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INTRAPOPULATION GENETIC DIVERSITY ASSESSED IN TEPHROSIA CALOPHYLLA BEDD., A RARE MEDICINAL PLANT USING RAPD MARKERS

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ABSTRACT

Tephrosia calophylla is a perennial under-shrub with a tuberous root and is known for its various therapeutic properties. Genetic diversity was analysed using RAPD primers among 10 accessions collected from the population of Talakona, Andhra Pradesh, India. Twenty random primers ,each with 10 bases generated a total of 47 polymorphic bands out of 70 total bands ,that showed 67.6% polymorphism . Genetic distance between each accession was calculated and dendrogram was constructed on the basis of the similarity matrix data by unweighed pair group method with average (UPGMA) cluster analysis. The analysis with RAPD markers reflected high level of diversity within the population in this species.

KEYWORDS: Conservation, genetic diversity, Random Amplified Polymorphic DNA (RAPD), *Tephrosia calophylla* (TC).

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INTRODUCTION

Medicinal plants play a vital role to perserve our health .From times immemorial ,plants are the most important components and have been an integral part of traditional medicines. In India inexhaustiable resources of drugs of plant origin are used in various in various systems ofmedicines in Ayurveda, Siddha, Unani etc. However , due to an indiscriminate use of these resources overtime and fragmentation of habitats, many of these species are increasingly threatened and face theriskofbeinggenetically improverished[1].*Tephrosia calophylla* is a rare medicinal plant that belongs to family Fabaceae .It is distributed in the states of Andhrapradesh ,Karnataka and Tamilnadu [2-3].It is rare and endemic to south India and it is rare in Talakona on hill plateau in shady localities often effected by soil erosion and summer fires [4]. The plant is known to elaborate a rich variety of flavanoids and isoflavanoids. Many flavanoid related phytoconstituents were reported from *Tephrosia calophylla* [5-6].The plant was also reported for hepatoprotective activity[7],antiprotozoal[8],antiulcer [9],antibacterial activity.[10]A viable conservation strategy is needed for preserving the dwindling genetic resources of this species. The determination of population genetic structure is of great importance in the development of strategies for collecting and conserving plant materials as genetic resources ,as well as in improvement for their utilization .Hence ,as a prerequisite the amount of genetic diversity within and among populations need to be investigated to guide strategies for their conservationandsustainable utilization.Despite the major position of *Tephrosia calophylla* as an important medicinal plant no data is available about the genetic structure of *Tephrosia calophylla* population .PCR based RAPD markers have been widely used in assessing genetic variation within a species. [11]In this study we have generated a series of RAPD profiles in order to assess genetic diversity within the population

MATERIALS AND METHODS

Population sampling

The study area was visited regularly during monsoon season and plant materials have been identified and verified by taxonomist Dr.K. Madhava chetty S.V.University,Tirupati. Tender leaves of the plant material were collected from a population near the temple area in Talakona, Chittoor district, Andhra Pradesh, India (Fig 1).A total of ten individuals from a population were included in the study .The distance between the plants collected was at least 10m to increase the possibility of detecting the variation potential within the population. The ten individual accessions selectedwerenumbered TC1,TC2,TC3,TC4,TC5,TC6,TC7,TC8,TC9,TC 10 respectively. (Table 1).

Table 1
The location of Tephrosia calophylla collection

Location	Accession No
Talakona (Chittor district)	TC1,TC2,TC3,TC4,TC5,TC5,TC6,TC7,TC8,TC9,TC10.

DNA Extraction

Total genomic DNA was extracted from leaves according to Murry and Thompson with slight modifications[12].Quality and concentration of total DNA was verified by UV spectrophotometry at 260nm and 280nm. Further quality of DNA was tested by horizontal ararose gel electrophoresis (1%) and visualized under UV light , gel documentation system.

RAPD analysis

Out of 20, Random decamer primers (Operon technologies, USA) of OPA series were used individually as primers for RAPD analysis. The PCR amplification was carried out in CG-

Palmcycler Germany. The PCR conditions were optimized in terms of concentration of template DNA, Taq DNA polymerase and $MgCl_2$ concentration varying concentration of template DNA. Maximum no. of reproducible bands was obtained with DNA concentration of 25 ng/25µl of PCR reaction mixture. The final amplification assay contained 25ng genomic DNA ,0.5 units Taq DNA polymerase, 100mM each of d NTPS, 5mM $MgCl_2$, 0.6 µm primers and 1 X Taq buffer in a PCR reaction volume of 25µl. PCR amplification was carried out with pre-denaturation at 94^0c for 3 min, denaturation at 94^0c for 1 min, primer annealing at 37^0c for 1 min and primer extension at 72^0c for 2 min followed by 40 cycles of amplification and final extension at 72^0c for 7 min. Gel electrophoresis was carried out on the amplified products using1% *agarose, stained with ethidium bromide and visualized under UV illumination. The 1kb DNA ladder was used as a molecular weight marker and the amplification were repeated twice to confirm the data.*

Figure 1
Talakona located in chittor district of Andhra pradesh,India.

Collected from: www.mapsofindia .com

Data analysis and scoring

Evaluation of fragment patterns was carried out by similarity index .Reproducible bands were scored manually as '1' or '0' for presence or absence of the bands . The data matrix was analysed to compute the Jaccard's coefficient using SPSS software. A dendrogram was constructed on the basis of the similarity matrix data by unweighed pair group method with average (UPGMA) cluster analysis (Table 3).

RESULTS

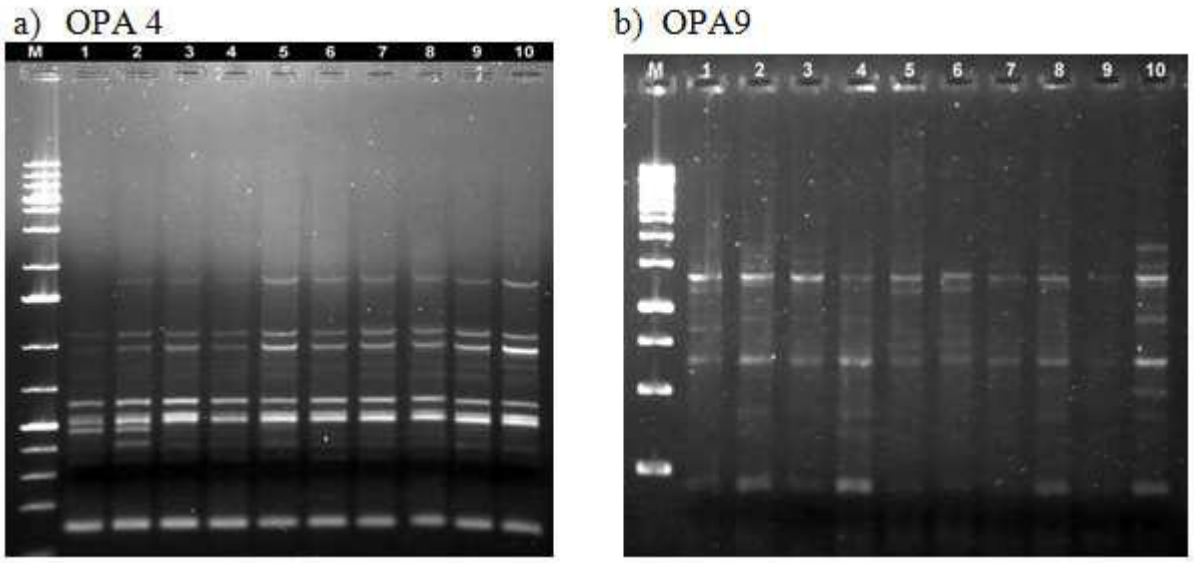
A total of 20 random primers of OPA series were screened to characterize the ten *Tephrosia calophylla* accessions. After screening 20 primers, 11 primers resulted in amplification of which 9 primers gave reproducible results. The levels of genetic similarity ranged from 0.52 to 0.83.The final numerical analysis included 70 total number of bands with 67.6% polymorphism resulted from 9 primers amplification.The RAPD profile of 10 accessions were compared individually for each primer and used for further analysis. The RAPD profiles of 10 accessions were separately compared to find out the differences among them by the occurrence of polymorphic bands.PCR amplification of the DNA isolated from 10 selected accessions yielded a total of

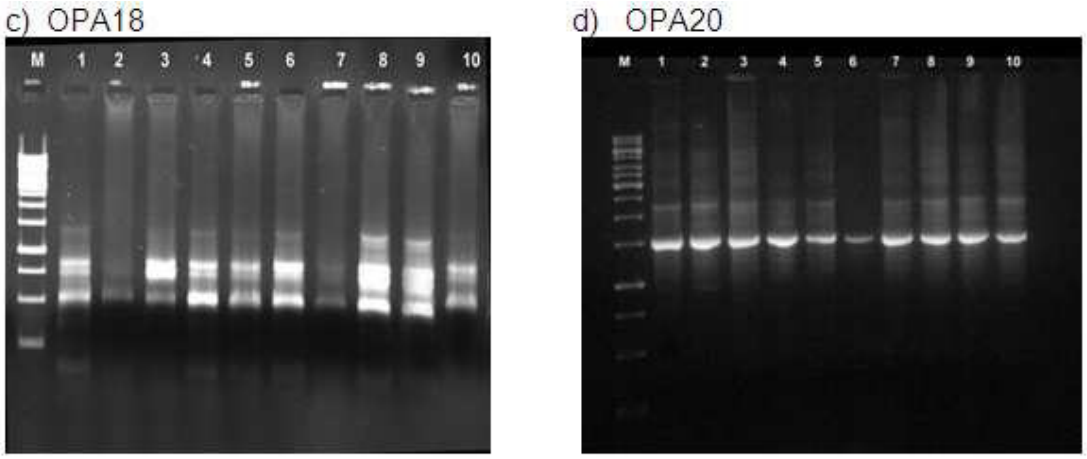
70 amplified products of which 47 were polymorphic and 23 were monomorphic. The total amplified DNA bands varied between 2 to 11 with an average of 7.7 bands per primer (Table 2) (Fig2)*A high level of polymorphism was detected with primer OPA 9 which showed 81.8% polymorphism whereas amplification with primers OPA 4, OPA 7 and OPA 20 detected the least polymorphism (50%).Primers OPA 18, OPA 2, OPA 13, OPA 3, OPA 10, OPA 4, OPA 7 and OPA 20 showed 80%, 77.7%, 71.4%, 66.6%, 66.6%, 50% of polymorphism respectively. The range of amplified fragments is from 250-9000bp. The maximum number of polymorphic bands was obtained with primer OPA 9 and the minimum number was obtained OPA 20. The polymorphic percentage range from 50% to 81.8%.*

Table 2
Polymorphism in Tephrosia calophylla(TC) using RAPD decamer primers.

Primer	Sequence 5 ´to 3´	Total no. of bands	No. of polymorphic bands	% of polymorphism
OPA 2	TGCCGAGCTG	9	7	77.7
OPA 3	AGTCAGCCAC	9	6	66.6
OPA 4	AATCGGGCTG	10	5	50.0
OPA 7	GAAACGGGTG	8	4	50.0
OPA 9	GGGTAACGCC	11	9	81.8
OPA 10	GTGATCGCAG	9	6	66.6
OPA 13	CAGCACCCAC	7	5	71.4
OPA 18	AGGTGACCGT	5	4	80.0
OPA 20	GTTGCGATCC	2	1	50 .0
	Total	70	47	67.6% (Avg)

Figure 2
Polymorphic bands generated by different RAPD decamer primers.



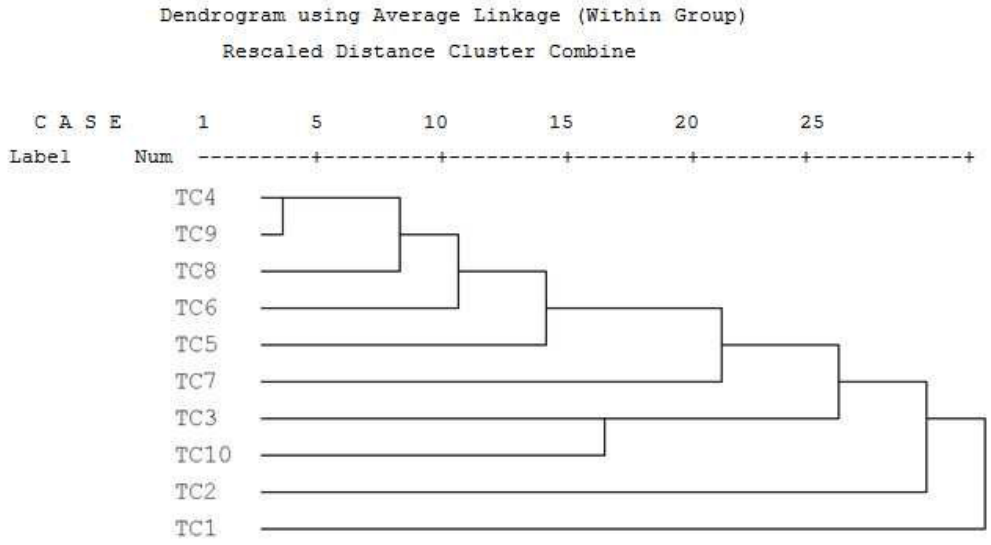


RAPD amplification profile of Tephrosia calophylla accessions with different RAPD primers.M-1 kb DNA ladder , Lane 1:TC1, Lane2:TC2,Lane 3:TC3, Lane 4:TC4, Lane 5:TC5, Lane6:TC6, Lane7:TC7, Lane8:TC8, Lane9:TC9, Lane10:TC10 .
a)OPA 4 , b)OPA 9, c)OPA 18, d)OPA 20. Profiles.

Table 3
Similarity matrix generated for Jaccard´s coefficient for Tephrosia calophylla accessions.

Case	Matrix File Input									
	TC1	**TC2**	**TC3**	**TC4**	**TC5**	**TC6**	**TC7**	**TC8**	**TC9**	**TC10**
TC1	1									
TC2	.650	1								
TC3	.632	.679	1							
TC4	.656	.672	.655	1						
TC5	.672	.690	.704	.754	1					
TC6	.695	.656	.667	.807	.768	1				
TC7	.525	.569	.667	.632	.745	.704	1			
TC8	.635	.625	.690	.797	.729	.750	.638	1		
TC9	.607	.623	.755	.836	.796	.786	.698	.807	1	
TC10	.571	.724	.774	.672	.719	.683	.655	.733	.737	1

Figure 3
UPGMA cluster analysis of RAPD data for 10 accessions of TC.



CLUSTER TREE ANALYSIS

RAPD data of ten accessions with 9 reproducible primers generated similarity coefficient with maximum similarity value of 83.6% between accessions of TC9 and TC4 followed by TC9 and TC8; TC6 and TC4(80.7%) whereas least similarity was found between TC1 and TC 7(52.5%) followed by TC1 & TC2(56.9%) and henceTC1,TC7&TC2 were most diverse accessions.The cluster Tree analysis (fig 3) showed that the 10 Tephrosia calophylla accessions were broadly classified into one main cluster .This cluster further divided into twosubclustershaving TC4,TC9,TC8,TC6,TC5 and TC7. Second sub cluster having TC3 and TC10. Whereas the accessions TC2 and TC1 did not resemble any other accessions forming a separate branch.

DISCUSSION

The RAPD technique had been successfully used in variety of taxonomic and genetic diversity studies and it was found suitable to use with *Tephrosia calophylla* genotype because of its ability to generate reproducible polymorphic bands. A number of studies on the assessment of genetic diversity in different species using a variety of DNA markers have been reported.PCR based markers are now being used widely . These techniques include RFLP, RAPD ,AFLP,etc ,In the present investigation ,RAPD a PCR- based technique ,has been reported to assess the variability at the intraspecific level.In this technique ,arbitrary short oligonucleotide primers targeting unknown sequences are used to generate amplified products that often show polymorphism within species[13-14].RAPD for example require far less material because only small amounts of DNA are needed. This RAPD technique has several advantages such as speed, low cost and usage of small amounts of plant materials.[15].The genetic structure of plant populations reflects the interaction of many different processes such as long- term evolutionary history of the species.(eg., shifts in distribution, habitat fragmentation, population isolation , mutation , genetic drift, mating system, gene flow and selection[16-17]. All of these factors can lead to complex genetic structuring within populations. Brown proposed that a fraction of about 10% is an appropriate sample size for sampling core entries from whole collection in germplasm respositories. The calculations using this theory has lead to the result that at least 70% of the existent alleles could be drawn with 95% certainity if 10% or more of the plants were sampled from the population[18].The present study shows an average polymorphism of 67.6% .It is higher in comparison with other plants like *Oroxylum indicum*(49.16%)[19],, *Paeonia suffruticosa*(22.5%),*Paeonia rockii*(27.16)[20] .The plant shows vegetative propagation through tubers with rare flowering and is an outcrossing plant. This amount of polymorphism may be attributed due cross pollination and more genetic drift.The herbs as opposed to long – lived trees experience a higher number of life cycles within a given period of time and may respond more quickly to the environment al changes[21].Low genetic diversity could result from self pollination,lack of geneflow,limited gene drift and reduced mutation rates.It is more likely that the different levels of intra populational diversity are due to the effects of population size rather than the influence of the ecological conditions[22]. The high diversity is the reflection of adaptation to the environment, which is beneficial to its propagation ,resources conservation. The high level of genetic variation discovered in natural populations indicates that this population has plenty of scope for evolution to occur[23]. The level of genetic diversity of populations as well as the degree of gene differentiation between the populations is important for genetic conservation. The maintenance of genetic diversity is considered crucial for long-term survival and the evolutionary response of population to adapt to the changes in the environment[24].

This study was an attempt to establish the genetic diversity background in *Tephrosia calophylla* with RAPD markers. High levels of polymorphism found in this work showed that RAPD markers as a suitable tool for genetic

diversity studies. This study could pave the way for detailed research to understand all the aspects of this divergence

CONCLUSION

In the present study we have developed a series of RAPD profiles for assessing genetic diversity within the population. The fundamental source of bioprospecting and for any plant improvement program is genetic diversity. The diversity within, or between these plants forms valuable genetic resource on the earth. Genetic variation holds the key to the ability of populations and species to persist over evolutionary time through changing environments. Several molecular techniques such as RAPD are very useful in detecting the genetic diversity. Genetic diversity is considered very important for designing conservation strategies. The present work helps in doing so.

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REFERENCES

1. Uma shaanker R.,Ganeshaiah KN., Mapping genetic diversity of *Phyllanthus emblica* :Forest gene bank as a new approach for insitu conservation of genetic resources. Current science,73:163-1658. (1997).
2. Sri Rama Murthy K., Sandhya Rani S., and T. Pullaiah. Systematic analysis and little known plants of Fabaceae: Faboideae in Eastern Ghats, India .In: Recent trends in plant Sciences. 77-87; Regency Publications, New Delhi. (2005)
3. Charyulu D.R., and Rao K.N .,*Tephrosia calophylla*- a rare medicinal plant.J.Econ.Taxon Bot 13:223-225. (1989).
4. Pulliah T ., and Ramamurthy K.S., Flora of Eastern ghats, Vol 2. (2001).
5. Reddy R.,, Khalivulla, S.I., Reddy MV,,Gunasekhar D., Deville A.,Bodo B.,Flavanoids from *Tephrosia calophylla*. Nat Prod Commun ;4(1):59-62. (2009).
6. Seru Ganapathy., Guttula Veera Kantha., Srilakshmi., Steve Thomas Pannakaj., Hafizur Rahman. Cytotoxic benzyl coumestan derivatives from *Tephrosia calophylla*.Phytochemistry;70(1):95-99. (2009).
7. Adinarayana K.,J ayaveera K.N.,Mallikarjuna Rao., P.Madhusudhana Chetty., C.,Sandeep., D.K.Swetha.,C and Mohamed Saleem T.S.M. Acute toxicity and hepato protective effect of the methanolic extract of *Tephrosia calophylla*. .Res J.Med.Plant 5:266-273. (2011).
8. Subhadra.S., Kancharapalli VR.,Vinod kombath Ravindran .,Sai Kumar Paree.,Sunitha chintala.,Rjeshwar Thatipally.,"Comparitive toxicity assessment of three *Tephrosia* species on Artemia Salina and animal cell lines". J Pharma Vol. 2, issue.3, page No.143-148(2011).
9. Anitha G., and sudarshanam, G. Studies on antimicrobial activity of *Tephrosia calophylla* against *xanthomonas citri* and *salmonella typhimurium*. *IJABPT. p.339-342*(2013).
10. Divya S., Haritha V., Prasad K.V.S.R.G., Evaluation of *Tephrosia calophylla* for anti ulcer activity. In experimental rats. Pharmacologyonline 3. P.573- 585. (2011).
11. Rosa Martinez.,Carolina A.,Sonsoles F . Genetic variability among *Alexandrium tamarense* and *Alexandrium minutum*

strains studied by RAPD banding pattern analysis .J.Harmful Algae,(5):599-607. .(2005)
12. Murray HG., Thompson WF., Rapid isolation of high molecular weight DNA. Nucleic Acids Res 8:4321-4325. (1980).
13. Rodriguez JM., Berke T., Engle L and Nienhuis, J, Variation among and within capsicum species revealed by RAPD markers. Theory .appl. genetics 99,147-156.(1999).
14. Jain, A., Bhatia ,S., Banga, SS.,Prakash CS and Lakshmi Kumaran. Potential use of random amplified polymorphic DNA (RAPD) technique to study the genetic diversity in Indian mustard (*Brassica junceae*) and its relationship to heterosis. *Theor.Appl.Genet*.88,116-122. (1994).
15. Williams JGK.,Kubelik AR.,Livak KJ.,Rafalski JA.,Tingey SV .DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res ., 18:7213-7218. (1990)
16. Schaal BA ., Hayworth DA .,Olsen KM .,Rauscher JT and Smith WA ,Phylogeographic studies in plants: Problems and prospects .Mol.Ecol.7, 465-474. (1998).
17. Slatkin M ., Gene flow and the geographic structure of populations.Science.236,787-792. (1987)
18. Brown A.H.D., The case of core collections ,In: The use of Plant Genetic Resources .Brown ,A.H.D., Frankel, O.H.,Marshell,D.R. and Williams, T.J. (eds)Cambridge University Press. .(1989).
19. Jayaram K., and Prasad M.N.V. ,Genetic diversity in *Oroxylum indicum* (L.)Vent (Bignoniaceae),a vulnerable medicinal plant by random amplified polymorphic DNA marker. African Journal of Biotechnology Vol,7(3),pp.254-263. (2008).
20. Pei Y.L., ZOU Y.P., YIN Z.,Wang., X.Q, Zhang., Z.X., Hong D.Y. Preliminary report of RAPD analysis in *Paeonia suffruticosa* susp. Spontanea and *P.rockii*, Acta phy.sin 33:350-356. (1995).
21. Comes H.P., and Kadereit J.W. The effect of quaternary climatic changes on plant distribution and evolution. Trends Plant Sci.3. (11), 432-438. (1998).
22. Suphiya Khan .,Vaishali and Vinay Sharma, Genetic differentiation and diversity analysis of medicinal tree *Syzygium cumini* (Myrtaceae) from ecologically different regions of India. Physio Mol Biol Plants, 16(2). (2010).
23. Narain, P. Genetic diversity – conservation and assessment. Current Science, Vol.79, NO. 2 (2000).
24. Nagananda.G.S., Chetana.G.R., Rajath.S., Sandeep suran and Sundara Rajan.Assessment of genetic variation among three species of Aerides, An epiphytic orchid from Western ghats, India using RAPD markers. Int J Pharm Bio Sci :682 – 688; (2012).