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## ORIGINAL RESEARCH

# DNA Barcoding of *Vanda* Species from the Regions of Shevaroy and Kolli Hills using *rbcL* gene

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## ABSTRACT

Orchidaceae is the largest flowering plant family. It consists of nearly 25000 species. The plants of orchidaceae are widely distributed throughout the world. DNA isolation of *Vanda* was carried out successfully using CTAB method and purified using sodium acetate-ethanol precipitant. The isolated genomic DNA was amplified through PCR using *rbcL* gene and the amplicons were obtained. The amplicons obtained were approximately at the length of 500-800bp for the *rbcL* gene region and sequencing were done. The new DNA sequences were identified at genus and species level using BLAST software tool (NCBI, USA) and deposited in NCBI. The DNA sequences that shown maximum similarity to query sequences were selected and analyzed using MEGA 6.0 (Molecular Evolutionary Genetics Analysis) for phylogenetic analysis. The phylogenetic tree was generated based on Neighbour joining method.

**KEY WORDS:** *rbcL*, *Vanda* species, Phylogeny, DNA sequencing and Orchids.

## INTRODUCTION

Orchidaceae is one of the largest families of flowering plants, comprising nearly 25000 species. The plants of orchidaceae are widely distributed throughout the world. Orchids have three main growth habit; soil dwelling (terrestrial), on other plants (epiphytic) and on rock surfaces (lithophytic) (Smith and read, 1997). The majority of orchids are photosynthetic at maturity. In traditional Asian medicines, a numbers of species have been used in treating fever, pains, dyspepsia, pleura, pulmonary tuberculosis, circulation disorders and tumours (Yoshikawa *et al.*, 1998; Tseng *et al.*, 2006; Watanab *et al.*, 2007; Gutierrez 2010). *Vanda* is the genus in the family orchidaceae with the most magnificent flowers which are very interesting for ornamentation. The genus has a monopodial growth habit with stems which vary

considerably in size from miniature to several meters in length. DNA barcoding is a taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species. Molecular phylogeny in that the main goal is not to determine patterns of relationship but to identify an unknown sample. The terms DNA barcoding as taxon was first proposed by Paul Hebert of University of Guelph in 2003. The large chain gene (*rbcL*) is part of the chloroplast DNA molecules in plants. It is the best characterized gene sequence. Most of the investigating groups tested its suitability in barcoding. It encodes the large subunit of ribulose 1,5 bisphosphate/carboxylase (RUBISCO). Phylogenetics is the study of evolutionary relationships. The evolutionary history inferred from the

phylogenetic analysis is usually depicted as branching, tree like diagrams. They represent that an estimated pedigree of the inherited relationship among molecules, organisms or both. The phylogenetic analysis can be used to analyze the genetic variation in orchid's family. The objects of the study include the collection of *Vanda species* from the regions of Eastern Ghats. From the collected plants isolation of genomic DNA has to be done. The isolated DNA sequences were amplified and the amplicons were sequenced using *rbcl* spacer Chloroplast DNA region and to construct the phylogenetic tree of DNA sequences.

## RESULTS AND DISCUSSION

DNA isolation of *Vanda* was carried out successfully using CTAB method and purified using sodium acetate-ethanol precipitant. The isolated genomic DNA was amplified using PCR and the amplicons were obtained. The amplicons obtained were approximately at the length of 500-800bp for the *rbcl* gene region using 100-1000bp as a DNA ladder. The amplicons were eluted and sequenced using both forward and reverse primers and the sequence chromatograms were successfully obtained for the samples. The five sequences were deposited in the NCBI and the accession numbers are from MF446877-MF446881.

### Phylogenetic analysis

The Phylogenetic tree obtained using MEGA 6.0 for *Vanda* from region based on *rbcl* genic region sequence was shown. The tree generated based on NJ joining method separated the *Vanda* from others into single clade

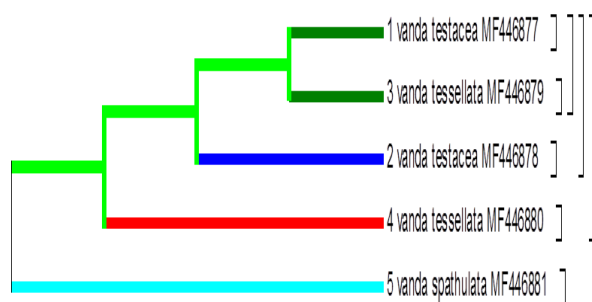


Figure 1: Phylogenetic analysis of *Vanda Species*

The evolutionary history was inferred using the Neighbor-Joining method (Saitou *et al.*, 1987). The bootstrap consensus tree inferred from 1000 replicates is taken to

represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 418 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013). Results clearly show that the *rbcl* region is a good choice of a particular region of the chloroplast genome for resolving phylogenies. DNA barcoding is a valuable tool for taxonomists working with *Vanda*. It can be used to identify species efficiently and accurately on the basis of standard region as a marker. Most of the phylogenetic studies suggest that *rbcl* is the best suited to reconstruct the relationship down to the generic levels. The *rbcl* gene is a valuable tool for accessing phylogenetic relationships at the interfamilial level (Chase *et al.*, 1993).

## CONCLUSION

The *rbcl* genic region of chloroplast DNA region was amplified and sequences for *Vanda* species from 2 different regions of Eastern Ghats. The amplicon sizes ranged were approximately from 500-800bp and the sequence obtained were analyzed. The phylogenetic tree obtained by MEGA 6.0 suggests that *Vanda species* from different region has no generic difference. Since *rbcl* genic region shows high variation among the plants this region could be a good candidate region for discriminating the plants.

## MATERIALS AND METHODS

### Collection of plant sample

The plant samples are collected from different accessions of species belonging to the genus *Vanda* (Leaf sample) from the regions of Shevaroy hills and Kolli hills.

### DNA Isolation, Amplification and Sequencing

A modified CTAB or chloroform extraction protocol is utilized for the extraction of genomic DNA from plants (Barnwell *et al.*, 1998). The *rbcl* region of each sample is amplified in 50 µl reaction volumes, each containing 38.2µl sterile distilled H<sub>2</sub>O, 5µl of 10X buffer, 0.5 µl of 50mM MgCl<sub>2</sub>, 2µl of 10mM dNTPs, 1µl of each of the primer, 0.3µl

of Taq DNA polymerase (Himedia) and 2µl of extracted genomic DNA. The PCR mixture undergoes Initial denaturation at 94 °C for 5 min, 35 cycles of 30 sec at 94°C, 1 min at 55°C, and 2 min at 72°C and final extension at 72° C for 10 min in Eppendorf thermal cycler. The sequence of the PCR amplicons was determined by the method developed by Sanger and colleagues. The fragments which vary in size were separated by electrophoresis on an acrylamide gel and the nucleotide order can be determined by scanning up the gel and analyzing the bands. The sequencing reaction was performed with ABI big dye cycle sequencing terminator reactions (Applied Bio systems) at Eurofins Genomics, Bangalore in a total volume of 20µl. The reaction contained 4µl of purified PCR product, 4µl of sequencing buffer and 4µl of either forward or reverse primer at a concentration of 10mM. The sequencing PCR program was run in 25 cycles for 3 minutes at 96°C, 15 seconds at 96°C, 10 seconds at 55°C and 4 minutes at 60°C. The ABI genetic analyzer (Applied Biosystems) was used to read the sequences and the obtained sequence was analysed.

#### Data analysis

The new DNA sequences were identified at genus and species level using BLAST software tool (Altschul *et al.*, 1990). The identified sequences were deposited in the NCBI. The DNA sequences that shown maximum similarity to query sequences were selected and analyzed using MEGA 6.0 (Molecular Evolutionary Genetics Analysis) for phylogenetic analysis (Tamura *et al.*, 2013). The phylogenetic tree was generated based on Neighbour joining method.

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