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Genetic diversity analysis of *Oroxylum indicum* (L) Vent, an endangered and important medicinal plant using Inter Simple Sequence Repeat (ISSR) markers

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Abstract
Oroxylum indicum (L) Vent (Bignoniaceae) is an important but endangered medicinal plant of Western Ghat region. It is also found in North east region and in foothills of Himalayas. In the present study, Inter Simple Sequence Repeat (ISSR) and Random Amplified Polymorphic DNA (RAPD) were employed to investigate the genetic diversity of the plant *Oroxylum indicum* L (Vent) collected from four different locations. 5 RAPD and 15 ISSR markers were used to compare and predict the genetic similarity or differences within accessions analysed. Eight ISSR primers together produced 64 bands across four genotypes, of which 28 bands were polymorphic showing 43.89 % polymorphism. The results show that the variation among populations or geographic region is more prominent and divided the plants region wise indicating the role of geographic isolation in shaping the present population genetic structure. This distributive pattern of genetic variation of *Oroxylum indicum* (L) Vent accessions provides an important baseline data and implies that the conservation efforts should aim to preserve this endangered species of Western Ghats.

Keywords: *Oroxylum indicum* (L) vent, ISSR and RAPD

1. Introduction

Advent of modern analytical tools necessitated development of new methods to gauge genetic diversity across different biomes. RAPD and ISSR markers are increasingly being employed for ensuring the quality control of herbal drugs sourced from different geographical regions. Oroxylum indicum Vent. (Family- Bignoniaceae) syn: Shyonaka, is an important herb in Ayurvedic medicine and indigenous medical system for over thousands of years [1].

Oroxylum indicum L. (Vent.) have been used as a single drug or as a component of well known Ayurvedic formulations like Chyavanprash, Dashmularista etc. [2]. The root bark and stem bark possess antiallergic properties & are used in treating allergic disorders, urticaria, jaundice, asthma, sore throat, laryngitis, hoarseness, gastralgia, diarrhoea, dysentery, erythema and measles [3].

While the plants belonging to same species may differ in their chemical or elemental profile [4, 5] if collected from two geographically distinct locations, but their DNA fingerprints would establish authenticity and add identity to the plant. Similarity of DNA fingerprints depends on genetic closeness of tested samples. DNA fingerprinting can distinguish plants from different families, genera, species, cultivars etc. Thus identification and characterization of germplasm is an important link and necessary for proper conservation and optimal utilization of plant genetic resources. The use of DNA fingerprinting for germplasm characterization is also useful in establishment of sovereign rights over indigenous plant genetic resources. Attempts were made to correlate *Oroxylum indicum* L. (Vent.) from collected from Karnataka and Assam state of India [6], but similar studies to correlate *Oroxylum indicum* L. (Vent.) from Maharashtra state and North East state is not reported in literature. Thus an attempt has been made to study the DNA fingerprint of this plant collected from these regions using random molecular markers such as Inter Simple Sequence Repeat (ISSR) and Random Amplified Polymorphic DNA (RAPD).

2. Material and Methods

Leaves of plants growing in Western Ghat (District Ratnagiri, Maharashtra, India), Sahydri hills (District Pune, Maharashtra, India) and two locations of North east India (District Lakhimpur, Assam and Dispur, Assam, India) were collected and freezed to -200C immediately after collection. Plant samples from western region were referred as WS2 and SY1, while plants sourced from North East were designated as NE1 (District Lakhimpur) and NE2 (Dispur, Assam). Total genomic DNA was isolated from young leaves of plants growing in respective habitat. The leaf tissue was powdered in liquid nitrogen and immediately transferred in 1 ml of extraction buffer (100 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, 2% CTAB, 0.2% β-mercaptoethanol (v/v) and 1% PVP (w/v) and incubated at 65oC for 60 min. An equal volume of chloroform: isoamylalcohol (24:1 v/v) was added to the extract and centrifuged at 10,000 rpm.

ISSR and RAPD marker amplification studies were carried out using five arbitrary decamer primers (RAPD) sequences from Operon Technologies, Alameda, CA, USA and fifteen ISSR Primer sequences from the Biotechnology Laboratory, University of British Colombia (UBC) were synthesized by Eurofins. PCR amplifications were performed in 15 µl volumes, using a VERITI 96 well thermal cycler (Applied Biosystems, USA). The reaction mixtures contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 10-20 ng genomic DNA, 10

pmol primer, 1.5 mM MgCl2, 200 µM of each dNTP (In vitrogen by Life technologies, USA), and 1U Taq DNA polymerase (Bangalore Genie). The temperature profile consisted of an initial denaturation step at 94°C for 4 min, followed by 40 cycles. The PCR products were separated on 1.5% agarose gel 1X TBE buffer (Tris-borate-ethylene di-amine tetra acetic acid) and was estimated with 100bp DNA ladders (Life technologies, USA). The gels were visualized under UV using ALPHAIMAGER EC gel documentation system. Fifteen ISSR and five RAPD primers were used for the initial testing of variability and reproducibility of the band. Eight ISSR primers were selected based on polymorphism level within the population and reproducibility were screened three times and band scored for diversity analysis for 20 min at room temperature (RT). The aqueous phase was aspired and placed in a new 2.0 ml tube. Equal volume of chilled isopropanol was added followed by centrifugation at 10,000 rpm for 20 min at RT. The pellet was washed with 70% ethanol by centrifuging at 10, 000 rpm for 8 min. The DNA was then treated with1 µl of RNase A (10 mg/ml) incubated for 1 h at 37^0 C, extracted twice with chloroform: Iso-amylalcohol (24:1). Then it was centrifuged at 12,000 rpm for 10 min and washed twice with 80% ethanol. DNA was dissolved in 100 µl of autoclaved Milli Q water.

Quantity and quality of extracted DNA is checked and estimated by visual assessment of band intensities in comparison to Lambda DNA marker on 0.8% agarose gel stained using Ethidium bromide. DNA samples were diluted with sterile Milli Q water to 5 ng/µl and used as templates for PCR amplification.

3. Results and Discussion

Eight ISSR primers together produced 64 bands across four genotypes of *Oroxylum inidcum* (L.) Vent, of which 28 bands were polymorphic showing 43.89% polymorphism (Fig 1, Table 1 and 2). Over 99% of the ISSR fragments were reproducible in the present experiment. The high reproducibility of ISSR markers may be due to the use of selective primers reported to be informative in one or more plant species in previous reports. To study the distribution of genetic variation in the populations, gene diversity statistics was calculated using Powermarker v. 3.23[7]. The results indicate that the variation among populations or geographic region is more prominent and divided the plants region wise (Table 3 and 4). The neighbour joining tree also conferred a clear grouping and differentiation based on origin of populations. Samples were grouped into two groups, plants originating from western region (WS2 and SY1) were grouped in one group and plants originating from North East (NE1 and NE2) in second group. Dendrogram constructed correlates with their geographical distribution (Fig. 2). Plant from Western ghat is more similar to plant in Sahyadri hill as compared to the plants from North east region. The tree simultaneously show high level of diversity within group showing 63% diversity between WS2 and SY1 from western region and around 80% diversity between NE1 and NE2. In general all four lines of *Oroxylum indicum* (L.) Vent are highly diverse showing 60 to 80 % diversity within group.

Table 1: List of informative ISSR primers used for diversity analysis of *Oroxylum indicum* (L.) Vent

Marker	Primer sequence (5'-- 3')	Total bands	Polymorphic bands	P(%)
UBC 807	AGAGAGAGAGAGAGAGT	**7**	**2**	**28.6**
UBC 808	AGAGAGAGAGAGAGAGC	7	3	42.9
UBC 810	GAGAGAGAGAGAGAGAT	9	5	55.6
UBC 818	CACACACACACACACAG	6	2	33.4
UBC 834	AGAGAGAGAGAGAGAGYT	10	5	50
UBC 845	CTCTCTCTCTCTCTCTRG	9	8	88.8
UBC 848	CACACACACACACACARG	8	3	37.5
UBC 888	BDBCACACACACACACA	8	0	0
Mean		8.25	3.5	42.8

Table 2: Binomial score of four genotypes of *Oroxylum indicum* L. (Vent.) scored using eight ISSR primers.

Primer No 807					
Sr. No	Band Size	WS2	SY1	NE1	NE2
1	1500	1	1	1	1
2	1000	1	1	1	1
3	500	1	1	1	1
4	470	1	0	0	0
5	350	1	0	0	0
6	320	1	1	1	1
7	300	1	1	1	1

Primer no 808					
Sr. No	Band Size	WS2	SY1	NE1	NE2
1	2000	1	1	1	1
2	800	1	1	1	1
3	700	1	1	1	1
4	600	0	1	1	1
5	500	1	0	0	0
6	450	1	0	1	1
7	400	1	1	1	1

Primer no 810					
Sr. No	Band Size	WS2	SY1	NE1	NE2
1	1300	1	1	0	1
2	800	0	1	1	0
3	520	0	1	1	0
4	500	1	1	1	1
5	450	0	0	1	0
6	400	0	1	1	0
7	380	1	1	1	1
8	280	1	1	1	1
9	200	1	1	1	1

Primer no 818					
Sr. No	Band Size	WS2	SY1	NE1	NE2
1	1000	1	1	1	1
2	650	1	1	1	1
3	550	1	1	1	0
4	450	1	0	1	0
5	400	1	1	1	1
6	380	1	1	1	1

Primer no 834					
Sr. No	Band Size	WS2	SY1	NE1	NE2
1	1100	1	1	1	1
2	950	1	1	1	1
3	900	1	1	1	1
4	750	1	0	0	0
5	700	0	0	1	1
6	550	1	1	1	1
7	520	0	0	1	1
8	480	0	0	1	1
9	420	1	0	0	0
10	400	1	1	1	1

Primer no 845					
Sr. No	Band Size	WS2	SY1	NE1	NE2
1	1400	1	1	0	0
2	1300	1	0	0	0
3	600	1	1	1	1
4	550	1	0	1	1
5	500	0	1	0	0
6	480	0	0	1	0
7	425	1	1	0	1
8	400	0	0	1	0
9	300	1	0	0	0

Primer no 848					
Sr. No	Band Size	WS2	SY1	NE1	NE2
1	1400	1	1	1	1
2	1200	1	1	1	1
3	700	1	1	0	0
4	650	1	1	-1	1
5	420	1	1	1	1
6	400	1	1	0	0
7	350	1	1	0	0
8	100	1	1	1	1

Primer no 888					
Sr. No	Band Size	WS2	SY1	NE1	NE2
1	700	1	1	1	1
2	600	1	1	1	1
3	550	1	1	1	1
4	500	1	1	1	1
5	400	1	1	1	1
6	300	1	1	1	1
7	100	1	1	1	1
8	80	1	1	1	1

Table 3: Jaccard similarity coefficient Matrix of four genotypes of *Oroxylum indicum* (L.) Vent

	WS2	SY1	NE1-1	NE2-1
WS2		0.74138	0.63492	0.70175
SY1	0.74138		0.7193	0.73585
NE1-1	0.63492	0.7193		0.80769
NE2-1	0.70175	0.73585	0.80769	

Table 4: Marker index of informative primers calculated using Powermarker Ver. 3.23

Marker	Major.Allele.Frquency	SampleSize	No. of obs.	AlleleNo	Availability	GeneDiversity	PIC
UBC807	0.7500	4.0000	4.0000	2.0000	1.0000	0.3750	0.3047
UBC808	0.5000	4.0000	4.0000	3.0000	1.0000	0.6250	0.5547
UBC810	0.5000	4.0000	4.0000	3.0000	1.0000	0.6250	0.5547
UBC818	0.5000	4.0000	4.0000	3.0000	1.0000	0.6250	0.5547
UBC834	0.5000	4.0000	4.0000	3.0000	1.0000	0.6250	0.5547
UBC845	0.2500	4.0000	4.0000	4.0000	1.0000	0.7500	0.7031
UBC848	0.5000	4.0000	4.0000	2.0000	1.0000	0.5000	0.3750
UBC888	1.0000	4.0000	4.0000	1.0000	1.0000	0.0000	0.0000
Mean	0.5625	4.0000	4.0000	2.6250	1.0000	0.5156	0.4502

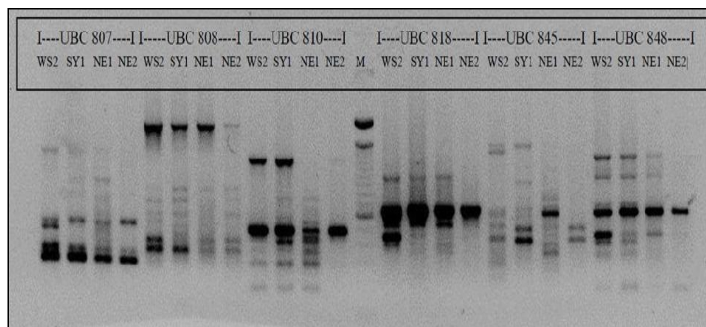

Fig 1: The PCR profile of *Oroxylum indicum* genotypes produced with six ISSR primer (Lane M is a 100 bp ladder).

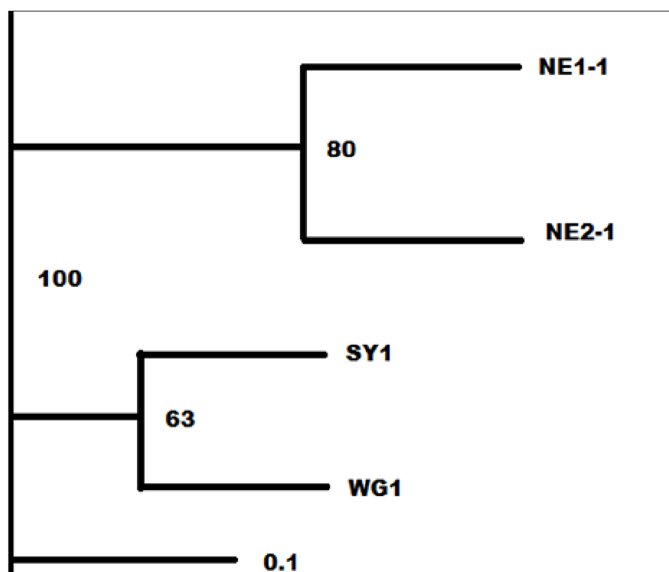

Fig 2: Dendrogram showing geographical distribution of four genotypes of *Oroxylum indicum* (L.) Vent

4. Conclusion

Plants collected from four different location grouped into two groups of two each based on their similarity index and genetic distance. The plant from Western Ghat (WS1) is more phylogenetically related with that of plant from Sahydri ranges growing at a distance of around 70 Km as compared to plants from North east India (900 Km). NE1 in turn is more closely grouped with another plant from North east region (NE2). ISSR and RAPD fingerprinting provides a powerful tool for the generation of potential diagnostic markers for cultivar analysis. Dendrogram derived by ISSR based phylogenetic analysis point towards the fact that there are certain region specific variations in *Oroxylum* lines. It may be because of the multiple generations of selection carried out after their introduction in the respective habitat.

5. References

1. Dinda B, SilSarma I, Dinda M, Rudrapaul P. *Oroxylum indicum* (L.) Kurz, an important Asian traditional medicine: from traditional uses to scientific data for its commercial exploitation. Journal of ethno pharmacology. 2015; 161:255-278.
2. Kritikar KR, Basu BD. Indian Medicinal Plants. Oriental enterprises. 2001; 4:1105-1107.
3. Harminder VS, Chaudhary AK. A review on the taxonomy, ethnobotany, chemistry and pharmacology of *Oroxylum indicum* vent. Indian journal of pharmaceutical sciences. 2011; 73(5):483.
4. Srinivas KS, Aparna AS. High Performance Thin Layer Chromatographic determination of chrysin in *Oroxylum indicum* vent. from different geographical regions of India. Journal of Chemistry. 2012; 9(1):313-317.
5. Srinivas KS, Saraf AA. Profile of Elemental Composition of *Oroxylum indicum* L. (Vent.) Collected from Different Geographical Regions of India. Pharmacognosy Journal. 2011; 3(24):50-54.
6. Rajasekharan PE, Kareem VK, Ravish BS, Mini S. Genetic diversity in *Oroxylum indicum* (L.) Vent. a threatened medicinal plants from India by ISSR analysis. Indian Journal of Biotechnology. 2017; 16(3):357-365
7. Liu K, Muse SV. Power Marker: an integrated analysis environment for genetic marker analysis. Bioinformatics. 2005; 21(9):2128-2129.