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ASSESSMENT OF GENETIC VARIATION AMONG THREE SPECIES OF *AERIDES*, AN EPIPHYTIC ORCHID FROM WESTERN GHATS, INDIA USING RAPD MARKERS

NAGANANDA. G. S*, CHETANA. G. R, RAJATH. S, SANDEEP SURYAN AND. S. SUNDARA RAJAN

Department of Plant Biotechnology, Genohelix Biolabs – A division of Centre for Advanced Studies in Biosciences, Jain University,127/2, Bull temple Road, Chamrajpet, Bangalore 560 019

ABSTRACT

The genetic diversity and relationship between the different species of genus *Aerides* i.e, *A.crispa, A. maculosa and A. ringens* was determined using randomly amplified polymorphic DNA (RAPD) analysis. All three species were collected from Western Ghats of India and analyzed using ten RAPD primers. Total of 159 RAPD fragments were generated of which 40 were polymorphic. The Jaccard's similarity coefficient values ranged from 0.5 between *A.crispa and A.ringens* to 0.66 between *A. crispa and A. maculosa.* The analysis included both total and polymorphic band scores. A dendrogram tree was constructed using UPGMA method depending on similarity coefficient values. In this study, the three different species of genus *Aerides* were successfully differentiated by RAPD method. The results obtained could help in better understanding of the genetic profile and variation among these species and can be used to develop strategies for conservation, population and evolutionary genetics studies of these species.

KEYWORDS: ***Aerides crispa, A. maculosa, A. ringens***, **RAPD, genetic variation**

NAGANANDA. G. S

Department of Plant Biotechnology, Genohelix Biolabs – A division of Centre for Advanced Studies in Biosciences, Jain University,127/2, Bull temple Road, Chamrajpet, Bangalore 560 019, Karnataka, India.

***Corresponding author**

INTRODUCTION

Orchidaceae, the largest families of flowering plants with approximately 20,000 species, grown in all terrestrial ecosystems except the poles and extremely dry desserts [1]. Orchid species are always pursued by amateurs and growers from all over the world because of their spectacular and fragrant flowers [1, 2]. They are marketed as both plant and cut flowers. India is one of the biodiversity hotspots [3] contributing to the world's biological resources from long stretches of Eastern Ghats, the greater Himalayan range on the northern plains and Western Ghats. However in case of Orchids, floriculture is picking up as an industry with a turnover of about, $ 47 million [4]. Orchids also have various medicinal properties. These qualities make orchids a valuable plant family in kingdom with Economical, Industrial as well as Medicinal importance.

Genus *Aerides* belongs to the family Orchidaceae also known as the Cat's tail Orchid or the Fox-Brush Orchid. It is a group of tropical epiphyte orchids that grows mainly in the warm low lands of the tropic of Asia- India, Nepal, Southern China, South-East Asia, The Philippines and New Guinea [5]. There are 25 monopodial epiphytic species in this genus [6].

In recent decades, however, habitat destruction, especially in the tropical and subtropical areas where most orchid species occur, macroclimatic changes, shifting cultivation and loss of host tree species in case of epiphytic orchids, tremendously threatened the lives of many orchid species and bound to result in the elimination of species. [1, 2, 7 and 8]. Comparative population studies using DNA based marker systems are emphasized to collect information on the level and pattern of genetic diversity of wild orchids, which is the first step to facilitate their conservation. Knowledge about genetic diversity is considered the baseline for conservation [9]. Although many studies have been carried out on Orchids, very little is known about their levels and patterns of genetic variation, particularly at DNA level [8].

The RAPD technique and DNA sequencing analysis of specific regions have been used for routine cultivar identification and genetic diversity studies of many plants [10]. Though RAPD has some limitations such as marker allele dominance and sometimes low reproducibility, which may have discouraged many investigators from using RAPD, However the major advantages of RAPD analysis outweigh its disadvantages. RAPD analysis can potentially provide a much higher number of reproducibility marker loci and high levels of polymorphism than allozyme analysis, and it costs much less and is faster and easier to perform than microsatellite analysis because no prior DNA sequence information for the target species is required [11]. In the present study we aimed to evaluate the genetic diversity and relationship between the three different species of *Aerides* i.e., *A. crispa, A.maculosa and A. ringens* found in the Western Ghats of India by RAPD analysis.

MATERIALS AND METHODS

Plant Material and Genomic DNA Extraction

The plant samples were collected from the Western Ghats of India and maintained in the Green House of Genohelix Biolabs. Leaf samples of *A.crispa, A.maculosa and A.ringens* were collected and stored at 4 °C in zip-lock plastic bag till they were processed for genomic DNA isolation. The Orchid genomic DNA was extracted from leaves by a modification of the Cetyltrimethyl ammonium bromide (CTAB) method [12]. One hundred milligrams of fresh leaf tissue was placed in a chilled mortar and nine hundred microlitres of extraction buffer (2% CTAB, 0.1M Tris HCl, 1.4M NaCl, 20mM EDTA, 1% β-Mercapto ethanol and 1% Polyvinyl pyrrolidone) was added and the tissue was homogenized and transferred to 2 ml vial. Ground homogenate was incubated at 60 °C for 10 min and refrigerated at 4 °C for 5 min. The supernatant was obtained by adding ice cold

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chloroform-isoamyl alcohol (24:1, v/v) and spinning at 10,000 rpm for 10 min at room temperature. The DNA present in supernatant was then precipitated by addition of 0.7 volume of ice- cold isopropanol and incubating at – 20 °C for 15 min. The DNA was then pelleted down and air dried after centrifugation at 14,000 rpm for 10 min at 4 °C and rinsing in 70% ethanol and spinning again at 5,000 rpm for 5 min. The dried pellet was later dissolved in 200 µl of 0.1x T.E buffer (10mM Tris HCl and 0.1mM EDTA, pH 8.0) followed by addition of 2 µl of RNAse and incubated for 30 min at 37 °C. 1/10th volume of sodium acetate and 2.5 volume of ice- cold absolute alcohol was added and allowed for precipitation for 20 min at – 20 °C. The precipitated DNA was then pelleted down by centrifugation at 14,000 rpm for 10 min at 4 °C. The DNA was rinsed in 70% ethanol by spinning at 5,000 rpm for 5 min and the DNA pellet was dried after discarding the supernatant. The dried DNA pellet was resuspended in 40 µl of 0.1x T.E buffer. The DNA quality was assessed by examination on a 0.8 % agarose gel stained with ethidium bromide.

RAPD Amplification

RAPD amplification was performed in a volume of 25 µl that contained: 50 ng of template DNA, 2.5 mM each of dNTPs (Chromus Biotech Pvt. Ltd), 5 pM of primer (Sigma- Aldrich), 10x *Taq* assay buffer (10mM Tris-HCl, pH -8.8, 500 mM KCl, 15 mM MgCl2, 0.1 % gelatin, 0.05 % Tween 20 & 0.05 % Nonidet P 40), 1 unit of *Taq* DNA polymerase (Chromus Biotech Pvt. Ltd) and sterilized water. Amplification was performed in a thermal cycler (MJ Research Inc.). Initial denaturation was performed at 94 °C for 3 min before beginning the cycling protocol followed by 1 min at 92 °C, 2 min at 36 °C and 2 min at 72 °C. A total of 40 cycles were performed. The cycling was terminated with a final extension at 72 °C for 6 min. The amplification products were analyzed on 1.2 % agarose gels with low range molecular weight marker The gel was visualized under UV transilluminator and documented using HEROLAB gel documentation system.

RAPD data analysis

The presence of amplified bands with different intensities and locations were detected using Gel-Compar II software. All calculations were done using computer based software program. A pairwise similarity matrices and dendrogram was generated by using Jaccard's similarity coefficient and UPGMA method respectively using Gel-Compar II software.

RESULTS AND DISCUSSION

As one of the largest families of flowering plants, the Orchidaceae constitutes up to 10% of all flowering plant species [13]. Although numerous population genetic studies have been conducted on plants using allozyme markers [14-15], relatively few reports are available on comparative studies of population genetic diversity in wild orchids using different markers. Population genetics of large number of plant species were studied using RAPD [16-18]. The level of genetic diversity of populations as well as the degree of gene differentiation between the populations is important for genetic conservation [19]. The maintenance of genetic diversity is considered crucial for long-term survival and the evolutionary response of population to adapt to the changes in the environment [20-21].

In the present study, we used RAPD analysis to examine the relationship between the three species of *Aerides*, i.e., *A.crispa, A.maculosa* and *A.ringens* from Western Ghats of India. The results presented describe the use of this phylogenetically informative DNA based method to address the interspecific genetic relationships. Additionally, a phylogenetic tree was constructed using RAPD results and used to compare their lineages. All three species exhibited unique polymorphism in this study. Ten primers were used for the present study and all of them successfully amplified polymorphic DNA bands [Fig. 1 and Table 1]. The total number of bands generated by ten

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amplifying primers was 159 with an average amplification of 15.9 bands per primer, of which 40 bands were polymorphic. The number of polymorphic bands per primer ranged from 1 to 7 with percentage polymorphism across all the samples varying from 6.66 % to 53.84 %. The average polymorphism generated by these bands was 28.79 %.

Table 1
List of primers showing total and polymorphic amplicons generated.

Primers	Total no. of bands	Total no. of Polymorphic bands	% of Polymorphism
OPL02	24	6	25 %
OPN16	27	3	11.1 %
OPAB16	18	3	16.6 %
OPA18	13	6	46.1 %
OPC12	14	2	14.2 %
OPW03	15	5	33.3 %
OPAB04	13	7	53.8 %
OPAB12	4	2	50 %
OPW19	15	1	6.6 %
OPP13	16	5	31.2 %

The pairwise similarity matrices were generated by Jaccard's coefficient [Table 2]. The similarity coefficient values ranged from 0.5 to 0.66. A dendrogram was constructed by using GelCompar-II software to show a phenetic representation of genetic relationship as revealed by the Jaccard's similarity coefficient [Fig.2]. The cluster generated in the dendrogram constructed, comprised of *A.crispa* and *A.maculosa* showing similarity of 66.67 %. However the other species *A.ringens* showed an average of 52.78 % similarity in comparison with *A.crispa* and *A.maculosa.*

Table 2
Jaccard's similarity coefficient among different species of Aerides.

	A.crispa (Sample 1)	*A.maculosa* (Sample 2)	*A.ringens* (Sample 3)
A.crispa	1		
A.maculosa	0.66	1	
A.ringens	0.5	0.55	1

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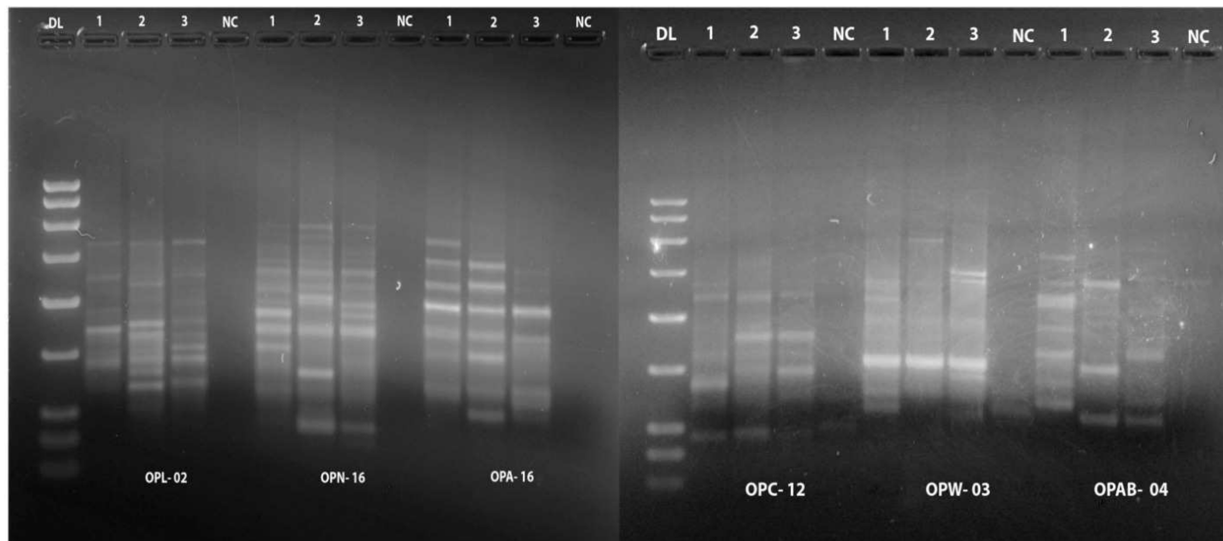

Figure 1
RAPD amplification profile of A.crispa (1), A.maculosa (2), A.ringens (3) with different RAPD markers. NC- Negetive Control, DL- Low range DNA ladder.

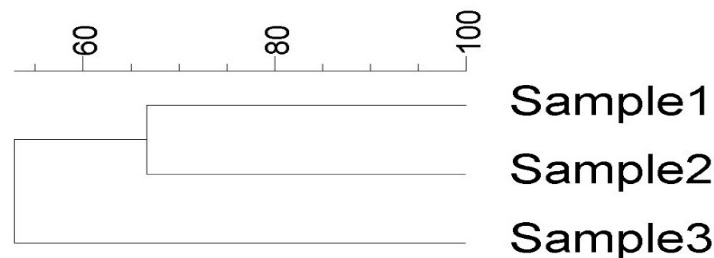

Figure 2
Dendrogram of Jaccard's similarity coefficient between the different species of Aerides. Key: A.crispa (Sample 1), A.maculosa (Sample 2), A.ringens (Sample 3)

From the present study it is known that *A.crispa* and *A.maculosa* are genetically more closely related with 66.67 % similarity where as *A.ringens* showed only 50% similarity with *A.crispa* and 55.56 % similarity with *A.maculosa.* The present study also indicates that the RAPD is sufficiently informative and powerful to access genetic variability of natural populations of three species of *Aerides.* RAPD, being a multi-locus marker with the simplest and fastest technology, have been successfully employed for determination of inter-species genetic diversity in several plant species [22, 23]. Thus, RAPD primers can provide a useful tool in the future design of collecting strategies for germplasm conservation.

The results demonstrated a significant correlation between the genetic differentiations among the three different species of *Aerides.* Identification of inter population diversity also forms a very essential pre-requisite for the promising genetic diversity analysis. The observation and interpretations of this investigation are interesting as a preliminary exploration analysis. The results of this study would help in better understanding of the genetic profile that can be used to develop strategies for conservation and sustainable utilization of epiphytic orchid. Also this study forms a starting point for future research on the population and evolutionary genetics of this species. The powerful capability of molecular

technique to distinguish closely related genotypes based on their RAPD patterns has been brought out by this study.

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