GENETIC DIVERSITY OF *PSORELEA CORYLIFOLIA* OF S OUTHERN ANDHRA PRADESH

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Abstract: *Psoralea corylifolia*is an important medicinal herb of Fabaceae is a rich source of many secondary metabolites, which are used as medicines. A wide variation in terms of genetic diversity and psoralen content has been recorded through RAPD among various chemotypes of *P. corylifolia* collected from southern Andhra Pradesh. A high degree of polymorphism (89.68%) was revealed among the 4 chemotypes studied. This study provides scientific basis for authentication ofrelatively high diversity in terms of genetic and biochemical constitution among different chemotypes of *P. corylifolia* collected from various regions of Southern India for detection of elite germplasm for commercial purpose.

Keywords: Genetic diversity, chemotype, *Psoralea corylifolia,*

Introduction: *Psoralea corylifolia* belongs to the family Fabaceae and comprises 120 species in the world. It is a rich source of medically useful bioactive compounds. The antibacterial action of the fruit inhibits the growth of *Mycobacterium tuberculae* (Wamer and Giles 1982). Besides, oral administration of *P. corylifolia* showed the positive effects on bone mineral density as well as bone formation (Hsiun et al. 2007). Triplex-formation oligonucleotides attached with a photo-reactive psoralen molecule can be used to induce site specific DNA damage and control gene expression. *P. corylifolia* is distributed almost throughout India and extensively used as herbal drugs in Indian traditional medicinal practices like Ayurveda, Siddha, Unani, etc. Availability of good quality raw material and itsauthentication regarding quality control parameters are the major concern for this taxon. Detection of the elite germplasm of *P. Corylifolia* would provide great emphasis on enhanced cultivation and conservation of the desired germplasm. DNA marker based finger-printing can distinguish species, ecotypes, chemotype, varieties and genotypes rapidly using small amounts of DNA and therefore can assist to deduce reliable information on their identification, characterization and phylogenetic evaluation. Random amplified polymorphic DNA (RAPD) is an inexpensive and rapid method not requiring any information regarding the genome of the plant which may facilitate the conservation and utilization of plant genetic resources, permitting the identification of unique chemotypes, varieties and genotypes. However, there are no reports on the genetic diversity analysis of different *P. corylifolia* chemotypes of India. Present word aims at achieving both the authenticity at the level of species among the different variants of *P. corylifolia* by using DNA fingerprinting (RAPD) of two groups of chemotypes collected from different locations of Southern India.

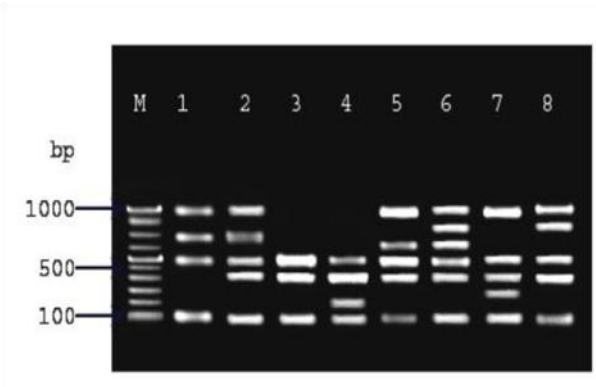

Fig 1. Representative RAPD profile of different chemo types of *P.corylifolia* collected from different locations of India using primer VAA-02. (Samples1-8asinTable1; LaneM:1 oobp DNA sizemarker).

Materials and Methods: Plant materials for HPLC and RAPD Analysis: Seeds of *P. corylifolia* were collected from two different zones southern Andhra Pradesh and were sown on different seed

beds in the month of March, in the, Department of Botany, Andhra Christian College, Guntur.All the plants set seed in the month of November.

Extraction of genomic DNA: Total genomic DNA from 2 g each leaf tissue of atleast5 mature plants of different chemotypes of *P. corylifolia* was extracted using modified CTAB (CetylTrimethyl Ammonium Bromide) method (Saghai- Maroof*et al*. 1984), individually. Further, the DNA was pooled with their respective chemotypes for genetic diversity studies. The quantity and quality of the RNase treated DNA of all the plant samples of *P.corylifolia* was determined spectrophotometrically and using agarosegel(0.8%)electrophoresis.

RAPD Analysis: For assessment of biodiversity, DNA samples of all eight zones were pooled to get a mixture of genomic DNA, prior to PCR. This was done for reducing the screening reactions with all the forty primers, VAA-01 to VAB-20 (VAA-01 toVAA-20 and VAB-01 to VAB-20) (Sigma Aldrich, USA). PCR amplification solutions (25 µl) included approximately 50 ngofgenomic DNA, 3 mM $MgCl_2$,0.1 mM each of dNTPs, 1U of Taq polymerase (Invitrogen Corporation, USA) and 2.5 µl of 10X PCR reaction buffer (500 mMKCl, 200 Mm Tris-HCl (pH8.4)] and 1 µlof 0.8 µM of RAPD primer (Sigma Aldrich, USA). DNA amplifications were carried out in a 2720 Thermal Cycler (Applied Biosystems). The amplification products were resolved on 1.2% (w/v) agarose gels containing ethidium bromide and gels were digitally photo-graphed under UV gel documentation system.

Analysis: RAPD markers across the 4chemotypes for genetic diversity were scored for their presence '1' or absence '0' of bands for each primer. By comparing the banding patterns of chemotypes for a specific primer, monomorphic and polymorphic bands were identified and faint or unclear bands were not considered. The binary data so generated were used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. The polymorphism information con-tent (PIC) was calculated by the formula: PIC = 2 Pi (1-Pi)where, Pi is the frequency of occurrence of polymorphic bands in different primers (Bhat 2002). Pair-wise similarity matrices were generated by Jaccard's coefficient of similarity by using the SIMQUAL (Similarity for qualitative data program) for-mat of NTSYS-pc (Jaccard 1908, Rohlf 2000). A dendrogram was constructed by using the unweighted pair group method with arithmetic average (UPGMA) with the SAHN (Sequentialagglomerative hierarchical and nested clustering method) module of NTSYS-pc to show a phylogenetic representation of geneticrelationships as revealed by the similarity coefficient (SneathandSokal1973).

Results: **Biodiversity: Selection of Chemotype Yielding High Psoralen Content:** Selection of the high yielding chemo type was done with respect to the optimum amount of psoralen detected in the form of peak from the seed samples of the respective chemo-types through HPLC analysis by comparing with the peak area of psoralen standard. Among all the seed samples, seeds of Seshachalm region yielded the highest amount of psoralen*i.e.*4763.00±1.29^{h}mg/g fresh wt., followed by other regions (4202.00±0.91^{g}mg/g fresh wt),

DNA Finger Printing: RAPD Analysis (DNA Profiling): The various chemotypes of *P. corylifolia* were analyzed using 40 random primers of which seven produced repro-ducible polymorphic banding patterns (Table 2). A total of 43bands were scored of which 38.5 (89.68%) were polymorphic. The number of bands generated per primer varied from 2 to 9.A minimum of 2 bands were generated by the primer VAA-19,while a maximum of 9bandswereobservedwithprimerVAA-02. The size of the amplified products varied from 100 bp to1000 bp. Five primers (VAA-01, VAA-04, VAA-19, VAB-05and VAB-06) generated 100% polymorphic bands (Table 2).RAPD profiles of a representative primer VAA-02 are shown in Fig. 2. The mean PIC calculated from the frequency of poly-morphicbandsacrossallchemotypeswas0.442(Table 2).The primerVAA-02revealedthehighestPICvalueof 0.498followed by the primers VAA-04; VAB-05 (0.497), VAA-01(0.484);VAB-06(0.455)and VAA-03(0.442).

Genetic Diversity: Thegenetic diversity among the 4 chemotypes of *P.corylifolia* was analyzed employing Jaccard's coefficient. Jaccard's coefficient of similarity varied from 0.512 to 1.000, indicative of high level of genetic variation among the chemotypes studied (Table3). The highest genetic similarity co-efficient (0.767) was observed between *P. corylifolia* chemotype accessions of Seshachalma forest and Chittoor dist.

Discussion: Authentication of species identity and prediction of thequantity of bioactive principles are the two major attributes ofquality control which are required for the trade of herbal plantsusedforpharmaceutical purposes (Kumar and Gupta 2008).Therefore, it has become imperative to develop approaches for ensuring

availability of raw materials of superior quality in terms of enhanced level of biologically active compounds, *exsitu* conservation, assessment and characterization of genetic diversity.(Gomez-Galera*et al*. 2007, Parast*et al*. 2011).

It is therefore, suggested that evaluation of bioactive compound is imperative to select the elite chemotype/genotype/ecotype/variety or clone prior venturing clonal propagation and for *invitro* germplasm conservation.

RAPD fingerprinting amplifies the genome at multiple loci randomly. Such multi- bandRAPD profiles have proved very useful in clustering of different genotypes, ecotypes, chemotypes, cultivars and varieties at intraspecies level (Choundhury et al. 2007, Zhang et al. 2012,2013). Such clusters can be correlated with different groups pertaining to different geographical regions, neutraceutiacl and metabolic compositions. Needless to say, the UPGMA clusteranalysis assumes a constant evolutionary rate among accessions and is thus considered most appropriate for diversity study with in species. The results presented here demonstrated the selection of high psoralen yielding chemotype amongst the eight chemo-types of *P. corylifolia* studied. The utility of RAPD markers in partitioning genetic variation amongst them could serve as potential source of unique genetic material for future germplasm conservation, to identify duplicated accessions within germplasm collections as well as for their exact characterization and for selecting diverse parental combinations inbreeding programs for crop improvement. This study further providesguidance for the future analysis of genetic diversity in other chemotypes or genotypes /accessions of *P.corylifolia* witheven more reliable molecular markers such as AFLP and microsatellites to facilitate *P. corylifolia* germplasm management and conservation for commercial purposes. There is also potentiality of developing authentic genetic fingerprints of all available chemotypes in India which will constitute a major step for-ward to our understanding of the specific level of genetic diversity prevailing in *P.corylifolia* chemotypes

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