J. Orchid Soc. India, 28: 79-82, 2014 ISSN 0971-5371

MOLECULAR STUDIES IN *DENDROBIUM* SPECIES

G Ramesh and S M Khasim

Department of Botany and Microbiology, Orchid Biology Laboratory
Acharya Nagarjuna University, Nagarjunanagar, Guntur, Andhra Pradesh, -522 510, India

Abstract

The genus *Dendrobium* Swartz belonging to tribe Dendrobieae, sub-tribe Dendrobiinae of the family Orchidaceae comprises about 1200 species. In India, it is represented by 102 species. Molecular analysis of genetic diversity has been investigated by using SDS-PAGE and RAPD markers *in Dendrobium herbaceum, D. microbulbon,* and *D. moschatum.* Samples collected from two different geographical areas *i.e.*, from (Kerala and Karnataka) showed variations in their molecular characters. The samples collected from Kerala at an elevation of 985 m showed few and lesser molecular weights of protein bands, whereas the samples collected from Karnataka at an elevation of 825 m showed more number of protein bands and maximum number of protein bands. The DNA protein bands were more in number and prominent from the samples collected from Karnataka. RAPD and SDS protein profile data indicate the inter-population diversity in between these two sites. This can be attributed to the ecological and climatic conditions prevailed in these two reference sites of South India.

Introduction

DENDROBIUM HERBACEUM Lindl. is known as grassy *Dendrobium*. *Stem* pendulous, 2-3 feet long flowers raceme with short, few flowered (Abraham and Vatsala, 1981); *D. microbulbon* A. Rich. is an endemic, epiphytic orchid, used in stomachache by the tribal people of Gujarat. *D. moschatum* (Buch. Ham.) Sw. *Stem* pendulous, long; *Leaves* narrow, oblong, acute, leathery to 8 cm long and 2 cm broad. The three *Dendrobium* species are epiphytic orchids grows on different tree trunks (Table 1). The maintanance of genetic diversity within and among the populations is very important for a long term conservation programme (Availa-Diaz and Oyama, 2007). In orchids, genetic diversity has varied from very low to high; wide spread species in general have higher levels of variation than the endemic species with a narrow geographic range and usually larger populations have more diversity (Availa-Diaz and Oyama, 2007; Gustafsson, 2000). The objective of this investigation was to assess the molecular diversity in *Dendrobium herbaceum* Lindl., *D. microbulbon* A. Rich. and *D. moschatum* (Buch.-Ham.) Sw. Throughout its geographical distribution in Western ghats of India, by SDS-PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis) and RAPD (Random Amplified Polymorphic DNA). RAPD is powerful tool to estimate the range of genetic variability and therefore, it is useful to evolve conservation strategies of a particular species.

Materials and Methods

Study Area

Two reference sites situated in the Southern Ghats of India were selected for the present study. The first site Kerala (ke) at an elevation of 985 m; and the second site

Table 1. List of populations collected from the two reference sites (Kerala and Karnataka).

Species	Place of Collection	Host Tree	Population
Dendrobium herbaceum Lindl.	Karuman code, Kerala	*Mangifera indica*	Ke[1]
D. microbulbon A. Rich.	Palavara, Kerala	*Terminalia bellitrica*	Ke[2]
D. moschatum (Buch.-Ham.) Sw.	Karuman code, Kerala	*Mangifera indica*	Ke[3]
D. herbaceum Lindl.	Khanapur, Karnataka	*Terminalia elliptica*	Ka[1]
D. microbulbon A. Rich.	Halsi, Karnataka	*Syzygium cumini*	Ka[2]
D. moschatum (Buch.-Ham.) Sw.	Hanbur, Karnataka	*Phoenix sylvestris*	Ka[3]

Received : August 15, 2014; Accepted : November 05, 2014

is Karnataka (ka) at an elevation of 825 m. The species *Dendrobium herbaceum, D. microbulbon* and *D. moschatum* growing on different host trees were collected from the above two reference sites.

Molecular Studies

At each reference site, three populations namely ke^1, ke^2 ke^3 and ka^1, ka^2 and ka^3 were selected. Leaf samples were collected from these six populations growing on different host trees *i.e., Mangifera indica, Phoenix sylvestris, Syzygium cumini, Terminalia bellirica* and *T. elliptica*.

SDS-PAGE

Fresh leaves of 2g were crushed in buffer containing 1.4M NaCl, 20 mM EDTA (Ethylenediamine tetraaceticacid) 100 mM Tris-Hcl (pH 8.0), 2% CTAB (N–Cetyl –N, N, N trimethyl ammonium bromide) and mercaptoethanol with mortar and pestle and it is subjected to SDS-PAGE (Shi and Jackowski, 1998). Protein banding pattern was observed and protein molecular weight ranging from 11 to 84 kDa was used for comparision.

RAPD Analysis

A modified CTAB technique (Doyle and Doyle, 1987) was used for the extraction of genomic DNA and PCR amplification. Only six random primers were used out of which only five responded, sequencing 5′ to 3′ GGTCGGGGAA, GTTTCGCTCC, GTAGACCCGT, AATCGGGCTG, AAGAGCCCGT and AAGCGGCAAC were used in this study. PCR was performed in a reaction volume of 25 ml containing 50 Mm Kcl, 100 M Tris Hcl, 100 ng genomic DNA and 1 unit of Taq DNA polymerase.

Amplified products were resolved elecrophoretically on 1.5% agarose gel run at 100 V visualized by staining with ethidium bromide. RAPD bands were scored as present or absent for each DNA sample and analyzed according to Nei and Li (1979) definition of genetic similarity, *i.e.*, Sij = 2a / (2a + b + c), where Sij is the similarity coefficient between two individuals (i and j); 'a' is the number of bands present in both i and j, 'b' is

Table 2. SDS-PAGE Protein bands and their molecular weight in *Dendrobium*

Population / Protein bands	Ke^1	Ke^2	Ke^3	Ka^1	Ka^2	Ka^3
1	69.048	69.918	77.897	74.490	72.857	84.872
2	65.538	67.959	75.436	72.224	67.959	77.439
3	62.780	64.848	72.974	69.265	63.700	71.333
4	59.222	54.804	70.923	66.000	55.768	67.641
5	50.964	51.388	68.462	57.110	52.717	62.586
6	49.555	49.103	65.020	49.555	50.664	60.208
7	48.835	40.458	58.372	48.156	49.103	57.046
8	47.294	36.668	54.178	43.173	47.648	53.428
9	38.363	32.115	52.035	41.358	43781	51.096
10	35.000	27.008	50.525	38.977	40.757	49.396
11	31.476	24.775	49.699	34.728	38.392	48.091
12	29.419	23.461	48.604	32.155	33.398	42829
13	26.912	22,.399	47.911	30.636	29.686	38.596
14	25.481	20.605	32.362	27.008	26.797	33.499
15	23.615	19.563	26.345	24.969	24.014	29.498
16	22.408	18.459	24.371	21.235	20.149	26.765
17	20.312	17.188	21.580	19.853	18.593	25.332
18	18.616	15.102	19.543	18067	17.880	21.418
19	15.770	13.755	16.916	16.352	16.236	16.213
20	13.550	11.673	14.964	14.219	13.878	14.275
21	12.050			11.673	11.796	

Table 3. Neils genetic similarity matrix of populations based on RAPD analysis.

	Ka^2	Ka^3	Ke^1	Ke^2	Ke^3
Ka^1	0.040	0	0	0.045	0
Ka^2	—	0.040	0.040	0.043	0.040
Ka^3		—	0	0.045	0
Ke^1			—	0.045	0
Ke^2				—	0.045

the number of bands present in i and absent in j, and 'c' is the number of bands present in j and absent in i. The matrix of similarity was clustered using UPGMA algorithm and constructed the dendrogram.

Results and Discussion

Protein Profile Based on SDS-PAGE

The SDS-PAGE protein profile showed different bands of diverse molecular weight of 69 KD to 84 KD that have been noted in different populations. All the 21 bands were observed in 5 populations and only 20 bands were observed in Ke^2 population.

The SDS-PAGE protein profile showed that higher molecular weights 74 KD, 72 KD and 84 KD were observed in (ka^1, ka^2 and ka^3) Karnataka collection, whereas lower molecular weight 69 KD, 69.9 KD and 77 KD were observed in (ke^1, ke^2 and ke^3) Kerala collection.

RAPD Analysis

The RAPD amplification profile showed variability amongst six populations (Fig. 1) collected from the two reference sites. The similarity matrix of pair wise combinations of samples was presented in Table 3. The similarity coefficient between the populations ka^1 and ke^2, ka^3 and ka^2, ke^1 and ke^2 and ke^3. The similarity coefficient values ranged from 0.0 to 0.045. The highest value of similarity coefficient found between the populations was 0.045. In order to analyze the relationship among the populations studied, the UPGMA based dendrogram was constructed using paired matrix values. From the dendrogram, it was evident that samples collected from two different geographical areas showed two clusters (Fig 2). The highest value of mean

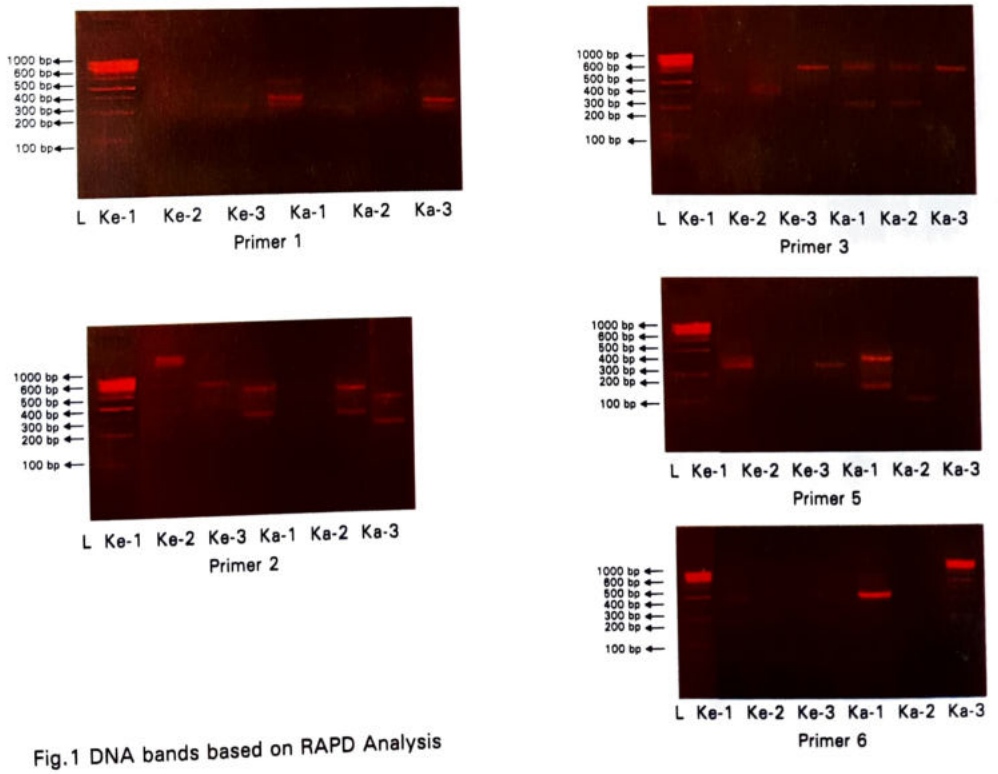

Fig.1 DNA bands based on RAPD Analysis

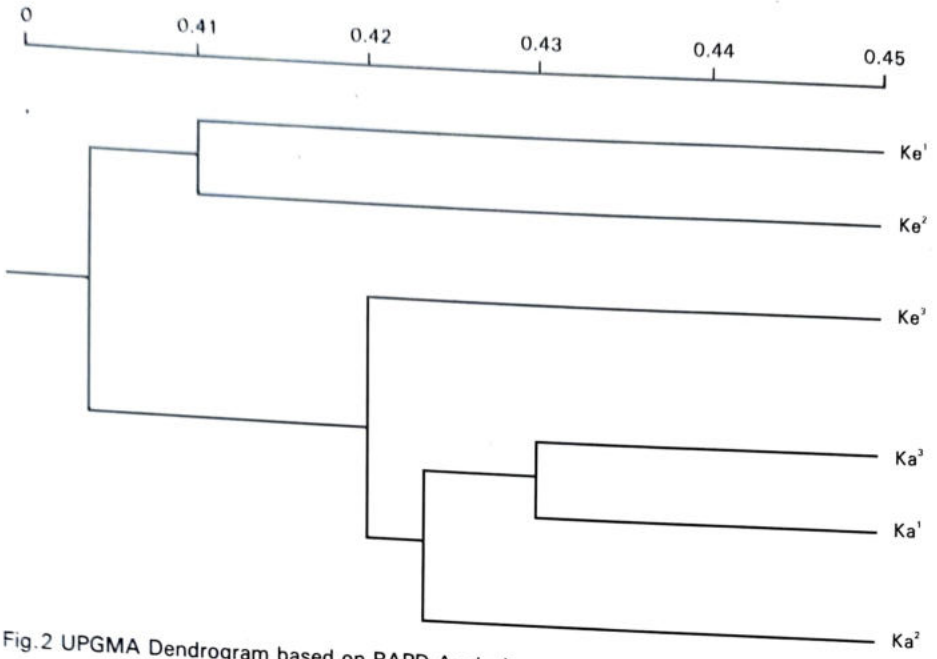

Fig.2 UPGMA Dendrogram based on RAPD Analysis.

similarity coefficient 0.045 was found in the populations ka and ka³.

The present investigation showed the genetic diversity amongs the populations of same location. Besides, there has been considerable variations found in samples collected from two distinct geographical locations. The gene flow was limited due to the greater distance between these two geographical sites. The isolation by distance, as well as climatic conditions and elevation from the sea level, brings about the considerable genetic variation (molecular and morphologically) (Raymond and Rousset, 1995).

According to Misra (1995), orchids are highly habitat specific, and therefore, they suffer due to the destruction of their delicate habitats. Basumatry *et al.* (2008), made similar studies in the epiphytic orchids and suggestedeffective conservation measures. Therefore, apart from molecular analysis, the studies on community dynamics and interaction with host trees are equally essential before evolving the conservation strategies of an epiphytic orchid (Khasim and Ramesh, 2010).

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