Genetic Diversity among Desmodium Gangeticum (L.) DL Accessions a Desmodin Synthesising Ethno-Medicinal Plant With SSR and Internal Transcribed Spacer Region for Species Conservation

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***Abstract*-In the present study simple sequence repeat (SSR) and intertranscribed spacer markers markers were used in *Desmodium gangeticum* to find out the genetic diversity analysis for the biodiversity conservation of the *Species* as the plant is being used in medicinal preparations as well as have many industrial applications. The study was aimed to find out the relationships among different accessions. We find intra-population mean genetic value of (1.835) and Shannon (0.641) among the accessions. in AMOVA analysis, we noticed small variation accessions as (*P* = 1).The dendrogram generated through distance matrix using the SIMQUAL-Dice Coefficient module of NTSYSpc ver matches to some extent with the PCA graph. In the barcode ITS marker analysis the UPGMA shows the accessions clustered into two groups. Blast results showed maximum E-value in DG2 accession. In conclusion the above based markers can be used for the population conservation and authentication of the above medicinally important plant.**

***Keywords:* *Desmodium gangeticum* ,SSR , intertranscribed spacer, PCA, Dendogram.**

I. INTRODUCTION:

Medicinal plant genetic resources are an important sector in the sustainable development of human therapeutic applications. The slaughter of biodiversity resources and, as a consequence, the genetic diversity they represent, is a common reality. It is for that reason we should develop adequate and effective strategies to conserve these genetic resources. *Desmodium gangeticum* is one of the multifaceted plant for which we have to go for its adequate conservation otherwise the plant in future will be in the red list species. The plant is a sub-tropical perennial herb that grows with angular branches, Leaves simple, flowers purplish or white. Its flowering–fruiting season is during the months of March to December, is found distributed throughout the warmer parts of India, tropics of Africa. The plant is being used in various medicinal applications such as antioxidant, anti-inflammatory activities (1).The roots shows antileishmanial and immunomodulatory activity (2),curability in premature ejaculation (3) and in treatment of ulcer (4). Different types of alkaloids have been extracted from the plant such as gangetin, gangetinin, desmodin, and desmocarpin (5); phospholipids, sterols, and flavones glycosides have also been extracted by (6), (7) and (8) which are being used by various pharmaceutical industries.

The aim of our study is to provide a rationale means for identifying the genetic diversity analysis in the above plant by using molecular markers viz, SSR and intertranscribed spacer markers as limited study has been done in the above threatened plant. Molecular markers are being used for understanding the basis of genetic diversity and different markers have been used by various authors such as Random Amplified Polymorphic DNAs (9),Simple Sequence Repeats (10), Restriction fragment length polymorphisms RFLP (11), Amplified Fragment Length Polymorphisms AFLP;(12),(13) and (14), SRAP (15),Single Nucleotide Polymorphisms, SNPs(16) etc. As for Simple sequence repeats (SSR) or microsatellites are concerned they are short, tandemly repeated motifs of DNA.some reports have described the levels of occurrence of microsatellites associated to transcribed regions (17), (18),(19) and (20).Simple sequence repeat markers are being used in genetic relatedness ,diversity identification for the conservation and utilization of germplasm resources (21), (22) and (23). The application of these markers include their simplicity, effectiveness, abundance, hypervariability, reproducibility, codominant inheritance, and extensive genomic coverage (24) With the increase of EST sequences in the public database, a large number of available sequences presents opportunities to identify and validate usefulness of these microsatellites at a low cost and in an efficient manner. There is another marker known as intern transcribed spacer markers or it is also called barcode. In plant genomes the nuclear ribosomal RNA (rRNA) genes occur in thousands of copies and are organized in tandem

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arrays while the 5S rDNA locus encodes the 5S rRNA genes, the 45S rDNA locus contains genes for 18S, 5.8S and 26S rRNA, which are transcribed as a single unit and then spliced.The 18S, 5.8S and 26S rRNA genes are separated by internal transcribed spacers ITS1 and ITS2 or ITS4, and the 45S transcription units themselves are separated from each other by non-transcribed intergenic spacers (IGS), extending from the 39 end of 26S rRNA to 59 end of the 18S rRNA coding regions (25).The ITS marker have been used in genetic variation studies before by (26) in genera of compositae plants. now a days it has been used in different plants like *Epimedium* (27),*Panax* (28) and *Asarum* plant (29) etc. The aim of our study were to assess of genetic variability and diversity among different accessions of *Desmodium gangeticum* using SSR and intertranscribed spacer marker so as to provide genetic data and a theoretical basis for protection of the species which will contributes to monitoring of germplasm in future.

II. MATERIALS AND METHODS

Plant material:

In the present study, different accessions covering a wide range of the morphological variation were collected from different biodiversity regions of the India Table 1.The collections were maintained under in vitro and Green house conditions.

DNA extraction:

DNA was extracted with Plant mini kit (Qigen), Quantity and quality was assessed by electrophoresis in (0.8%) agarose gel stained with ethidium bromide (Amershco) using 0.5x TBE electrophoresis buffer at (95 V for 40 min).Quantitative estimates of sample DNA were made by nanodrop method (Thermo scientific).

Primer Sequence:

SSR primer sequences were available from the information available in NCBI. The sequences of SSR primers were selected on the basis of their proper properties of their broad coverage of the genome and their high levels of polymorphism .The inter transcribed spacer primers were chosen according to (26) to amplify the concerned region of the ribosomal DNA, which encompasses the 5.8S gene and both ITS-1 and ITS-4 regions. The primers were synthesised from Integrated DNA Technologies (USA).

SSR PCR Amplification:

The PCR reaction mix up contained (30- 45 ng) DNA, 1 unit Taq DNA polymerase (New England biolabs), 2Mm of dNTPs mix (NEB),10 pmol primers and 10X buffer containing $MgCl_2$ (NEB) in a total mix volume of 10 µl reaction with following cycling conditions; $94C^0$ for 5 min, 32 cycles of $93C^0$ for 1min,50 C^0 for 1 min, $72C^0$ for 1min, and a final extension temperature of $72C^0$ for 5 min. The amplified products were separated on 1.8% (w/v) agarose gel at a constant voltage of 80v for 2 hours and were visualised in Bio-rad gel doc system

Internal transcribed spacer assay:

The entire ITS region, comprising ITS1, 5.8S, and ITS4, was amplified from genomic DNA from all the accessions with primers ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') in a 100µl reaction in 10 times the reaction mix up used in SSR analysis PCRs were performed with a Eppendorf PCR System using he conditions of 5 min at 95 C, followed by 32 cycles of 30 s at 94 C, 50 s at 55 C, and 1 min at 72 C, with a final extension step for 5 min at 72 C. PCR products were electrophoresed in 1.5% and purified with the Gel Extraction Kit (QIAGEN) according to the manufacturer's manual. The purified products were sequenced at http://www.scigenom.com/.

Searches for similar sequences were done using the BLASTN & X algorithm available in the National Centre for Biotechnology Information (NCBI,http://www.ncbi.nlm.nih.gov/BLAST/index.htm.

III. DATA ANALYSIS

Simple Sequence Repeat (SSR):

Most of the SSRs show multiple bands which indicates that for a given SSR more than one allele was observed, which may reflect heterozygosity and/or segregation at the respective SSR marker. All the genotypes were scored for the presence and absence of the SSR bands. Some SSR were repeated twice and only the reproducible bands were scored and the data were entered into a binary matrix as discrete variables, 1 for presence and 0 for absence of the character and this data matrix was subjected to further analysis and imported in the form of excel file. POPGENE version 32 (30) was also used to estimate Gene diversity and Shannon's information index (I) for co-dominant data markers.

Principal Coordinates Analysis (PCoA) was performed with the software NTSYS version 2.2 (31). The allelic SSR matrix was used for the analysis of molecular variance (AMOVA) using GenAlEx 6.41 (32). AMOVA was used to estimate the partition of the genetic variation within and among accessions. The significance of the variance components was determined with a permutation test (999 replicates). SAHN module plot of NTSYS version 2.2 (31) was used for plotting the dendrogram to observe genetic relatedness and the robustness of the tree was tested using bootstrap analysis (1000 iterations).

Intertranscribed Spacer:

Based on studies showing that BLAST outperforms of NCBI's web-based algorithm using the default settings was used to identify the query sequences. Each identification was made manually, taking E-value and maximum identity. Evolutionary analyses were conducted in MEGA6 (33)

Results and Discussion:

SSR based marker analysis used in this study revealed small genetic divergence between different accessions. The 10 microsatellite primers (Table 2) were used in *D. gangeticum* and a total of 46 polymorphic bands sizes were

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generated , some primes gave distinct polymorphic products highlighted in bold in (Table 2) and rest of markers could not be amplified or produce faint bands. One reason for lack of amplification could be rapid genome modification. Microsatellite fragments ranged from 150 bp to 750 bp in size (Fig.1). Examination of intra-population mean genetic diversity revealed the value of (1.8357) and mean Shannon (0.6411), (Table 3), Principal coordinates analysis (PCoA) and UPGMA cluster analysis (Fig. 2, 3) was done to display the spatial allocation to examine relationships between different accessions in populations and helps us in understanding the relationships between them. Based on AMOVA analysis, we did not noticed any variation among *Desmodium gangeticum* accessions indicating statistically non significant genetic differentiations among populations ($P = 1$) which may perhaps also due to small no of sample size. Here we can state that the marker SSR, used in this present study displayed a relatively weak genetic diversity among *Desmodium gangeticum* populations. Variability turned out to be in the same range in between the accessions which is probably the result of obligatory cross-pollination in this dioecious species. The genetic fragmentation of this species despite of its fragmented and isolated habitats did not happened yet .There is growing concern about diminishing populations, loss of genetic diversity, DNA barcoding have been extensively used for species identification, diversity Barcode phylogeneny has been validated for conservation and identification purposes in many other medicinal plants species like *Panax* (28),*Asarum* plant (29), *Epimedium* (27, 34),Gesneriaceae (35), (36), (37) and (38). In our *Desmodium gengaticum* Species the phylogenetic tree (Fig.4) obtained with the Neighbor-Joining (NJ) algorithm, 4 accessions of *Desmodium* were differentiated into two clusters viz (Dg1&2) and (DG3&4) which is in accordance due to geographical locations. BLAST Results showed maximum E-value in DG2 and maximum identity in DG4 (Table 4). The above results of both the SSR and intertranscribed work could have through implications for the conservation of this threatened species.

IV. CONCLUSION

Indeed, management measures for the conservation and restoration of the above species require knowledge in natural habitats also .The plants are not necessarily rare, but they could quickly become critically endangered if they are harvested in an unsustainable way. Additional field studies together with the people collecting these plants combined with a more taxon specific approach could give insight into whether these endangered plants enter the markets as well and if the plant collectors are aware of the differences in morphology and abundance between these plants which indicate that there are species that are locally overexploited or endangered. Considering the medicinal importance, the above discussed markers may provide productive application to the cultivation, conservation of genetic resources, assessment of genetic relationships and sustainable utilization of this natural gifted species.

Author's Contribution:

Haji RF performed the experiment, wrote the manuscript and analysed the data ; VV gives comments and suggestions that helped in improving the manuscript.

Competing Interests: The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS:

The authors acknowledge to the Dabur Research Foundation New Delhi India, UGC-India for providing the financial assistance and SMVD University were the work was being carried in.

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