International Research Journal of Plant Science (ISSN: 2141-5447)
Vol. 12(1) pp. 01-03, January, 2021
Available online @ https://www.interesjournals.org/plant-science.html
DOI: http:/dx.doi.org/10.14303/irjps.2021.001


*Full Length Research Paper*

# Phylogenetic analysis on different species of *syzygium*

**V. Kavitha* and T.V. Poonguzhali**

Department of Botany, Queen Mary's College, Chennai.

Correspondence email: kavichipli@gmail.com

**Abstract**

*Syzygium* (family:Myrtaceae) is a genus of plants with many benefits in economic and health. Information and publications on the classification of *Syzygium* still few in number. Grouping several plant species in this group is still problematic, among other there is overlapping of *Syzygium* and Eugenia and the position in the classification of some plants are yet unknown. Our study demonstrates that phylogenetic analysis can provide an efficient method for species level identifications and contribute powerfully to taxonomic and biodiversity research.

**Keywords:** *Syzygium*, MEGA, rbcl , Phylogenetic analysis

## INTRODUCTION

*Syzygium* is the most species rich genus of woody plants in Southeast Asia with around 1000 species but little is known of the genus in Wallacea, the biogeographically important transition zone between the Asian and Australian continental areas. The *Syzygium* is considered to be difficult with respect to higher level classification, reflecting a predominance of "local" taxonomic treatments, while there are few morphological characters which can consistently relate species into species groups.

## MATERIALS AND METHODS

### Collection of samples

Two different species of *syzygium* plants were collected from Madurai in Tamil Nadu. They were identified as species of *syzygium*. The plant specimen were shade dried and powdered for further use.The voucher specimens were deposited at the herbarium of the Department of Botany,Queen mary's College, Chennai-600 004.

### Genomic DNA isolation

### Isolation of genomic DNA:

The genomic DNA was isolated from plant sample by following the method (Figures 1 and 2) (Moller et al., 1992).

### PCR analysis

PCR amplification was performed using a 50 µL reaction mixture containing 100 ng of template DNA, 20 µmol of rbcl primers, 200 µM of dNTPs, 1.5 mM of MgCl2, 1U of *Taq* DNA polymerase (MBI Fermentas) and 10 µL of 10x *Taq* polymerase buffer. The sequences of rbcL primers used were as follows.

rbcLa-F: (5'-ATGTCACCACAAACAGAGACTAAAGC-3') - Forward

rbcLa-R: (5'-GTAAAATCAAGTCCACCRCG-3') - Reverse

Amplification was carried out with an initial denaturation at 98°C for 45 sec followed by 35 cycles of denaturation at 98°C for 10 sec, annealing at 55°C for 30 sec, extension at 72°C for 40 sec and final extension at 72°C for 10 min using a thermocycler (iCycler; Bio-Rad Laboratories, CA). PCR products were analyzed on 1% agarose gel for rbcL amplicons in 1x TBE buffer at 100 V (Kimura M1980).

### Sequence analysis of PCR products

The rbcl amplified fragments were purified using the QIAquick gel extraction kit (Qiagen, Valencia, CA) from the agarose gel and sequencing using automated DNA sequencer (Model 3730, Applied Biosystems, USA). The sequences were analyzed using the option Basic Local Alignment Search Tool (BLAST) software available in NCBI.

### Phylogenetic analysis

The sequences of these rbcl genes were compared against the sequences available from GenBank using the BLASTN program and were aligned using CLUSTAL W software Thompson 1994. Phylogenetic trees were constructed using the maximum parsimony method (Eck RV and Dayhoff MO 1966). Bootstrap analysis was done based on 1000 replications (Felsenstein J 1985). The MEGA4 package was used for all analyses.


**Figure 1. Genomic DNA from plant samples**

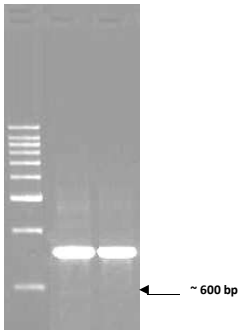


**Figure 2. PCR amplification of rbcl gene of plant samples**

# RESULTS AND DISCUSSION

Conditions: 1% agarose gel electrophoresis

(Lane M: 100 bp DNA Ladder; Well-2: S1; Well-3: S2)

### Sequences of the sample

>S1_RBCL_S10215

TCGTCAGTACACAGCGAGACTAAGCGTGSATGSAGC-
CAAARKAGCTAGGCRAGCAAAGAAGCTGATAGCAAGGAGC-
CWCCGAGAACTCCTCAACCTGGRGTTCCTGCTGAGGAGC-
WRGGGRGACGGKRGCTGCTGTATCTTCTACTGGTACATG-
GACAACTGTGTGGACCGATGGGCTTACCAGCCTTAATC-
GTTATAAAGGAAGATGCTACGGCATCGAGCCTGTTGCTG-
GAGAAGAAAATCAATATWTATGTTATGTAGCTTACCCTT-
TAGACGTTTTTGAAGAAGGTTCTGTTACTAWTATGTT-
TACTTCCATTGKGGCTAATGTATTTGGGTTCAAACCCCTGC-
SCGCTCTACGTCTGGAGGATCTGCGAATCCCTACTTCCTATA-
CRAAAACTTTCCAAGGCCCGCCTCATGGCATCYAAKTTGAGA-
GAGATAAATTGAACARGTATGGCCGTCCCCTATTGGGATG-
TACTATTAAACCTAARTTGGGGTTATCCGCTAAGAACTACG-
GTAGAGCAGTTTATGAATGTCTTCGYGGTGGACTTGATTT-
TACCCAT

>S2_RBCL_S10215

CTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGAT-
TATAAACTGACTTATTATACTCCTGACTATGAAACCAAA-
GATACTGATATCTTGGCAGCATTCCGAGTAACTCCT-
CAACCTGGAGTTCCTCCTGAGGAAGCAGGGGCTGCGG-
TAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTG-
GACCGATGGGCTTACCAGCCTTGATCGTTATAAAGGAA-
GATGCAACCACATCGAGCCTGTTGCTGGAGTAGAAAAT-
CAATATATATGTTATGTAGCTTACCCTTTAGACCTTTTT-
GAAGAAGGTTCTGTTACTAATATGTTTACTTCCATTGTGGGTA-
ATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTGGAG-
GATCTGCAATCCCTACTTCCTATACGAAAACTTTCCAAGGCCC-
GCCTCATGGCATCCTAGTTGAGAGAGATAAATTGAACAAG-
TATGGCCGTCCCCTATTGGGATGTACTATTAAACCTA-
AATTGGGGTTAACCGCTAAGAACTACGGTAGAGCAGTTTAT-
GAATGTCTTC

The evolutionary history was inferred using the Maximum Parsimony method (Eck RV and Dayhoff MO 1966). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein J 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The MP tree was obtained using the Close-Neighbor-Interchange algorithm (Saitou N and Nei M 1987) with search level 3 (2, 3) in which the initial trees were obtained with the random addition of sequences (10 replicates) (Figures 3 and 4). The codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option). There were a total of 461 positions in the final dataset, out of which 3 were parsimony informative. Phylogenetic analyses were conducted in MEGA4 (Tamura K et al., 2007).

The evolutionary history was inferred using the Maximum Parsimony method (Eck RV and Dayhoff MO 1966). Tree #1 out of 130 most parsimonious trees (length = 121) is shown. The consistency index is (1.000000), the retention index is (1.000000), and the composite index is 1.000000 (1.000000) for all sites and parsimony-informative sites (in

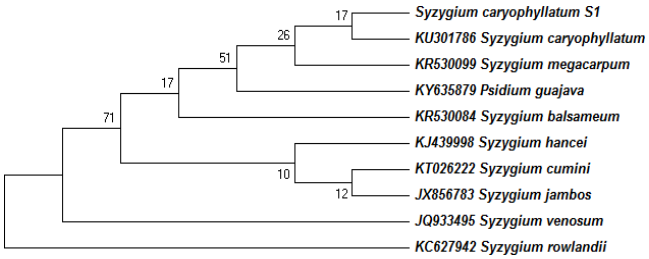


**Figure 3. Phylogeny tree analysis of the sample 1**

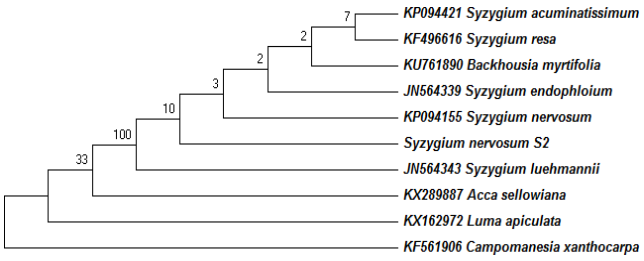


**Figure 4. Phylogeny tree analysis of the sample 2**

parentheses). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein J 1985). The MP tree was obtained using the Close-Neighbor-Interchange algorithm (Nei M and Kumar S 2000) with search level 3 (2, 3) in which the initial trees were obtained with the random addition of sequences (10 replicates) (Figures 3 and 4). The codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option). There were a total of 560 positions in the final dataset, out of which 1 were parsimony informative. Phylogenetic analyses were conducted in MEGA4 (Tamura K et al., 2007).

# SUMMARY

1. In the present study two different plants of syzygium namely were collected from in and around Chennai, Tamil nadu.
2. The genomic DNA was isolated from all the two samples in good condition. The isolated DNA was amplified and PCR products of appropriate size were recovered for all the samples.
3. In order to confirm the genus of the collected samples PCR amplification was performed using rbcLa (Forward primer) and rbcLa (Reverse primer) primers.
4. Molecular evolutionary genetic analysis (MEGA) electropherogram based on these sequences illustrated the levels of divergence between the morphologically identified genera.
5. Phylogenetic analysis using Maximum parsimony method resulted in well resolved phylograms.
6. Thus, it can be concluded that our finding are having separate evolutionary entities.

Based on the BLAST analysis and phylogeny analysis clearly revealed that the given samples were belongs to the taxa as follows:

S1: Syzygium caryophyllatum

S2: Syzygium nervosum

# REFERENCES

Eck RV, Dayhoff MO(1966). Atlas of Protein Sequence and Structure. Sys Bio. 16: 262–263.

Felsenstein J(1985). Confidence limits on phylogenies: An approach using the bootstrap. Evol. 39:783-791.

Kimura M(1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evolution. 16:111-120.Moller E.M, Bahnweg G, Sandermann H and Geiger H (1992). A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nuc Acids Res. 20: 6115-6116.

Nei M, Kumar S (2000). Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Saitou N & Nei M (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol. 4:406-425.

Tamura K, Dudley J, Nei M, Kumar S(2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol. 24:1596-1599.