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RESEARCH ARTICLE

USE OF ISSR MARKERS FOR THE STUDY OF GENETIC POLYMORPHISM AMONG POPULATIONS OF CO*MIPHORA WIGHTII* (ARNOTT.) BHANDARI

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A B S T R A C T

Effective conservation and management of plant genetic resources is imperative and can only be brought about by a thorough understanding of the species in concern, both at physiological and molecular level. In particular, an adequate knowledge of the existing levels of genetic diversity within and between populations is a fundamental requirement for both basic and applied research. C*ommiphora wightii* has been as a deemed critically endangered species owing to its heedless exploitation for gum resin and fuel wood. The study was conducted to assess the genetic diversity among the different populations of this endangered medicinal plant, in Rajasthan, using ISSR markers. UPGMA dendrograms constructed using the ISSR profiles give an indication that polymorphism can be attributed to variables like edaphic factors.

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INTRODUCTION

Commiphora wightii (*Arnott.*) Bhandari, locally known as "Guggal", belongs to the family Burseraceae. Six species of the genus *Commiphora* are found in the Indian sub continent[1] and three species (*C. wightii*, *C. agallocha* and *C. berryi*) occur in India[1,2]. The plant is a shrub or small tree with thin papery bark. The plant grows wild in arid and semi arid climate and unfertile soils of Rajasthan, Karnataka and Gujarat. The plant exudes oleo-gum resin when mechanical or natural injury is inflicted on the bark. This oleo-gum resin comprises of several plant sterols, diterpenes and steroids[3]. However, Its main components are guggulsterones E and Z[4], which are involved in anti-cholesterol activity[5]. Guggal gum is known to hypolipidemic, hypocholestremic and an anti-obesity agent [5, 6, 7, 8]. The hypolipidemic activity of guggal gum is attributed to its antagonistic effect against farnesoid X, a nuclear hormone receptor that is activated by bile acid [8].

C.wightii has been included in the IUCN Red data list and UNDP has listed this species as critically endangered[9]. The guggal gum is extracted from the plant by mechanical injury this has resulted in the decline of its population from its natural habitat in Rajasthan. In addition, the natural propagation by seeds is very poor; this is attributed to the low seed viability. Observations have revealed that this species is under threat in its entire range of distribution at all areas they inhabit in Rajasthan and Gujarat, and as per a survey in Rajasthan during 2007 the species was present only in 2% of sample plots confirming its rarity [10]. The study by Reddy et al., 2012[10] has revealed that *C wightii* is facing severe threat and risk of extinction due to various factors like over-exploitation for guggul gum, narrow extent of occurrence, severe fragmentation of population, small area of occupancy, very low regeneration capacity and invasion of alien species. Therefore, there is urgent need to acknowledge the threats and problems encountered by the plant, to plan effective conservation strategies.

For effective conservation, management and efficient utilization of plant genetic resources, it is very important to understand the molecular basis of the essential biological phenomena in plants. In particular, an adequate knowledge of existing genetic diversity within and between populations at molecular level is fundamental interest for basic science and applied aspects like the efficient management of the plant genetic resources. The Present study is an attempt to assess the molecular basis of genetic diversity among the population of an endangered medicinally important plant *Commiphora wightii inhabiting* the diverse locations in Rajasthan, using ISSR markers.

MATERIALS AND METHODS

Plant Material

Plant material for genomic DNA isolation was collected from identified wild populations of *C. wightii* as mentioned in table 1. Leaves were brought in the laboratory in liquid nitrogen and stored at - 20ºC in zip lock bags. The leaves were subjected to the extraction of genomic DNA by minor modifications in CTAB method[21].

DNA Isolation Protocol

Fresh green leaves weighing 0.5 g were de-veined and grinded to a fine powder in mortar pestle using liquid N_2. A 60 ml homogenization buffer stock was prepared by adding

9ml 150 mM Tris-Cl, 3 ml 25mM EDTA, 18ml 1.5 M NaCl (all at pH 8.0) to 30 ml of DDW, and warmed at 65ºC. 2.1g CTAB and 1.8g PVP was added to the pre-warmed solution, 180 µl Beta Mercaptoethanol was added prior to the process of homogenization. The fine leaf powder was then suspended in 3 ml of pre-warmed CTAB solution. This 3 ml suspension was transferred to a sterile centrifuge tube & 20 µl of RNase was added to it. The solution was incubated for 45 minutes at 65ºC with gentle inversions. The tube was then cooled to room temperature & 3 ml of Chloroform: IAA ratio (24:1) was added to it. The tube was inverted gently 20-25 times to form an emulsion. The emulsion was centrifuged at 10,000 rpm for 10 min. at RT. The upper aqueous layer was pipette out, transferred into sterile centrifuge tubes without disturbing the interphase. 3 ml of 3M NaCl was added to the aqueous phase and once again subjected to centrifugation at 10,000rpm at RT. 0.6 volumes (1.8 ml) Isopropyl alcohol was added to the aqueous phase, mixed well and incubated for 30 min. at RT. The solution was centrifuged at 10,000 rpm for 15 min. at RT. The supernatant obtained was gently poured off. The pellets obtained were washed thoroughly with 750 µl of 70% ethanol & spun at 10,000 rpm for 5 minutes. The supernatant was discarded and the white pellet obtained was air dried (~45 min), & then re-suspended in 30 µl of TE (10 mM Tris HCl+ 0.1 mM EDTA; pH 8.0) at 4ºC. 3M sodium chloride solution was again added to the T.E. Buffer + DNA solution and re-precipitation was done with ethanol. The sample was centrifuged at 10,000 rpm for 15 minutes and pellet was re-dissolved in TE buffer. The process was repeated three times. This method allowed recovery of good quality DNA, suitable for complete restriction digestion was amplifiable in PCR as compared to other methods.

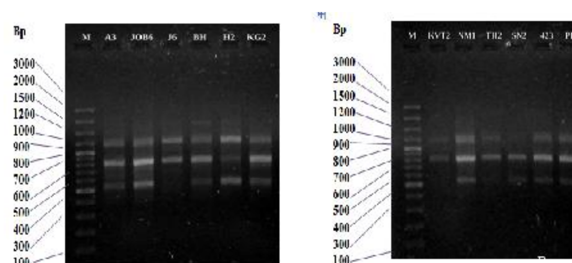

Figure 1 ISSR profile of *C. wightii* generated by the primer UBC 881. **M** – Represents 100bp ladder. Samples collected from **A**- Ajmer (A3), JOB6 (Jobner), Jodhpur (J6), Bheem(BH), Gomti Chouraha (H2), Kirwa Ghaat(KG2), Kavita village (KVT2), **B** - Nimach Mata (NM1), Thoor village (TH2), Sri Nagar village(SN2), Mangaliyawas (423) and Pandu beri, Pushkar (PB1).

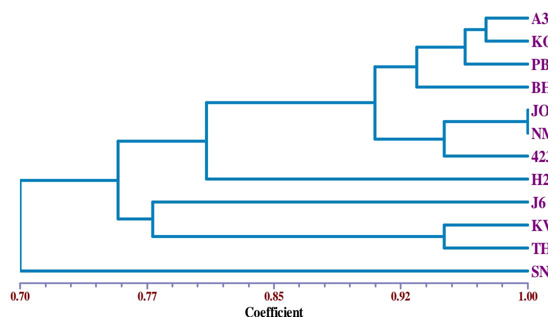

Figure 2 Dendrogram generated using un-weighted pair of group method with arithmetic average analysis (UPGMA), showing relationships between different populations of *C.wightii,* using all the data obtained from the various ISSR primers used.

samples were diluted to final concentration of 5ng/µl and 10ng/µl for use in PCR analysis. DNA samples which were the best in quality as evident on agarose gel and an OD_{260}/OD_{280} ratio nearer to 1.7-1.8 were used for further analysis.

Table 1 Location of the sampled population of *C. wightii* with terrains and average climatic conditions

S.No.	Area	Terrain	Population Code	Sample size	Sample code	Climatic Conditions
Ajmer Region						
1.	Ajmer	Hilly	A	7	A3	Hot, Semi Arid
2.	Pushkar	Hilly	PB	5	PB1	Hot, Semi Arid
3.	Srinagar	Rocky	SN	5	SN2	Hot, Semi Arid
4.	Mangaliyawas	Hilly	M	10	423	Hot, Semi Arid
Jaipur Region						
1.	Jobner	Plains	JOB	6	JOB6	Hot, Semi Arid
Jodhpur Region						
1.	Kailana	Plains	J	7	J6	Hot, Arid climate
Rajasamand Region						
1.	Bheem	Rocky	BH	1	BH	Sub-tropical dry climate
2.	Gomti Chouraha	Rocky	H	6	H2	Sub-tropical dry climate
Udaipur Region						
1.	Kirwa Ghat	Hilly	KG	4	KG2	Tropical climate
2.	Kavita	Hilly	KVT	2	KVT2	Tropical climate
3.	Neemach Mata	Hilly	NM	2	NM1	Tropical climate
4.	Thoor	Hilly	TH	5	TH2	Tropical climate

DNA Quantification

DNA concentration was estimated using spectrophotometric method (UV-Vis Spectrophotometer, Pharmaspec UV-1700, Shimadzu, Japan). Absorbance of the solution was measured at wavelengths 260 nm and 280 nm. The DNA concentrations were calculated using following formula:

$$\text{DNA concentration } (\mu g/ml) = \frac{50 \times OD_{260} \times \text{Dilution Factor}}{1000}$$

The ratio of OD_{260}: OD_{280} was calculated. The same procedure was followed for quantifying all the samples. DNA

ISSR Amplification

Ten inter simple sequence repeats (ISSR) primers (Eurofin Genomics, Bangalore, India) namely: 811P, 814P, 815P, 818P, 826P, UBC-810, 842, 897, 880, 881 were used in a final volume of 25 µl containing 50 ng of template DNA, 2.5 µl of 10X assay buffer, 25mM (2.0 µl) $MgCl_2$, 200µM (0.5 µl) dNTPs mix (10 mM stock), 2.0 µl (20 pmol) ISSR primer (10 pmol/µl stock), and 1.0 unit (0.2 µl) of Taq DNA polymerase (5U/ µl stock). All the reagents of the reaction

mixture were procured from Bangalore Genei Pvt. Ltd., Bangalore, India, except primers. The ISSR amplifications were performed in a thermal cycler (BIO-RAD T100, USA)

First cycle consisted of initial denaturation of template DNA at 94ºC for 04 minutes, followed by 35 cycles of denaturation at 94ºC for 01 minute, primer annealing at melting temperature TmºC , ranging between 50.4ºC to 58.8ºC as mentioned by the supplier for 01 minute, primer extension at 72ºC for 02 minutes; and final extension at 72ºC for 07 minutes.

Table 2 Extent of polymorphism determined in different samples of *C. wightii* using ISSR primers, and their sequence used.

ISSR Primer	Sequence 5'–3'	Total Loci amplified	Polymorphic loci	Percentage polymorphism (%)	Range of amplicons (bp)
811P	$(GA)_8C$	06	02	33.33	800-200
814P	$(CT)_8$ A	03	01	33.33	1200-500
815P	$(CT)_8G$	03	00	00	1200-600
818P	$(CA)_8G$	05	01	20	1000- < 300
826P	(AC)8C	06	01	16.66	1500-500
UBC 842	(GA)8YG	07	00	00	1100-200
UBC 857	(AC)8YG	03	01	33.33	100-500
UBC 880	(GGAGA)3	08	01	12.50	1500-200
UBC 881	(GGGTG)3	06	03	50	1300-500
Total		47	10	22.11	-

Table 3 Similarity matrix for Nei and Li's Coefficient of twelve populations of *C.wightii*

Sample	A3	JOB6	J6	BH	H2	KG2	KVT2	NM1	TH2	SN2	423	PB1
A3	1											
JOB6	0.92	1										
J6	0.8	0.77	1									
BH	0.92	0.9	0.82	1								
H2	0.77	0.85	0.67	0.75	1							
KG2	0.97	0.95	0.82	0.95	0.8	1						
KVT2	0.77	0.7	0.77	0.7	0.6	0.75	1					
NM1	0.92	1	0.77	0.9	0.85	0.95	0.7	1				
TH2	0.82	0.75	0.77	0.75	0.6	0.8	0.95	0.75	1			
SN2	0.65	0.72	0.55	0.62	0.62	0.67	0.72	0.72	0.67	1		
423	0.85	0.92	0.75	0.82	0.82	0.87	0.77	0.92	0.72	0.8	1	
PB1	0.92	0.9	0.77	0.9	0.75	0.95	0.75	0.9	0.8	0.72	0.87	1

Data Analysis

The data was scored as "1" for the presence and "0" for the absence of band for each primer sample combination for ISSR analysis. A dendrogram was constructed using the un-weighted pair group method with arithmetic average (UPGMA) with the SAHN model of the NTSys-PC software to show a phenotypic representation of the genetic relationships as revealed by similarity coefficient (Sneath and Sokal, 1972)[11].

RESULTS

Commiphora wightii populations were analyzed using 25 ISSR primers, out of which 09 generated amplified products. Out of the 09 ISSR markers utilized, 07 produced reproducible polymorphic banding patterns. In the 800 P series of ISSR primers, 811 P produced 02 polymorphic loci, while primers 814P, 818P and 826P generated 01 polymorphic locus each. A total of 23 loci with band range size between 200bp to 1,500bp were generated from these primers of series 800P. 811P and 814P both generated 33.33% polymorphism, while primer 818P and 826P generated 20% and 16.66% polymorphism respectively. out of the four primers from the UBC series that were tested three of them generated polymorphic bands. In this series of ISSR primers, the highest percentage of polymorphism i.e. 50% with 03 polymorphic loci was generated by UBC 881; the band size for these loci ranged between 500bp to 1300bp. UBC 842 generated 07 loci while UBC 857 and UBC 880 generated 03 and 08 loci respectively; also the latter two generated 01 polymorphic locus each. UBC 857 generated 33.33% of polymorphism while UBC 880 generated 12.5%, with 01 polymorphic locus each. A total of 24 loci within a band range of 100bp-1500 bp were amplified by the primers of UBC series. A total of 47 loci were amplified from all the ISSR primers used, out of these, 10 were found to be polymorphic in nature. The dendrogram Figure 2 has been constructed using un-weighted pair group method with arithmetic averages or UPGMA with a SAHN module of the NTSYS software. It shows a phenetic representation of genetic relationships as revealed by the similarity coefficient for all the ISSR profiles that have been generated for *C.wightii.* The dendrogram reveals three clusters with outliers. Cluster I comprises of A3, KG2, PB1 and BH. Cluster II is composed of JOB6, NM1 and M423; with H2 being an outlier for these 2 clusters. KVT2, TH2 and J6 form cluster III and SN2 is outlier in the picture.

Jaccard's pair-wise similarity coefficient values among all the populations of *Commiphora wightii* using all the ISSR primer profiles generated vary between 0. 55 and 1.00 as shown in table 3. 55% similarity which happens to be the least in terms of similarity coefficient data for all ISSR profiles has been observed between J6 and SN2. H2-KVT2 and TH2-H2 show 60% similarities while 62% similarity is observed between: BH-SN2 & H2-SN2. A similarity of 65% has been recorded between 3 sample populations' pairs namely: J6-H2, SN2-KG2 and TH2-SN2. 77% similarity has been seen in many sample populations like A3-H2, KVT2-A3, JOB6-J6, J6-KVT2, NM1-J6, TH2-J6, KVT2-M423 and J6-PB1. 85% is observed in A3-M423¸JON6-H2 and H2-NM1. 92% similarity is observed in A3-JOB6, BH-A3, JOB6-M423, A3-PB1 and NM1-423. A similarity of 97% is observed between A3 and KG2 of the sample populations. The highest similarity coefficient of 1.00 or 100% similarity is observed between NM1 and JOB6.

DISCUSSION

Evaluation of genetic diversity among populations is routinely assessed at molecular level using a number of techniques that involve DNA analysis through which the level of variation can be measured directly. Molecular markers like ISSR and/ or RAPD have been employed for the analysis of polymorphism and diversity in a number of spice plants (15, 16,17), Jatropha species (18,19,20) and citrus species (12,13,14). Long term observations have revealed that *C. wightii* is under threat in its entire range of distribution in Rajasthan due to over exploitation and very low regeneration capacity, the present investigation is an attempt to assess the polymorphism and genetic diversity of the species for its effective conservation, management and to utilize this plant genetic resource efficiently by using ISSR markers. As per our field observations the plant inhabits a diverse range of climatic zones such as hot arid, hot semi arid, sub tropical dry and tropical zones in Rajasthan. A recent study by Harish et al, 2014 (22) assessed polymorphism in *C. wightii* and reported 73 to 100% polymorphism but as per our observations, an average polymorphism of 22.11% was observed with the series of ISSR primers we selected of the study. In the present study, we also analyzed genetic diversity by generating a UPGMA dendrogram of all the ISSR profiles. The dendrogram comprised of all the sample populations being distributed into three major clusters; the sample population SN2 was an outlier and shared 70% similarity with the other sample populations.

Several studies on genetic diversity of plants by molecular markers have established the correlation between geographical distances and genetic similarity between individuals. (21,22,23). In the present study the dendrogram for the all the 12 sample populations using ISSR markers showed an intriguing pattern formation of clusters.

The major cluster comprises of 11 samples divided into smaller sub clusters and sample population SN2 from the Sri Nagar village, being an outlier for all these. This can be attributed to the change in edaphic factors, as the area where the plant population grows, is a low ditch, which caused accumulation of water and nutrients for the plants to thrive well, in comparison to the other zones.

References

1. Bhatt AD, Dalal DG, Shah SJ, Joshi BA, Gajjar MN, Vaidya RA, Vaidya AB and Antarkar DS (1995) Conceptual and Methodologic Challenges of Assessing the Short-Term Efficacy of Guggulu in Obesity: Data Emergent from a Naturalistic Clinical Trial, *Journal of Postgraduate Medicine*, Vol. 41, No. 1, pp. 5-7.
2. Chen, K.; Ren, P.; Ying, C.; Jiang, Q.; Jia, X. Genetic relationships among of Jatropha curcas L. clones from Panzhihua, China as revealed by RAPD and ISSR. Afr.J. Agric. Res., 2011, 6(11), 2582- 2585.
3. Coletta Filho, H. D., Machado, M. A., Targon, M. L. P. N., Moreira, M. C. P. Q. D. G. and Pompeu, J., Jr. (1998). Analysis of the genetic diversity among mandarins (Citrus spp.) using RAPD markers. Euphytica, 102, 133±9
4. Corazza-Nunes MJ, Machado MA, Nunes WMC, Cristofani M, Targon MLPN (2002) Assessment of genetic variability in grapefruits (Citrus paradisi Macf.) and pummelos (C. maxima Burm. Merr.) using RAPD and SSR markers. Euphytica. 126: 169–176
5. El Ashry ES, Rashed N, Salama OM, Saleh A (2003) Components, therapeutic value and uses of myrrh. *Pharmazie*, *58*, 163-168.
6. Fang, D.Q., R.R. Krueger, and M.L. Roose. 1998. Phylogenetic relationships among selected Citrus germplasm accessions revealed by inter simple sequence repeat (ISSR) markers. J. Amr. Soc. Hort. 123:612-617
7. Ganesh Ram S, Parthiban KT, Senthil Kumar R, Thiruvengadam V, and Paramathma M (2008) Genetic diversity among Jatropha species as revealed by RAPD markers, Genet. Res. Crop Evol., DOI 10.1007/s10722-007-9285-7
8. Gupta S, Srivastava M, Mishra GP, Naik PK and Chauhan RS (2008) Analogy of ISSR and RAPD markers for comparative analysis of genetic diversity among different Jatropha curcas genotypes, Afr. J. Biotechnology, 7, 4230-4243.
9. Haque I, Bandopadhyay R, Mukhopadhyay K (2010) Population genetic structure of the endangered and endemic medicinal plant Commiphora wightii. Mol Biol Rep 37:847–854
10. Harish, Gupta AK, Phulwaria M, Rai M K and Shekhawat NS. Conservation genetics of endangered medicinal plant *Commiphora wightii* in Indian Thar Desert. Gene. 2014; 535(2) : 266-72.
11. Lal H and Kasera PK (2010) Status and Distribution Range of Guggal: A Critically Endangered Medicinal Plant from the Indian Thar Desert, *Science & Culture*, Vol. 76, No. 11-12, pp. 531-533.
12. Ramawat KG, Mathur M, Dass S and Suthar S (2008) Guggulsterone: A Potent Natural Hypolipidemic Agent from *Commiphora wightii*-Problems, Perseverance, and Pros- pects, In: K. G. Ramawat and J. M. Merillon, Eds., *Bio- active Molecules and Medicinal Plants*, Springer, Hei- delberg, , pp. 101-121. doi:10.1007/978-3-540-74603-4_5
13. Ranade SA, Rana TS, Srivastava AP, Nair KN. 2006. Molecular differentiation in Murraya Koenig ex L. species in India inferred through ITS, RAPD and DAMD analysis. Current Science India 90: 1253- 1258.
14. Reddy CS, Meena SL, Krishna PH, Charan PD and Sharma KC (2012) Conservation Threat Assessment of *Commiphora wightii* (Arn.) Bhandari-An Economically Important Species, *Taiwania*, Vol. 57, No. 3, pp. 288-293
15. Satyavati GV, Dwarakanath C, Tripath SN (1969) Experimental studies on the hypocholesterolemic effect of Commiphora mukul Engl. (Guggul). Indian J Med Res 57:1950–1962
16. Singh S, Panda MK, Nayak S (2012) Evaluation of genetic diversity in turmeric (Curcuma longa L.) using RAPD and ISSR markers. Industrial Crops and Products Ind. Crops Prod. [Internet]. 37(1):284 - 291.
17. Sneath PHA and SOKAL RR (1973) Numerical taxonomy. W. H. Freeman, San Francisco
18. Suthar, S., Thul, S., Kukreja, A. K. and Ramawat, K. G. (2008) RAPD markers reveal polymorphism in *Commiphora wightii*, an endangered medicinal tree,

Journal of Cell and Tissue Research Vol. 8(2) 1477-1480

19. Tripathi SN, Sastri VVS and Satyavati GV (1968), Experimental and Clinical Studies on the Effects of Guggul (*C. mukul*) in Hyperlipidemia and Thrombosis, *Journal of Research in Indian Medicine*, Vol. 2, No. 2, p. 10
20. UNDP (2008) Rajasthan Red Listed Medicinal Plants, pp. 22-23. http://www.frlht.org
21. Urizar N L, Moore D D (2003) Gugulipid: a natural cholesterol lowering agent. Annul Rev Nutrition 23:303–313
22. Verma N, Singh SK, Gupta RC (1998) Simultaneous determination of the stereoisomers of guggulsterone in serum by high-performance liquid chromatography. J Chromatogr 708:243–248
23. Verma S and Rana TS (2011). Genetic diversity within and among the wild populations of Murraya koenigii (L.) Spreng., as revealed by ISSR analysis. Biochemical Systematics and Ecology 39(2):139-144

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