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MOLECULAR CHARACTERIZATION OF *DIOSCOREA VEXANS* PRAIN & BURK AN ENDEMIC MEDICINAL PLANT SPECIES OF ANDAMAN ISLANDS USING RAPD MARKERS

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ABSTRACT

Dioscorea vexans Prain & Burk (Family: Dioscoreaceae) is an endemic medicinal plant species of Andaman and Nicobar Islands having anti-fertility properties. Recurrent survey was conducted across 12 locations of South Andaman Islands for characterization of different accessions of *Dioscorea vexans* using random primers (RAPD markers). The RAPD-PCR profiling was used to assess the variation at genomic level and it resulted in reproducible patterns of amplicons by using specific combination of accession and primers. Among 32 decamer primers used, 18 showed appreciable amplification and all of them were polymorphic in nature. The entries were grouped into two major groups having variation of 95% in between them. UPGMA analysis revealed that the accessions from Sippighat and Port Mout showed maximum similarity of 36%. Accession from Chidiyatapu was found distinct from other samples showing 99% variation from others. The primers used in this study could clearly demarcate the entries into well-defined distinct groups as there was at least 64% variation among all the collected accessions. Though the collected accessions were morphologically similar, yet they were found to be different from each other as revealed by decamer primers from the clustering pattern. The study reveals that RAPD is an efficient and cheap tool, easy to handle and reliable molecular marker in finding out the genetic diversity in *Dioscorea vexans*, which will be useful in future conservation measures to protect this rare and endemic medicinal plant as well as other such species.

Key words: *Dioscorea vexans*, RAPD-PCR, genetic diversity, medicinal plants, conservation strategies

INTRODUCTION

Andaman and Nicobar Islands are situated in the Bay of Bengal between 6° & 14° N latitude and 92° & 94° E longitude covering an area of 8249 km^2. These islands occupy a phyto-geographically strategic position among mainland India, Thailand, Malay Peninsula and Java-Sumatra. It represents comparatively undisturbed patch of tropical evergreen forests which are rich in flora and fauna. High endemism and unique genetic richness is the characteristic mainstay of these islands which possess about 2500 angiosperm species, of which 10% (245 species) is said to be endemic (Mandal and Elanchezhian 2001, Mandal *et al*. 2007). Several plants of these islands were already under threatened category of the 1997 IUCN Red list of threatened plants. However, these islands can play a vital role as a reservoir of genetic material with appropriate conservation strategies. The Ministry of Environment and Forests, Govt. of India has already declared several parts of these islands as Biosphere reserve, national parks, based on the 'Project Document (Balakrishnan *et al*. 1989). The primitive people living in these islands exclusively depend on forest resources and sea products for most of their sustenance (Awasthi 1987, Awasthi 1988, Dagar and Dagar 1999, Elanchezhian *et al*. 2007). A large number of plant species of these islands possess medicinal values, which are being used by the tribes and aboriginals of this remote Union Territory. Among the endemic species, 52 plants are used in medicaments to cure diverse ailments. Since they are rare and endemic to these islands, they are having immense potential in genetic resource conservation (Mandal *et al*. 2007). Though these islands are rich in species biodiversity; yet the habitats are extremely fragile owing to prevalence of intense biotic and abiotic stresses. The endemic and threatened species were found to be vulnerable to extinction

owing to smaller land area of these islands which act as "death traps" for threatened species (Nayar 1995). Under such scenario, study of the variation prevailing in a given species per unit area may help in devising appropriate conservation strategies for future. The genetic diversity in many species including medicinal plants was assessed using PCR based RAPD marker (Myburg *et al*. 1997, Nebaur *et al*. 1999, Padmesh *et al*. 1999, Hosokawa *et al*. 2000, Raina *et al*. 2001, Neeraj *et al*. 2003, Vieira *et al*. 2003).

Dioscorea vexans (Fig. 1) is also one among the endemic medicinal plant species of Andaman and Nicobar Islands used by those aboriginals. It is a twining climber with slender herbaceous, moderately soft, yellowish green stem with 1.2-1.5 cm circumference; spines present at the base of stem. Leaves dark green above and grayish beneath, coriaceous, obovate, alternate/ oppositely paired, at each node there is tendril. Leaf blade hastate at base, acuminate at apex, basal nerves 5 in number and ~6 cm long twisted petiole angled at the base. Venation was found to be reticulate in the plant leaf. The plant generally grows in inland shade in forest and propagated vegetatively through aerial tubers. The tubers are collected and consumed by the tribes of Bay Islands in curing diverse ailments. It is known to have profound anti fertility properties as evident from the folk knowledge of Nicobari tribes. It is reported to be given to patients to relieve arthritis, asthma, eczema, and to regulate metabolism. It has also been reported to be used for curing chronic cough, diarrhoea and diabetes. This plant is already under threatened category of the 1997 IUCN Red list of threatened plants compiled by World Conservation Monitoring Committee. In the present study *Dioscorea vexans* plants were collected from 12 different places of Bay Islands and profiled involving RAPD markers to assess genetic diversity at genomic level.

MATERIALS AND METHODS

Survey was conducted across South Andaman Islands for collection and characterization of different accessions of *Dioscorea vexans*. The collection sites were namely Saithankari (D1), Habdripur (D2), Caddlegunj (D3), Farrergunj (D4), Lalpahad (D5), Sippighat (D6), Port Mourt (D7), Chouldhari (D8), Tushnabad (D9), Ograbraj (D10), Manjeri (D11) and Chidiyatapu (D12). Collected accessions were proof checked for phyto-authenticity with Botanical survey of India, Port Blair circle. Later these accessions were planted in mini gene garden of CARI, Port Blair (lat. 11°41'13.04"N; long. 92°43"30.16"E) for further characterization.

Isolation of DNA was done from young leaf tissue collected from plants by following CTAB method (Murray and Thompson 1980). Isolated DNA was purified using Sephaglas™ BandPrep Kit. DNA yield and quality of sample DNA was checked by electrophoresing with known standards e.g. lambda DNA (Pharmacia-Biotech, Cat No.27-0620-01). It was also checked spectrophotometrically from the absorbance data of sample DNA at 260nm (1 OD 260=50ìg of DNA) and purity of DNA sample was calculated from OD_{260}/OD_{280} ratio. The random amplification was performed following a modified PCR method (Williams *et al*. 1990). The reaction was carried out in a thermal cycler (MJ Research, PTC 200) where a total of 32 primers procured from OPERON Technologies (Alameda, CA, USA) were used. Eleven primers from OPQ series (OPQ 1, 2, 3, 4, 5, 6, 9, 10, 13, 14, 17), eleven primers from OPX series (OPX 1, 6, 7, 11, 12, 13, 14, 15, 17, 19, 20) and ten primers from OPD series (OPD 1, 3, 5, 7, 9, 11, 13, 15) were used in the characterization.

The PCR reaction mixture consisted of Taq polymerase assay buffer (2 µl) with 1.5mM $MgCl_2$, 10mM dNTP mix (1 µl), 1µl of control template DNA (200 ng/µl), 1µl of random primer (250 ng/µl), 1 unit of Taq DNA polymerase (Finnzymes, Finnland) and the solution was mixed gently after making the final volume to 20 µl with sterile distilled water. Amplification for at least 30 cycles was carried out with initial heat denaturation of the DNA at 94° C for 1 minute. For thermal cycling, temperature regimes of 94^0C for 1 minute, 37^0C for 2 minutes followed by 72^0C for 3 minutes were used. The final extension step at 72^0C for 10 minutes was followed by cooling to 8^0C to complete the PCR. The amplification products were separated on 1.2 % agarose gel (Sigma-Aldrich, St. Louis, USA) on 1X TAE buffer (40mM Tris acetate, pH 8.2, 1mM EDTA) and the gel was stained using ethidium bromide. Further the gel was photographed in a Gel documentation system (Vilber Lourmat, France, Cat. No. Bio1D ++ ver. 99.04) using Molecular analyst software. Electrophoretic patterns were scored according to the presence or absence of clear

visible and reproducible bands (Williams *et al*. 1990). The results were analysed with the principle that the band is considered monomorphic if present in all the accessions and polymorphic if not present in all but present in some accessions. The most intense band from each accession with primer was used as reference for calibration and bands were scored if the intensity of band was at least 10% of the reference band. The similarity index of bands in common between two accessions was estimated using the formula of Nei and Li 1979. A similarity matrix involving the accessions was generated with NTSYS-pc (Numerical Taxonomy System, Applied Biostatistics, New York, USA) software version 2 using dice coefficient. Dendrograms were constructed using an Unweighted pair group method with arithmetical averages (UPGMA) and analysis was carried out.

RESULTS AND DISCUSSION

The RAPD-PCR profiling followed in the study resulted in reproducible patterns of amplicons by using specific combination of accession and primers. Only those primers that displayed reproducible, scorable and clear bands were considered for analysis. Among 32 decamer primers used, 18 primers showed amplification in the form of discernable bands in the agarose gel. The result of RAPD-PCR was presented in Table 1 and Fig 2.

Table 1: Primers that showed amplification in RAPD-PCR and their sequences

S. N.	Primer	Sequence 5'-3'	Accession* showing amplification	Total number of accessions amplified
1	OPQ-05	5' CCGCGTCTTG 3'	1,4,7,9,10,11	6
2	OPQ-06	5' GAGCGCCTTG 3'	3,4,7,8,10,12	6
3	OPQ-09	5' GGCTAACCGA 3'	4,6,7,8,9,10	6
4	OPQ-10	5'TGTGCCCGAA 3'	1,3,4,10	4
5	OPQ-13	5' GGAGTGGACA 3'	4,6,7,8,9,10,11	7
6	OPQ-14	5' GGACGCTTCA 3'	6,8,9,10,11	5
7	OPQ-17	5'GAAGCCCTTG 3'	1,2,3,4,5,6,7,8,9,10,11	11
8	OPX-01	5'CTGGGCACGA 3'	2,3,4,5,7,8,10,11	8
9	OPX-06	5'ACGCCAGAGG 3'	3,4,5	3
10	OPX-07	5'GAGCGAGGCT 3'	1,2,3,4,5,6,7,8,9,10	10
11	OPX-11	5'GGAGCCTCAG 3'	1,2,3,4,5,6,7,8,9,10,11,12	12
12	OPX-12	5'TCGCCAGCCA 3'	2,3,7,9,10	5
13	OPX-13	5'ACGGGAGCAA 3'	3,4,5,6,7,9,10,11,12	9
14	OPX-14	5'ACAGGTGCTG 3'	2,3,5,6,7,10,11	7
15	OPX-15	5'CAGACAAGCC 3'	2,3,4,5,6,7,8,10,12	9
16	OPX-17	5'GACACGGACC 3'	10,11,12	3
17	OPX-19	5'TGGCAAGGCA 3'	2,3,6,7,8,9,11	7
18	OPD-17	5'TTTCCCACGG 3'	1,3,6,7,8,10,11	7

*Number in this column denote the locations (see text) from where the accessions were collected

Fig.1: *Dioscorea vexans* plant (left) with aerial tubers (right)

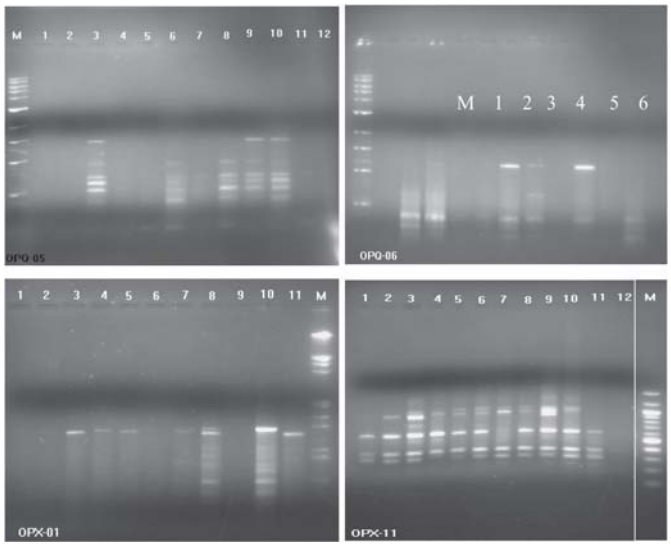

Fig. 2: RAPD profile of *Dioscorea vexans* accessions collected from Andaman Islands

Fig. 3: Dendrograms showing genetic relatedness among 12 accessions of *Dioscorea vexans* collected from Andaman Islands through RAPD-PCR analysis.

The eighteen primers amplified altogether 358 bands among the accessions and all of them were polymorphic in nature. The maximum number of bands was 56 amplified in OPX-11 (Fig 2) and least was 7 in OPX-12, OPX-17. Dendrogram was constructed based on similarity index (Dice coefficient) (Fig 3). The entries were grouped into three major clusters having least similarity in between them (5%). The upper cluster (UC) contains 5 accessions collected from Saithankari (D1), Habdripur (D2), Caddlegunj (D3), Farrergunj (D4), Lalpahad (D5) and middle cluster (MC) contains 6 accessions from Sippighat (D6), Port Mourt (D7), Chouldhari (D8), Tushnabad (D9), Ograbraj (D10) and Manjeri (D11). The lower luster (LC) contained only one accession collected from Chidiyatapu (D12) which was found entirely distinct from other samples with 99% variation. There was clear cut distinction among the all the accessions at 36% similarity level barring D6 and D7 of the middle cluster which showed the maximum similarity of 36%. Further the accessions of upper cluster were subdivided into three sub clusters having 18% similarity in between them. The upper sub cluster 1 (USC1) had a solitary collection from D1, while the upper sub cluster 2 (USC2) had two accessions (D2 and D3) and upper sub cluster 3 (USC3) had two accessions (D4 and D5). Similarly the middle cluster also exhibited three sub clusters at 18% similarity. The middle sub cluster 1 (MSC1) had four accessions while middle sub cluster 2 (MSC2) and middle sub cluster 3 (MSC3) had one accession each. The primers used in this study clearly demarcated the entries into well-defined distinct groups at 36% similarity. Appreciable molecular polymorphism was displayed by these primers used in this study. As reported earlier (Mandal *et al*. 2007, Virk *et al*. 1995) morphological similarities were found to be different than the clustering pattern as revealed by decamer primers. Even though the percent genome surveyed by these different oligonucleotide primers was less, the extent of polymorphisms was found to be high. Similar results were also reported in another medicinal plant species *Costus speciosus* collected from Andaman and Nicobar Islands (Mandal *et al*. 2007). The dendrogram also established genetic relatedness and dissimilarity features, which might have occurred in the genome during the course of evolution. The study reveals that RAPD is an efficient tool in studying the genetic diversity, which was derived from *Dioscorea vexans*. In this regard, the RAPD profiles in individual accessions were found to be different from each other and ample polymorphism was discernible in respect of this 18 oligonucleotide primers used in this study. This study also prospects ample scope of RAPD to be used in molecular profiling of such endemic rare medicinal plants which are otherwise similar at morphological level. This suggests that entries having morphological similarity can have divergence at DNA level. Further these classifications will be useful in future conservation measures to protect these rare and endemic medicinal plant species.

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