Analysis of genetic diversity of Lagenandra spp. (Araceae) of Kerala (South India) using ISSR Markers

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Analysis of genetic diversity of *Lagenandra* spp. (Araceae) of Kerala (South India) using ISSR Markers

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KEYWORDS : Araceae, variation, ISSR, cluster analysis, dendrogram

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ABSTRACT

The Present study reveals the molecular variations in different species of Lagenandra, an aquatic plant collected from different geographical areas of Kerala State, India. Molecular analysis was carried out using ISSR markers. Out of the 18 primers screened, a total of 66 scorable polymorphic markers were generated. The genetic distance between the population ranged from 0.0016 to 0.0271 and the genetic identity ranged from 0.9732 to 0.9984. The standard deviation of Gene diversity, Shannon's Information index and effective number of alleles are about 0.1468, 0.0719 and 0.0866 respectively. It is evident that that there is distinct genetic variability among Lagenandra species, occurring in Kerala

Introduction:

The genus *Lagenandra* coming under the angiosperm family Araceae consist of 15 species, of which six species were reported from Kerala which include *Lagenandra keralensis* Sivd & Jaleel, *Lagenandra meeboldii*(Engl.) C.E.C.Fisch., *Lagenadra nairii* Ramam. & Rajan, *Lagenandra ovata* (L.) Thwaites, *Lagenandra toxicaria* Dalz.var. *toxicaria* Hook, *Lagenandra toxicaria* Dalz. Var. *barnesii* Fischer. The genus is endemic top Bangladesh, Sri Lanka and India (Sivadasan *et al*, 2001). The plants are perennial, all species are ecologically very much alike, and they are usually in and along streams and rivers in forest and plantations but also in irrigation ditches around rice fields. In wet season they remain submerged and pollination is entomophilious. Many species are cultivated for decoration (Cook, 1996).

Among the six species, *L. ovata* (Pop. 1,2,3,4) are comparatively larger plants. *Lagenandra* spp. have certain medicinal values; it was evaluated for antibacterial activity against gram positive bacteria. The rhizome is used locally in the treatment of kidney disorders, heart diseases and swelling. *L. toxicaria* var. *toxicaria* (Pop. 11,12,15) and *L.toxicaria* var. *barnesii*(Pop. 13,14) are very much alike, except a few differences such as in *L. toxicaria* var. *barnesii* the petiole is about 3.5 cm long, the spathe is about 12 cm long opening with wide gap and outer surface is greenish pink. While in *L. toxicaria* var. *toxicaria* the petiole is about 9-10 cm long, spathe 6 cm long, open by a narrow slit and outer surface is greenish purple. These plants are traditionally used as water purifying agent because they absorb heavy metals, so they are often grown in well. Members of the species *L. meeboldii* (Pop. 5, 7) have some variations in leaf characters but resemble in reproductive characters. In one population (Pop. 7) the leaves are purple colored and the petiole is 13-14 cm long. While in another population (Pop. 5) of the same species, leaves are green pale green with white markings and the petiole is 4-5 cm long. *L. nairii* (Pop 8,9,10) are comparatively smaller plants than other species. It is a very rare plant reported only from the Athirapilly waterfalls of Thrissur district of Kerala. *L. keralensis* (Pop. 6) resembles *L. meeboldii*, but is smaller in size and has differences in the shape of spathe.

Molecular markers have been widely used to characterize population genetic structure of plants. These include allozymes and polymerase chain reaction (PCR) based markers like RAPD (Mirali & Nabulsi, 2003), ISSR (Wang *et al*, 2004), AFLP (Rottenberg & Parker, 2003), and SSR (Rosetto *et al*, 2004). Inter Simple Sequence Repeats (ISSR) is a molecular marker that has been widely used in the studies of cultivar identification, genetic mapping, gene tagging and genetic diversity analysis.

Studies on its level of genetic diversity and pattern of genetic structure of *Lagenandra* species found in Kerala region were not undertaken. To conserve a species, it is important to have such basic information. Therefore we decided to examine the genetic variation within and among natural populations of *Lagenandra* species using ISSR markers. The fact that no DNA sequence information is known for the species is one of the main reasons for choosing ISSR technique in this study. On the other hand, the ISSR markers have recently become widely used in population studies because they are highly variable, and require less investment in time, money, and labor than other methods.

Materials and Methods

Sample collection: Fresh young leaves collected from 15 populations of five *Lagenandra* species and six varieties collected from various localities of Kerala were used in the present study. The accessions (Table: 01) were also maintained in the Aquatic plant conservatory (Aquagene) of Malabar Botanical Garden Calicut, Kerala.

Table. 01: Species collected for the analysis with Accession code and place of collection

Population Name	Accession Code	Collection Localities
L. ovata (L.) Thwaites	Pop 1	Pariya, Kasarkode
L. ovata (L.) Thwaites	Pop 2	Madathara, Kollam
L. ovata (L.) Thwaites	Pop 3	Munnar, Idukki
L. ovata (L.) Thwaites	Pop 4	Pala, Kottayam
L. meeboldii(Engl.) C.E.C.Fisch	Pop 5	Peechi, Thrissur
*L.keralensis*Sivd& Jaleel	Pop 6	Boothathankettu, Ernakulam

L. meeboldii (Engl.) C.E.C.Fisch	Pop 7	Nelliyampathy, Palakkad
*L. nairiii*Ramam. &Rajan	Pop 8	Athirappally, Thrissur
*L. nairiii*Ramam. &Rajan	Pop 9	Athirappally, Thrissur
*L. nairii*Ramam. &Rajan	Pop 10	Athirappally, Thrissur
*L. toxicaria*Dalz. var. toxicaria Hook	Pop 11	Padinjarathara, Wayanad
*L. toxicaria*Dalz. var. toxicaria Hook	Pop 12	Chalakkudy, Thrissur
*L. toxicaria*Dalz. var. toxicaria Hook	Pop 13	Narikkuni, Kozhikode
*L. toxicaria*Dalz. var. barnesii Fischer	Pop 14	Dhoni forest, Palakkad
*L. toxicaria*Dalz. var. barnesii Fischer	Pop 15	Aruvikkara, Thiruvananthapuram

ISSR analysis:

15 populations of five species and six verities of *Lagenandra* were used for the present study. Total genomic DNA was isolated from the fresh leaf samples following modified Murray and Thompson (1980) method using CTAB. ISSR reaction was carried out in 25 µl volume containing 50 ng of Template DNA, 2.5 µl of 10x reaction buffers, 50 µM of each dNTP, 20pmol of random primer, 0.75U Taq DNA Polymerase. Sterile distilled water was added to the reaction mixture to make the volume to 25 µl. The PCR reaction was carried following an initial denaturation for 5 min at 95° C, followed by 40 cycles of 1 min. at 95° C, 30 sec. at 50° C and 1 min. at 72° C and a final extension at 72° C for 5 min. Primers were used (Biogen USA) for the amplification and 25 µl of the amplicon was resolved in 1.2 % agarose gel (Sigma USA). The gel was stained using ethidium bromide and the DNA bands were visualized under UV illuminator. Bands were scored as present (1) and absent (0). A dendrogram was constructed based on the Nei's mean of distance (Nei, 1972) by UPGMA method.

Results:

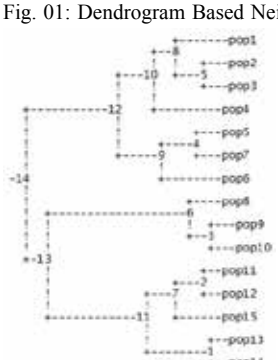
Diversity analysis using Inter-Simple Sequence Repeat (ISSR) analysis using 18 primers including 15 accession of *Lagenandra* species from different districts of Kerala revealed the extend of diversity between the populations. Cluster analysis based on UPGMA reveals 2 well differentiated clusters.In cluster one, *L. ovata,* accessions of *L. meeboldii* and *L. keralensis* were clustered together with 99.51% genetic identity.This cluster includes two sub clads, one with 4 accessions of *L. ovata* populations grouped together in a single clad with 99.46% similarity. In the other sub clad 2 the 2 accessions of *L. meeboldii* and *L. keralensis* were clustered with 99.55% genetic identity.

The second major cluster also include two subclads. The accessions of *L.nairii* and accessions of *L. toxicaria* with two varieties were also included in this major cluster with 99.41% genetic identity. Here one subclad with 3 accessions of *L. nairii* were grouped with 99.35% genetic identity. The second subclad includes two clads one with *L. toxicaria* Dalz. var. toxicaria and another with *L. toxicaria* Dalz. var. barnesii. The former variety grouped with a mean genetic identity of 99.67% and later with 99.84%.

Table No. 02: Nei's Original Measures of Genetic Identity (above diagonal) and Genetic Distance(below diagonal).

pop ID	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15
01	****	0.9945	0.9953	0.9917	0.9901	0.9842	0.9905	0.9827	0.9803	0.9774	0.9871	0.9871	0.9866	0.9823	0.9884
02	0.0055	****	0.9975	0.9958	0.9952	0.9908	0.9940	0.9837	0.9771	0.9758	0.9823	0.9830	0.9829	0.9786	0.9855
03	0.0047	0.0025	****	0.9930	0.9956	0.9909	0.9927	0.9851	0.9781	0.9774	0.9831	0.9818	0.9815	0.9779	0.9860
04	0.0083	0.0042	0.0070	****	0.9912	0.9810	0.9902	0.9820	0.9760	0.9735	0.9788	0.9781	0.9791	0.9732	0.9772
05	0.0100	0.0048	0.0045	0.0089	****	0.9942	0.9975	0.9867	0.9808	0.9804	0.9863	0.9858	0.9825	0.9800	0.9885
06	0.0159	0.0093	0.0091	0.0191	0.0058	****	0.9949	0.9846	0.9799	0.9789	0.9845	0.9851	0.9791	0.9767	0.9905
07	0.0096	0.0060	0.0074	0.0098	0.0025	0.0051	****	0.9913	0.9876	0.9863	0.9916	0.9918	0.9884	0.9856	0.9931
08	0.0174	0.0165	0.0150	0.0182	0.0134	0.0155	0.0087	****	0.9965	0.9964	0.9863	0.9849	0.9829	0.9802	0.9865
09	0.0199	0.0231	0.0221	0.0243	0.0194	0.0203	0.0125	0.0035	****	0.9977	0.9883	0.9865	0.9806	0.9777	0.9865
10	0.0229	0.0245	0.0229	0.0269	0.0198	0.0213	0.0138	0.0036	0.0023	****	0.9864	0.9849	0.9819	0.9796	0.9860
11	0.0129	0.0178	0.0170	0.0214	0.0138	0.0156	0.0085	0.0138	0.0118	0.0137	****	0.9984	0.9926	0.9907	0.9956
12	0.0130	0.0171	0.0184	0.0222	0.0143	0.0150	0.0082	0.0152	0.0136	0.0152	0.0016	****	0.9953	0.9928	0.9960
13	0.0134	0.0173	0.0186	0.0211	0.0176	0.0211	0.0116	0.0173	0.0196	0.0182	0.0075	0.0047	****	0.9984	0.9931
14	0.0178	0.0217	0.0223	0.0271	0.0202	0.0236	0.0145	0.0200	0.0225	0.0206	0.0093	0.0072	0.0016	****	0.9936
15	0.0116	0.0146	0.0141	0.0231	0.0116	0.0095	0.0069	0.0136	0.0136	0.0141	0.0044	0.0041	0.0069	0.0064	****

Fig. 01: Dendrogram Based Nei's (1972) Genetic distance



Cluster analysis

Lagenandra populations are grouped into 3 clusters (Fig. 01). Cluster I comprises of populations Pop. 1, 2, 3, 4, 5, 6 & 7 collected from different localities of Kerala. Cluster II interestingly grouped together the populations Pop. 8, 9 & 10 from Trichur district and the cluster III again comprises populations 11, 12, 13, 14 & 15from different districts of Kerala.

Discussion

Knowing the genetic variation is the first step for designing a plant conservation or genetic improvement program. This study demonstrates that ISSR-PCR offered a suitable method for the detection of genetic variability in *Lagenandra* species. These are semi-aquatic herb found in the marshes and along water courses, often growing in semi-evergreen forests.

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The genus *Lagenandra* comprises of two endemic species (*L. naririi & L. meboldii)* Both medicinal and taxonomic significances are taken into account for the present diversity analysis using ISSR. The ISSR has been widely used to investigate clonal diversity and population genetic structure. ISSR data generated in the genus *Lagenadra* collected from different localities of Kerala showed interesting pattern of genetic diversity existing in the genus. Under cluster I, Pop. 1 and Pop. 2, Pop.3 and Pop.2 and pop. 4 and Pop. 2 showed great genetic diversity between each other. All the populations are coming under the same species *Lagenandra ovata* and are morphologically very similar but are collected from different climatic areas. Pop.7 and Pop. 5 showed interesting genetic diversity as both these populations are coming under the same species *Lagenandra meeboldii* but have some morphological variations. In population 7 leaves are variegated with pale green markings on dark green leaves and the petiole is short. While in population 5 the leaves are purple cloured and petiole is long (13- 14 cm).

In cluster II- Pop. 10 and Pop. 9 showed high genetic diversity, but both populations belong to same species *L. nairii* . It is an endemic species reported only from Trissur district. Both the populations are collected from same locality and have no morphological dissimilarity.

Under cluster III -Pop.12 and Pop. 11, Pop. 13 and Pop. 11 showed great genetic diversity. But all the populations are under the same species *Lagenadra toxicaria* var. *toxicaria* and are morphologically so similar but are collected from various geographical areas.

Knowledge of the variation between and within populations of rare and endangered species play a significant role in the formulation of appropriate management strategies directed towards their conservation (Milligan *et al.,* 1994). These population genetic structure characteristics have significant implication for conservation strategies. Low genetic diversity may reduce the potential of species or population to survive in a changing environment (Ellstrand and Elam, 1993). There is an urgent need to take effective measures to protect these species against further loss of genetic diversity. This investigation provides valuable information about the nature and pattern of genetic variation exist within different populations of the genus *Lagenandra* collected from different geographical areas.

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Fig. 3: *Lagenandra* species used in the present study. (a) *L. ovata* (L.) Thwaites (Pop. 1,2,3,4). (b) *L. nairii* Ramam. & Rajan (Pop. 8,9,10). (c) *L. meeboldii* (Engl.) C.E.C. Fisch (Pop. 5). (d) *L. meeboldii* (Engl.) C.E.C. Fisch (Pop. 7). (e) *L. toxicaria* Dalz. var. *barnesii* Fischer (Pop. 14, 15). (f) *L. toxicaria* Dalz. var. *toxicaria* Hook (11, 12, 13) . (g) *L. keralensis* Sivad. & Jaleel (pop. 6)

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