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Genetic Identification of *Ceriops decandra* (Chiru Kandal) using tRNA (Leu) Molecular Marker

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Abstract: *Ceriops decandra* is a glabrous shrub belongs to a family Rhizophoraceae distributed in a region including Southeast India, East Africa and Australia. The present study aimed to identify a tool in identifying the mangrove at the molecular level. The chloroplast *trnL* region was amplified from extracted total genomic DNA using the Polymerase Chain Reaction (PCR) and sequenced. Sequence of the principal agarose gel band revealed that *Ceriops decandra* and deposited in NCBI with the accession No. JN871232.

Key words: Chiru kandal, *trnL*, Rhizophoraceae

INTRODUCTION

Wetland ecosystems are imperative part of the natural resource pedestal in India, which includes salt marshes, mangroves, coral reefs, seaweed and sea grass. Of these, mangroves are predominantly important in view of its central role both from the ecological and economical development. The total area of mangroves in India is estimated to be 6,740 km^2 and in Tamil Nadu, mangrove coverage is about 150 km^2 (Sathya and Sekar, 2012). *Ceriops decandra* (Griff.) Ding Hou is a mangrove shrub belongs to the family Rhizophoraceae, growing in drier mud near the inland limits of normal tides. It is originated in a region including East Africa to Australia through Madagascar, India, Bangladesh, Sri Lanka, Burma, Thailand and Malaysia. Recent research has shown that the range of *C. decandra* is restricted to the east coast of India and Bangladesh, Southwestern Thailand and Western part of the Malay Peninsula. Economically *C. decandra* barks has huge amount of non-timber forest product of tannins widely used in the fishing nets, leather and chemical industries (Datta *et al*., 2011). Also it has therapeutically potential triterpenoids (Prasad *et al*., 2011). The middle and Southern part of its range is now considered to be *C. zippeliana* (Sheue *et al*., 2009). Also few researchers reported anti-nociceptive, hepatitis, anti-diabetic and anti-oxidant property of *C. decandra* (Uddin *et al*., 2005; Magwa *et al*., 2006; Nabeel *et al*., 2010; Krishnamoorthy *et al*., 2011).

Genetic analysis of mangroves is urgently necessary to combat biotic and abiotic stress. Number of molecular markers such as randomly amplified polymorphic DNA, Amplified fragment length polymorphism, Inter simple sequence repeat and simple sequence repeat studies have been used mainly for genetic diversity analysis of plants (Bandyopadhyay, 2011). Among these RAPD, RFLP, Protein and ISSR markers had been used to study diversity of *C. decandra* (Schwarzbachl and Ricklefs, 2001). Nevertheless, these molecular markers are dominant and they do not permit the differentiation of heterozygous from homozygous accessions. These traits make them extremely suitable to study diversity in supposedly related populations. Hence, the present study was taken up to identify a tool in identifying the medicinally potential mangrove species *Ceriops decandra* at the genetic level using *trnL* marker.

MATERIALS AND METHODS

Plant material and DNA extraction: Fresh leaves of *Ceriops decandra* were collected from Pichavaram mangrove forest (Lat 11.42, Log 79.79) Tamil Nadu, India. The specimen was botanically certified and a voucher specimen (AUCASMB06) deposited in the Herbarium of Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, India. Plant DNA was isolated by Cetyl trimethylammonium bromide (CTAB) protocol with modification described by Gurudeeban *et al*. (2011). The yield of DNA per gram of leaf tissue extracted was measured using a UV Spectrophotometer (Perkin Elmer, USA) at 260 nm. DNA concentration and purity was also determined by running the samples on 0.8% agarose gel.

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Polymerase chain reaction and DNA sequencing: The chloroplast *trnL-F* regions were amplified from extracted total genomic DNA using the Polymerase Chain Reaction (PCR) method. The universal primers 1, 2, 3 and 4 (Helini Biomolecules, Chennai) of Taberlet *et al.* (1991) were used to amplify *trnL*:

- *trnL* 5'-3': GGTTCAAGTCCCTCTATCCC
- 3'- 5': ATTTGAACTGGTGACACGAG

Polymerase chain reaction parameters were an initial denaturation of 2 min at 97°C; 30 cycles of 94°C for 1 min, annealing at 48°C for 2 min and elongation at 72°C for 1 min, followed by an elongation step of 72°C for 5 min. EtBr (Ethidium bromide) stained agarose gel was visualized under a transilluminator. The fragment of interest was excised with a clean razor blade. After removing the excess liquid, the agarose fragment was placed in the spin column. The tube was centrifuged at 5500 rpm for 40 sec for the elution of DNA. The eluent was checked by running on an agarose gel and observed on a transilluminator and the DNA fraction was subjected for sequencing using *trnL*-F specific primers (Helini Biomolecules, Chennai). Sequencing reactions were carried out with ABI PRISM Dye Terminator Cycle Sequence Ready Reaction Kit (Applied Biosystems Inc., USA). The obtained sequence was compared to the sequences in NCBI using the BLAST algorithm to search for close evolutionary relatives (Altschul *et al*., 1997).

RESULTS AND DISCUSSION

The genomic DNA of the *C. decandra* was subjected for the isolation of the DNA coding for *trnL* (Fig. 1) by using Polymerase chain reaction. The bands were cut and eluted and the DNA so obtained was subjected for sequencing. The sequence analysis demonstrated that all the corresponding bands on agarose gel belonged to *C. decandra*. Upon sequencing of the amplified DNA, the data obtained corresponds to 550 bases for *C. decandra* and deposited in NCBI with the accession No. JN871232 (Fig. 2) and the folded structure of tRNA L sequence shown in Fig. 3.

Phylogenetic association of *C. decandra*: Sequence of the foremost agarose gel bands revealed that *C. decandra* in tested leaves was 100% similarity to the *trn* (Leu) sequences of *C. decandra* found in NCBI Genome Databank. The phylogenetic tree was shown in Fig. 4. The parsimony informative of dataset consisted of 550 base-pairs with manually aligned matrix. Gaps represents as binary characters, missing data had no affect on the

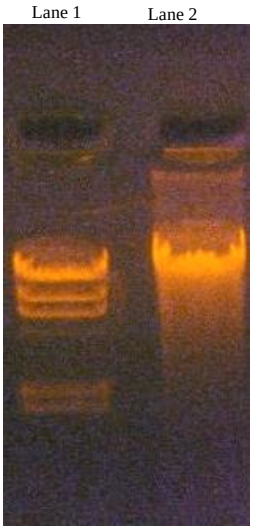

Fig. 1: Genomic DNA of the *C. decandra*, Lane-1: Lamda DNA, Lane-2: Plant DNA

topology. The 100% bootstrap consensus tree is shown in Fig. 2. The *trnL* gene sequence was of *C. decandra* blasted against nr database and the top hits were taken and aligned by multiple sequence alignment. The picture shows the phylogenetically related sequences. Our sequence is the first entry in Genbank, there was no homologous sequence for *C. decandra* in India. The topology is consistent with the maximum parsimony tree, which is more resolved within the clades of Rhizophoraceae.

The chloroplast DNA *trn*T-F region in land plants consist series of conserved *trn* genes *trnT* (UGU), *trnL* (UAA) and *trnF* (GAA) arranged in tandem and separated by noncoding spacer regions. The region is positioned in the large single copy region, approximately 8 kb downstream of *rbcL* (Besendahl *et al*., 2000). The apparent absence of gene rearrangements in the *trn*T-F region made the design of plant universal primers possible. As a result, the *trnL*-F region, comprising the *trn*L intron and *trn*L-F spacer, has become one of the most widely used chloroplast markers for phylogenetic analyses in plants (Quandt *et al*., 2004). The increased number of *trn* sequences from a wide range of plants has allowed further study of structures, functions and evolution in diverse orders of flowering plants, in basal angiosperms, in land plants (Bakker *et al*., 2000; Borsch *et al*., 2003).

The combination of *trnL* sequences with chloroplast markers *rbcL* and *matK* for phylogenetic reconstruction in the tropical flowering plants reported morphological character evolution, classification (Richardson *et al*., 2004), biogeography and molecular

> AUCASMB06
GGATTGAGCCTTGGTATGGAAACTTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATAAAAAATGGGTAATCCTG
AGCCAAATCCTGTTTTACGAAAACAAACAAAAGTTTATAAAGATATAATAAAAGAAAAAAGGGATAGGTGCAGAGACTCAAT
GGAAGTTGTTCTAACAAATGGAGTTGGCTGACTTTCATTAGTAAAGTAAAAGTAAAAGTAAAGGAATCCTTCTGTCAAAATT
AAAAATAAAATGCCAGAAAAGATAAAATAAAGGATAACCCTATATACATACGTATACGTACTGAAATAATATATCAAATGAT
TAATGATAACCCGAATTTTTCTTTTTGGATATTTTTTATTTATATAAAAAACGAAATAAAATAAAAATAATTGTTTTGAATC
GATTCCAAGTTGAAGAAAGGATCGAATATTATAATTAATCAAATCAGTCACTCCATAGTCTGATAGATAATTGATTAATTGG
ACGAGAATAAAGATAGAGTCCCATTGTACATGTCAATATCGACAACAAGGAAATTGAT

Fig. 2: Partial chloroplast tRNA-Leu *trnL* gene sequence of medicinal mangrove *Ceriops decandra*

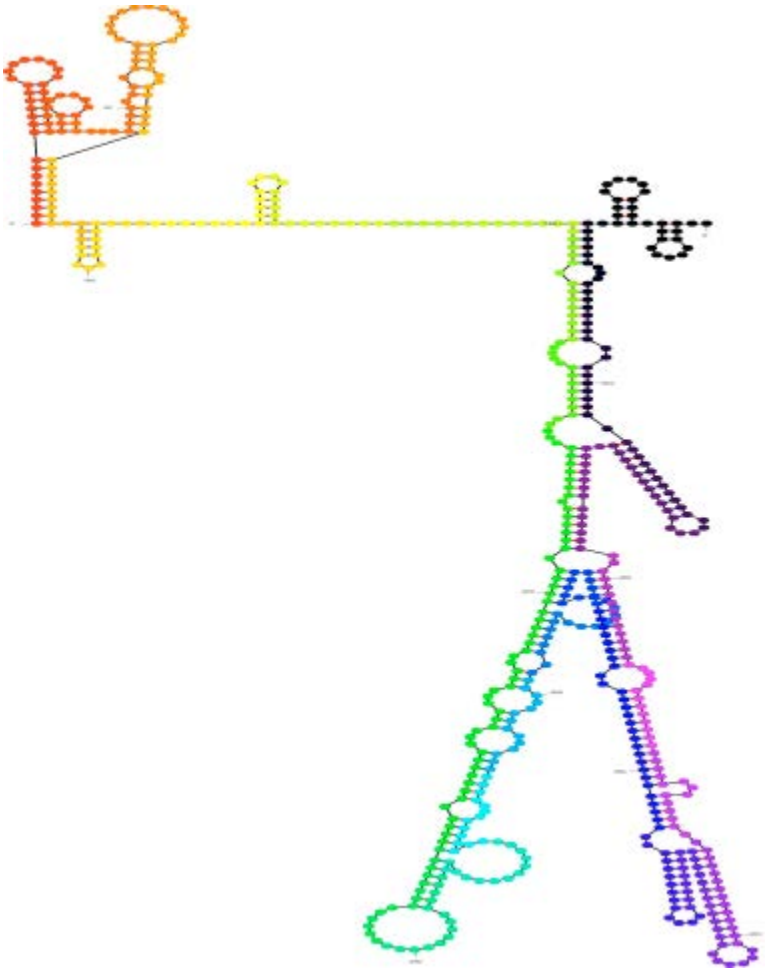

Fig. 3: Folded structure of tRNA-Leu *trnL* gene sequence of *Ceriops decandra*

dating (Richardson *et al*., 2004; Pirie *et al*., 2006; Pirie and Zapata, 2004). These markers appeared to contain complementary phylogenetic signals, as is expected from different sequences sampled from the plastid genome (Chase and Cox, 1998) and were thus applied in combined analyses. The combined analyses yielded better resolved phylogenies, subject to higher levels of support, than those derived from individual markers.

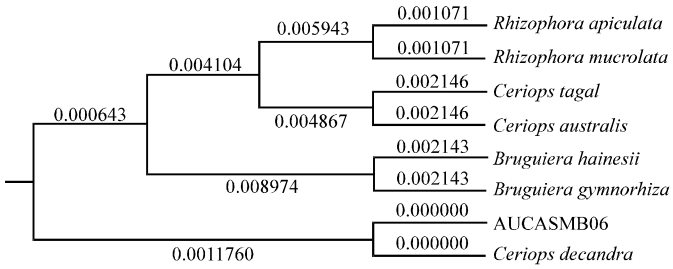

Fig. 4: UPGMA method of phylogenetic analysis of *trnL* gene sequence of *C. decandra* compared with Rhizophoraceae clades

CONCLUSION

The present study can be utilized in identifying the mangrove species directly by using any part of the tissue in live form or even from the fossil specimens. The gene sequences of *trnL*-F will be the same in any part of the plant because of the presence of chloroplast and its genome is same throughout. In India, there was no sequence available in the database for medicinal mangrove till now and hence it can be exploited for the identification of mangrove species up to the level of varieties as the sequences will be more or less conserved with minor variations.

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