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GENETIC DIVERSITY ANALYSIS AMONG THE INDIGENOUS ORCHIDS OF BAY ISLANDS USING RAPD MARKERS

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Abstract

Eight indigenous orchids were collected from different locations in the Andaman Islands. The accessions were fingerprinted using 80 random amplified polymorphic DNA (RAPD) markers. Among the 80 primers, 40 showed amplification and a Similarity coefficients were calculated based on 400 selected bands and UPGMA clustering analysis. The result showed that the green orchid *Eulophia andamensis* (Pretty Green Bay) forms a major cluster from all other species with a similarity of 67%, Both the Cymbidiums exhibited the highest similarity between them to about 77%. *Pholidota imbricata* exhibited higher similarity with *Dendrobium secundatum* than with other species of *Dendrobium*.

Introduction

THE ORCHIDACEAE with nearly 25000 species is one of the largest families of flowering plants. About 1300 species are estimated to occur in India. Their economic importance lies mainly in their ornamental value, but many orchids are used in the traditional system of medicine for curing a number of ailments. The Bay Islands (Andaman and Nicobar), situated 1200 km away from mainland, support tropical warm and humid climate, and are well known for their wide range of plant diversity and floristic richness. A total of 132 wild species of orchids from 59 genera have been reported from here. As many as 30 species are endemic to the Islands (Singh *et al*., 2003). There is a wide range of variations in the host preference and distribution of orchids in the island ecosystem. Wide variations are also found to occur in the size, shape, colour and attractiveness of the leaf as well as flower and its structure, besides growth habit and quality and number of flower/spike, as well. Wide genetic diversity present in the wild orchids of Andaman and Nicobar Islands may be utilised in the breeding programme for producing new hybrids free of pest and diseases, besides conserving the rare, endemic and threatened species. The indigenous species have been characterized on the basis of morphological traits which vary with the environmental conditions. This has resulted in a lot of confusion in the characterization of species. With the advent of molecular marker techniques, the variation in the nucleotide sequence in DNA can be easily measured with high reproducibility (Karp *et al.*, 1997). Among these techniques, RAPD is very widely used to measure genetic diversity in many crops (Vidal *et al.*, 1999). In this study an attempt has been made to assess genetic relatedness among some of the indigenous orchids in Bay Islands.

Material and Methods

Plant Material

Eight indigenous species of orchids (*Cymbidium aloifolium, Cymbidium bicolour, Dendrobium crumenatum, Dendrobium formosum, Dendrobium secundum, Eulophia andamanesis, Pholidota impricata, Rhynchostylis retusa,*) from the Bay Islands were collected from different locations, and studied for their diversity at Division of Horticulture and Forestry, Central Agricultural Research Institute, Port Blair. Out of the 14 cultivars, eight were collected from Havelock Island and others from Port Blair.

DNA Extraction

DNA was extracted from the young, leaves using a modification of CTAB method described by Doyle and Doyle (1990). The fresh leaves (3 g) were finely grounded in liquid nitrogen. To the homogenate, 15 ml preheated extraction buffer was added prior to further grinding. The extraction buffer consisted of 2 % (w/v) CTAB, 1.4 M NaCl, 0.1% (w/v) 2-mercaptoethanol, 20 mM EDTA, and 100 mM Tris- HCl (pH 8.0). The homogenate was subsequently incubated at 65°C for 30-45 mins and extracted twice with chloroform: isoamylalcohol (24:1) solution. The DNA was precipitated in cold Isopropanol and treated with RNase. After electrophoresis with a standard DNA on 0.8 % agarose gel DNA concentrations were determined by comparison of the intensity of staining with ethidium bromide.

DNA Amplification

The RAPD primers were obtained from Bangalore Genei Pvt Ltd, India. Sixty primers were initially screened using four clones. DNA amplification was carried out by modified protocol (Levi and Rowland, 1997).

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Table 2. RAPD markers generated for eight different species of orchids by random 10 mer primers.

Primer	Primer sequence (5'-3')	G + C	Scorable Content (%)	Polymorphic bands	
				bands	number size (bp)
OPA-03	AGTCAGCCAC	60	4	3	118-970
OPA-04	AATCGGGCTG	60	5	4	850-1090
OPA-07	GAAACGGGTG	60	5	4	460-990
OPA-08	GTGACGTAGG	60	7	6	100-1010
OPA-09	GGGTAACGCC	70	5	3	281-889
OPA-11	CAATCGCCGT	60	7	5	365-908
OPA-14	CTCGTGCTGG	70	4	3	190-834
OPA-20	GTTGCGATCC	60	5	3	528-878
OPC-02	GTGAGGCGTC	70	5	4	428-967
OPC-06	GAACGGACTC	60	8	7	327-1057
OPC-08	TGGACCGGTG	70	7	5	459-1185
OPC-13	AAGCCTCGTC	60	6	4	538-991
OPC-20	ACTTCGCCAC	60	5	3	424-1120
OPD-03	GGTCTACACC	60	5	3	587-899
OPD-16	AGGGCGTAAG	60	4	3	437-965

Amplifications were performed in 25 μl reaction mixture consisting of 40 ng of genomic DNA, 10 x reaction buffer with $MgCl_2$ 15 mM, 10 mM each of dATP, dCTP, dGTP and dTTP, 0.2 mM primer, 1U Taq DNA polymerase (Bangalore Genei Pvt. Ltd). PCR amplification was carried out on thermal cycler well blocks (MJ Research Inc., USA.) in 0.2 ml micro-centrifuge tubes. The cycling program was started with a 4 min initial denaturation at 94° C followed by 45 cycles of 1 min at 94°C for denaturation, 1 min at 37° C for annealing, 2 min at 72° C for extension and ended with a final 10 min extension at 72°C. Amplification products were maintained at 4° C until electrophoresis.

The reaction products were resolved by electrophoresis in a 1.2% agarose gel using 1x TBE buffer at 8v/ centimeter for 3 hrs. A 100 bp DNA ladder (Bangalore genei pvt ltd.,) was included as molecular size marker, which resolved DNA fragments ranging from 100bp-1kb.

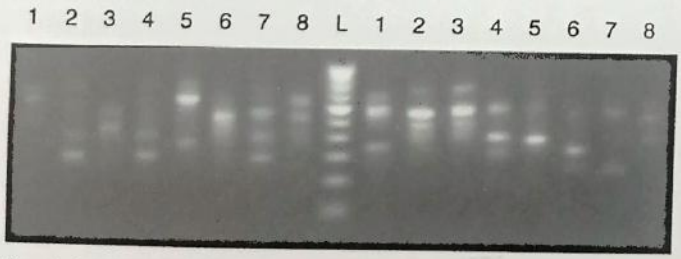

Fig. 1. RAPD patterns of eight indigenous orchids of Bay Islands generated by primers OPA3 and OPA4. (1, *Eulophia andamanesis*; 2, *Cymbidium aloifolium*; 3, *Dendrobium formosum*; 4, *Cymbidium bicolour*; 5, *Dendrobium crumenatum*; 6, *Pholidota imbricata*; 7, *Rhynchostylis retusa*; 8, *Dendrobium secundum*; L, 100 bp DNA ladder).

Data Analysis

Bands from selected primers were scored as 1 (presence) or 0 (absence) for 8 accession tested. Then, pair wise difference matrix between cultivars was determined by the index of (Nei and Li, 1979), and the unweighed pair-group method using arithmetic averages (UPGMA) cluster analysis was performed using a personal computer programe NTSYS 2.0 (Rohlf, 1997).

Results and Discussion

Of the 80 RAPD primers screened 40 showed amplification and among them 30 were selected for analysis due to their reproducibility and polymorphicity.

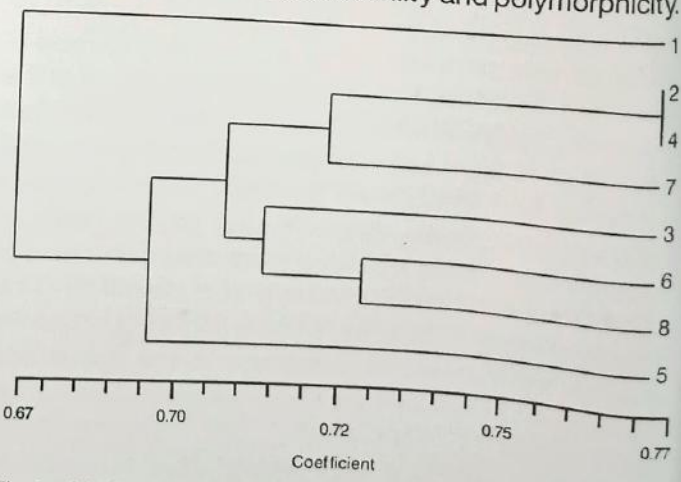

Fig.2. UPGMA dendrogram showing genetic relationships in different orchids. (1, *Eulophia andamanensis*; 2, *Cymbidium aloifolium*; 3, *Dendrobium formosum*; 4, *Cymbidium bicolour*; 5, *Dendrobium crumenatum*; 6, *Pholidota imbricata*; 7, *Rynchosytylis retusa*; 8, *Dendrobium secundum*).

According to the banding pattern obtained from 30 polymorphic primers (Fig. 1) all the orchid species tested in this study could be distinguished from each other indicating that PCR analysis using RAPD primers is an effective tool for cultivar identification.

Similarity coefficients were calculated based on 400 selected bands and UPGMA clustering analysis (Fig. 2) was performed. The green orchid *Eulophia andamensis* formed a major cluster distinct from all other species with a similarity of 67 per cent, Both the cymbidiums exhibited the highest similarity between them to about 77 per cent. *Pholidota imbricata* exhibited higher similarity with *Dendrobium secundatum* than with other dendrobes. Thus from the analysis, the genetic diversity of the different indigenous orchid species could be established.

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