **(Figure 3a, 3b)**.

RAPD and ISSR analyses have been carried out to assess the phenetic relationships between the intra- and inter-varietal genomes, using UPGMA clustering. The second objective of this study was to see if they indicate a possibility that the cultivated variety has diverged from the wild type. The RAPD data generated out of ten random oligonucleotide primers revealed 62 polymorphic products that corresponded to 54.3% polymorphism. Maximum number of amplified products was obtained from two primers but none of the primers produced 100% polymorphism. This clearly indicated qualitative differences among the genomic

DNA between the strains of *Mucuna pruriens* L. and therefore, genomic diversity and heterogeneity might be suggested **[2, 6, 8]**.

The high level of polymorphism detected in self-pollinated accessions of *Mucuna pruriens* may be attributed to the broad genetic base of the species and in the process of speciation novel gene combinations might have been acquired for better adaptability in the changing environmental conditions. Observing the relationship, it may be suggested that strain IV of variety *pruriens* is distantly related to strain I and II of the variety *utilis*. It is also noted that strains I and II are most closely related as the distance between them is lesser than that between strains I and III or between strains II and III. This indicated the genetic diversity of these strains for better adaptability to different climatic conditions **[2, 6, 8]**. The high number of polymorphic products generated by certain RAPD primers may be attributed to the fact that in RAPD even small divergence between two strains can result in distinct patterns as polymorphism may be the result of any of the various reasons such as (1) single nucleotide change within the primer binding site, (2) insertion or deletion with the amplified region so that part of the primer binding site in one of the strand is missing, (3) complete absence of complementary sites and (4) the region between the binding sites on opposite strands is beyond the normal amplifiable length.

The ISSR fingerprinting method has been reported to produce more complex marker patterns than RAPD **[21, 22]**, which is advantageous when differentiating closely related strains or cultivars. In addition, ISSR markers are more reproducible than RAPD markers **[23]**, because ISSR primers, designed to anneal to a micro-satellite sequence, are longer than RAPD primers, and this method has been utilized for cultivar identification of different plant species **[24, 25]**. In this study using four ISSR primers, 57.4% polymorphism has been observed out of total 47 amplified fragments. Moreover, three primers produced high number of polymorphic bands. ISSR fingerprinting has therefore been more efficient than RAPD assay, as it detected 57.4% polymorphic fragments in these four strains of *Mucuna pruriens*, as compared to 54.3% with RAPD fingerprinting. Similar results were obtained with other plant species **[26, 27]**. ISSR markers are also more informative than RAPD markers because of their greater ability to detect polymorphism. Analyzing the distance matrix, the ISSR study also

supports the RAPD results confirming that strain IV of var *pruriens* is distantly related to the strain I and II of var *utilis*. The genomic diversity and the phylogenetic relationship between these strains, as exhibited by chromosome analysis **[4]** and genetic markers (proteins) **[28]**, have also been confirmed by the molecular study as well (RAPD and ISSR). The disparity in intra-specific polymorphism could arise because natural populations tend towards isolation via self-pollination. It may, therefore, be suggested that the cultivated varieties might have originated from the wild variety of *Mucuna pruriens*. It is also suggested that ISSR assay have the potential of being used as diagnostic tools for cultivar or strain identification and it could be developed into cultivar-specific Sequence Characterized Amplified Region (SCAR) markers **[22]**.

Table 1: Sequence of the RAPD and ISSR primers used in the present study.

Primer Type	Primer Name	Primer Sequence (5'- 3')
RAPD	B 01	TCGAAGTCCT
RAPD	B 02	AGATGCAGCC
RAPD	B 08	TCACCACGGT
RAPD	B 10	CAGGCACTAG
RAPD	OPA 07	GAAACGGGTG
RAPD	OPA 09	GGGTAACGCC
RAPD	OPA 15	TTCCGAACCC
RAPD	OPB 04	GGACTGGAGT
RAPD	OPP 09	GTGGTCCGCA
RAPD	OPP 11	AACGCGTCGG
ISSR	1	CACACACACACACACAAT
ISSR	2	GCTCACACACACACACAC
ISSR	3	CACACACACACACACC
ISSR	4	GAGTCTCTCTCTCTCTCTC

Table 2: Quantitative and qualitative estimations of total genomic DNA following UV spectrophotometry in different *in vivo* strains of *Mucuna pruriens* L.

Variety/ Strain	Total genomic DNA content (µg/g of tissue)*	A_{260}/A_{280}
I	221.25 ± 1.74	1.82
II	173.25 ± 1.71	1.89
III	160.50 ± 1.04	1.59
IV	268.50 ± 1.90	1.71

*** Data represent Mean ± S.E. from 5 replicates**

Table 3: Number of monomorphic and polymorphic bands obtained using different RAPD primers in varieties of *Mucuna pruriens*

Primer	No. of monomorphic bands	No. of polymorphic bands
B 01	05	04
B 02	10	03
B 08	2	6
B 10	2	12
OPA 07	3	6
OPB 04	5	9
OPA 09	6	2
OPA 15	8	7
OPP 09	6	3
OPP 11	5	10

Table 4: Number of monomorphic and polymorphic bands obtained using different ISSR primers in varieties of *Mucuna pruriens* (*in vivo*)

Primer	No. of monomorphic bands	No. of polymorphic bands
ISSR 1	**1**	**7**
ISSR 2	**7**	**7**
ISSR 3	**5**	**3**
ISSR 4	**7**	**10**

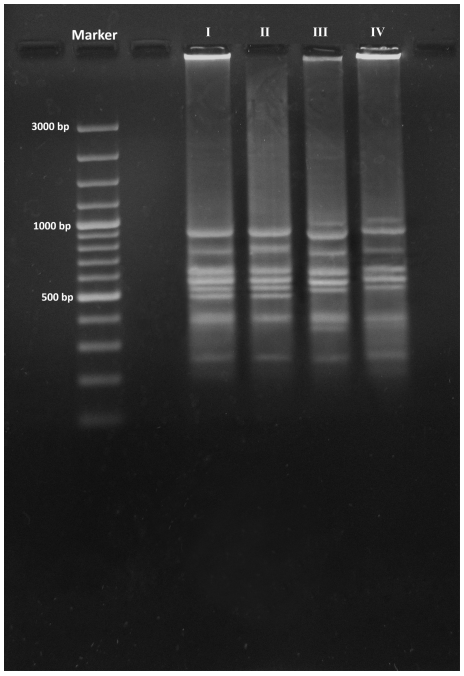

Figure 1: RAPD profile of four different strains of *Mucuna pruriens* using primer B 01

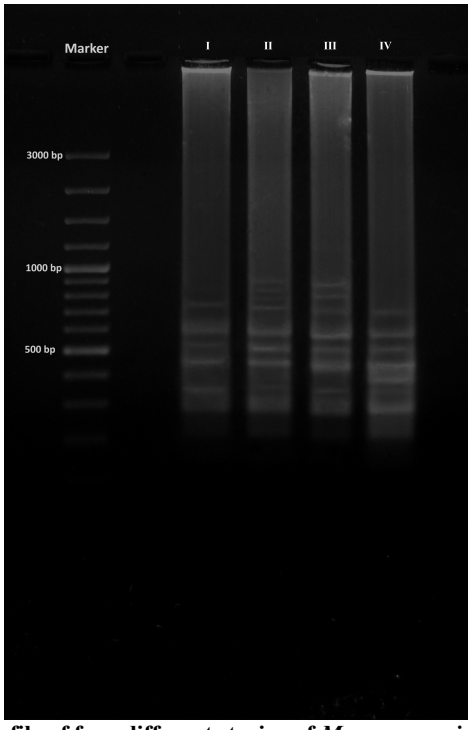

Figure 2: ISSR profile of four different strains of *Mucuna pruriens* using primer 3.

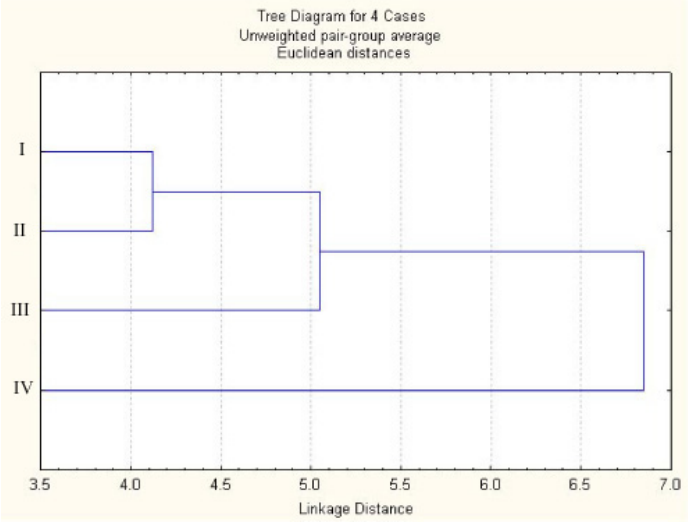

Figure 3 (a): UPGMA cluster analysis of RAPD data four different strains of *Mucuna pruriens*.

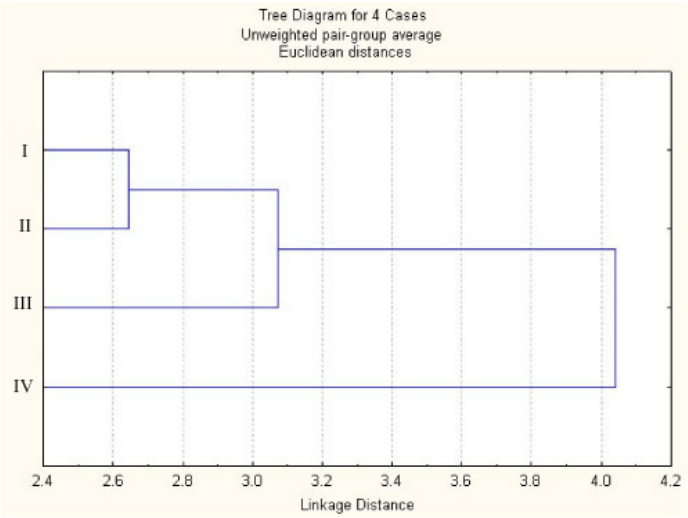

Figure 3 (b): UPGMA cluster analysis of RAPD data four different strains of *Mucuna pruriens*.

CONCLUSION:

From the present investigation it is revealed that RAPD analysis addresses mostly unique genome fragments, while ISSR analysis is used to study inter-micro satellite sequences, and both the methods allow a detailed characterization of the genomes under study. In conclusion, our results indicate that both RAPD and ISSR markers are useful for distinguishing and characterizing *Mucuna pruriens* germplasms. The genetic relatedness among these genomes could provide useful information for conservation.

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REFERENCES:

[1] Duke JA, Handbook of legumes of world economic importance, New York, USA: Plenum Press, 1981, http://dx.doi.org/10.1007/978-1-4684-8151-8

[2] Capo-Chichi LJ, Weaver DB, Morton CM, The use of molecular markers in the study of genetic diversity in *Mucuna*, Tropical and Subtropical Agroecosystems, 1(2-3), 2003, 309-318. https://www.redalyc.org/articulo.oa?id=93911288032

[3] Wilmot-Dear CM, A revision of *Mucuna* (Leguminosae-Phaseoleae) in China and Japan, Kew Bulletin, 39(1), 1984, 23-65.

[4] Lahiri K, Mukhopadhyay MJ, Mukhopadhyay S, Karyotype analysis and in situ 4C nuclear DNA quantification in two varieties of *Mucuna pruriens* L., Journal of Tropical Medicinal Plants, 11(2), 2010, 219-225. https://www.cabidigitallibrary.org/doi/pdf/10.5555/20113191452

[5] Caetano-Anollés G, DNA analysis of turfgrass genetic diversity, Crop Science, 38(6), 1998, 1415-1424. https://doi.org/10.2135/cropsci1998.0011183X003800060001x

[6] Sathyanarayana N, Mahesh S, Leelambika M, Jaheer M, Chopra R, Rashmi KV, Role of genetic resources and molecular markers in *Mucuna pruriens* (L.) DC improvement. Plant Genetic Resources, 14(4), 2016, 270-282. doi:10.1017/S1479262116000071

[7] Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV, DNA polymorphisms amplified by arbitrary primers are useful as genetic markers, Nucleic Acids Research, 18(22), 1990, 6531-6535. https://doi.org/10.1093/nar/18.22.6531

[8] Padmesh P, Reji JV, Jinish Dhar M, Seeni S, Estimation of genetic diversity in varieties of *Mucuna pruriens* using RAPD, Biologia Plantarum, 50(3), 2006, 367-372.

https://doi.org/10.1007/s10535-006-0051-z

[9] Mailer RJ, Scarth R, Fristensky B, Discrimination among cultivars of rapeseed (*Brassica napus* L.) using DNA polymorphisms amplified from arbitrary primers, Theoretical and Applied Genetics, 87(6), 1994, 697-704. https://doi.org/10.1007/BF00222895

[10] Yang X, Quiros CF., Construction of a genetic linkage map in celery using DNA-based markers, Genome, 38(1), 1995, 36-44. https://doi.org/10.1139/g95-005

[11] Crouzillat D, Lerceteau E, Petiard V, Morera J, Rodriguez H, Walker D, Phillips W, Ronning C, Schnell R, Osei J, Fritz P, *Theobroma cacao* L.: a genetic linkage map and quantitative trait loci analysis, Theoretical and Applied Genetics, 93(1), 1996, 205-214. https://doi.org/10.1007/BF00225747

[12] Maughan PJ, Saghai Maroof MA, Buss GR, Huestis GM, Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance, and near-isogenic line analysis, Theoretical and Applied Genetics, 93(3), 1996 392-401. https://doi.org/10.1007/BF00223181

[13] Spooner DM, Raul CT, Reexamination of series relationships of South American wild potatoes (Solanaceae: *Solanum* sect. *Petota*): evidence from chloroplast DNA restriction site variation, American Journal of Botany, 84(5), 1997, 671-685. https://doi.org/10.2307/2445904

[14] Ghislain M, Zhang D, Fajardo D, Huamán Z, Hijmans RJ, Marker-assisted sampling of the cultivated Andean potato *Solanum phureja* collection using RAPD markers, Genetic Resources and Crop Evolution, 46(6), 1999, 547-555. https://doi.org/10.1023/A:1008724007888

[15] Williams JG, Hanafey MK, Rafalski JA, Tingey SV, Genetic analysis using random amplified polymorphic DNA markers, In: Methods in Enzymology, New York, USA: Academic Press, Elsevier, 218, 1993, 704-740. https://doi.org/10.1016/0076-6879(93)18053-F

[16] Skroch P, Nienhuis J, Impact of scoring error and reproducibility

RAPD data on RAPD based estimates of genetic distance, Theoretical and Applied Genetics, 91(6), 1995, 1086-1091. https://doi.org/10.1007/BF00223923

[17] Halldén C, Hansen M, Nilsson NO, Hjerdin A, Säll T, Competition as a source of errors in RAPD analysis, Theoretical and Applied Genetics, 93(8), 1996, 1185-1192. https://doi.org/10.1007/BF00223449

[18] Doyle JJ, Doyle JL, A rapid DNA isolation procedure for small quantities of fresh leaf tissue, Phytochemical Bulletin, 19, 1987, 11-15.

[19] Doyle JJ, Doyle JL, Brown AH, Grace JP, Multiple origins of polyploids in the *Glycine tabacina* complex inferred from chloroplast DNA polymorphism, Proceedings of the National Academy of Sciences, 87(2), 1990, 714-717. https://doi.org/10.1073/pnas.87.2.714

[20] Lahiri K, Micropropagation, cytomolecular and chemical analysis of different varieties and regenerated plants of *Mucuna pruriens* L., Ph.D. Thesis, University of Calcutta, 2010.

[21] Parsons BJ, Newbury HJ, Jackson MT, Ford-Lloyd BV Contrasting genetic diversity relationships are revealed in rice (*Oryza sativa* L.) using different marker types, Molecular Breeding, 3(2), 1997, 115-125. https://doi.org/10.1023/A:1009635721319

[22] Pharmawati M, Yan G, Finnegan PM, Molecular variation and fingerprinting of *Leucadendron* cultivars (Proteaceae) by ISSR markers, Annals of Botany, 95(7), 2005, 1163-1170. https://doi.org/10.1093/aob/mci127

[23] Goulão L, Oliveira CM, Molecular characterisation of cultivars of apple (*Malus× domestica* Borkh.) using microsatellite (SSR and ISSR) markers, Euphytica, 122(1), 2001, 81-89. https://doi.org/10.1023/A:1012691814643

[24] Joshi SP, Gupta VS, Aggarwal RK, Ranjekar PK, Brar DS, Genetic diversity and phylogenetic relationship as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus *Oryza*, Theoretical and Applied Genetics, 100(8), 2000, 1311-1320.

https://doi.org/10.1007/s001220051440

[25] Arnau G, Lallemand J, Bourgoin M, Fast and reliable strawberry cultivar identification using inter simple sequence repeat (ISSR) amplification, Euphytica, 129(1), 2003, 69-79. https://doi.org/10.1023/A:1021509206584

[26] Raina SN, Rani V, Kojima T, Ogihara Y, Singh KP, Devarumath RM, RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification, and phylogenetic relationships in peanut (*Arachis hypogaea*) cultivars and wild species, Genome, 44(5), 2001,763-772. https://doi.org/10.1139/g01-064

[27] Nagaoka T, Ogihara Y, Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers, Theoretical and Applied Genetics, 94(5), 1997, 597-602. https://doi.org/10.1007/s001220050456

[28] Lahiri K, Mukhopadhyay MJ, Mukhopadhyay S, Biochemical marker-based comparative genomic characterization of *in vivo* varieties and *in vitro* regenerates of *Mucuna pruriens* L., an important medicinal plant, International Journal of Botany Studies, 3(3), 2018, 1-10. https://botanyjournals.com/assets/archives/2018/vol3issue3/3-2-50-151pdf