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Molecular phylogeny of *Sesuvium portulacastrum* using 18S nuclear ribosomal gene sequence

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ABSTRACT Mangroves are halophyte plants which exist in conditions of high salinity, extreme tides, strong winds and high temperature, muddy and anaerobic soils. *Sesuvium portulacastrum* is a mangrove species in the genus Sesuvium of the family Aizoaceae. Nuclear 18S rDNA gene is one of the most important molecular markers, used in diverse applications such as molecular phylogenetic analyses and biodiversity screening. In the present study Nuclear 18S rDNA gene of *Sesuvium portulacastrum* was amplified and sequenced. The sequence was submitted to NCBI genebank with accession no KC 146551.The length and GC percent of the Nuclear 18S rDNA gene was also calculated .It was found to be 1638 base pairs (bp) in length where as GC% found 51.2. There are 838 GC out of 1638 bases. From Phylogenetic tree it is inferred that *Sesuvium portulacastrum* is closely related to *Pereskia aculeate* and *Basella alba*. Along with these two species *Sesuvium portulacastrum* forms monophyletic group with other closely related species such as *Opuntia sp, Claytonia perforliata, Portulaca grandiflora.*

KEYWORDS : Sesuvium portulacastrum, Phylogenetic, Nuclear 18S rDNA gene Accession no., KC146551

Introduction:

Mangrove plant extracts have been used for centuries as a popular method for treating several health disorders.[1] Mangrove and its associates contain biologically active antiviral, antibacterial and antifungal compounds.[2] The extreme conditions like high salinity and other environmental stress enable them to yield secondary metabolites as chemical defense for their lives.[3]They are biochemically unique, producing a wide array of novel natural products. Because of the adaptive characteristics, the ecology of mangroves has been extensively studied.[4] However, molecular aspects of mangroves are only little studied. The genetic diversity has an impact on the higher levels of biodiversity.[5]

Sesuvium portulacastrum is a halophyte in the genus Sesuvium of the family Aizoaceae. *Sesuvium portulacastrum* has a long history of use in folk medicine it has been used for the treatment of epilepsy, conjuctivitis, dermatitis haematouria, leprosy and also to cure toothache.[6]The essential oil extracted from the fresh leaves of *Sesuvium portulacastrum*, exhibited antibacterial, antifungal and antioxidant activity .[7]The plant also has remarkable ability to survive under different stress conditions. Phylogenetic analysis of Sesuvium genus from Aizoaceae family at molecular and morphological basis. [8] revealed variation among the species of the genus Sesuviumon basis of their geographical location.S

In this study emphasis is given on phylogenetic analysis of *Sesuvium portulacastrum* from Aizoaceae family with other families like Cactaceae,Basellaceae,Portulacaceae,Phytolacaceae,Nyctaginacea e and Molluginaceae. The evolutionary relatedness can be established between different plants and can be represented by using phylogenetic tree. When events like hybridization, horizontal gene transfer, recombination, or gene duplication and loss are believed to be involved, phylogenetic networks have a useful role in understanding them. The18S rDNA gene is one of the most frequently used genes in phylogenetic studies and an important marker for random target polymerase chain reaction (PCR) in environmental biodiversity screening.[9] In general, rDNA gene sequences are easy to access due to highly conserved flanking regions allowing for the use of universal primers.[9,10] (Meyer, *etal,* 2010). The 18S gene is part of the ribosomal functional core and is exposed to similar selective forces in all living beings. The 18S rRNA gene is one of the most important molecular markers, used in diverse applications such as molecular phylogenetic analyses and biodiversity screening.Clearly, the molecular studies published so far have not adequately addressed the question of the phylogenetic relationships of *Sesuvium portulacastrum* using nuclear 18S rDNA as a marker. Moreover this study gives the accession no of the first submitted 18S partial gene sequence to NCBI .

Materials and Methods:

Collection of Plant Material:

Sesuvium portulacastrum was collected from Kelva beach, Thane District identified and authenticated . Other 18S gene sequences were retrieved from NCBI genbank. Name and their family of retrieved species are given in following table.

Table No 1: Name of species names and its family

Sr no.	Plant Name	Family	Sr no.	Plant Name	Family
1	***Sesuvium portulacastrum***	**Aizoaceae**	7	*Corbichonia decumbens*	Molluginaceae
2	*Pereskia aculeata*	Cactaceae.	8	*Ercilla volubilis*	Phytolaccaceae
3	*Basella alba*	Basellaceae	9	*Phytolacca americana*	Phytolaccaceae
4	*Opuntia sp*	Cactaceae	10	*Tetragonia expansa*	Aizoaceae
5	*Claytonia perfoliata*	Portulacaceae	11	*Bougainvillea glabra*	Nyctaginaceae
6	*Portulaca grandiflora*	Portulacaceae			

Isolation of Genomic DNA

Total genomic DNA was isolated from fresh leaves using a modified genomic DNA isolation protocol[11] Plant material was collected during rainy season. 0.5-1 gm of leaf tissue was used to isolate genomic DNA. The DNA was then resuspended in TE buffer (10 mM: 1 mM) and stored at -20°C.Quality and quantity of DNA was checked by loading 2µl genomic DNA and 3 µl of gel loading dye on 1.5% agarose gel. The amount of DNA obtained ranged from 20 µg to 60 µg.

PCR amplification and DNA sequencing

The entire 18S region of *Sesuvium portulacastrum* was PCR amplified in a thermal cycler (Eppendorf) using Forward primer (GTAGTCATATGCTTGTCTC) and Reverse primer (GAAACCTTGTTAC GACTT). Reaction volume was 50 µL and contained, 1X Taq DNA Polymerase buffer (Bangalore genei, India), 1.5 mM MgCl2 (Bangalore Genei, India), 200 µmolar each deoxynucleotide triphosphate (Bangalore Genei, India), 10 pmol oligonucleotide primers (Bangalore Genei, India), 1.0 unit of Taq DNA polymerase (Bangalore Genei, India), and ~25–60 ng of genomic DNA. PCR was performed in a thermal cycler (Eppendorf) and consisted of initial denaturation of 5 min at 94°C, 35 cycles of 1 min at 94°C for template denaturation, 1 min at 50°C for primer annealing, 1 min at 72°C for

primer extension, followed by a final extension of 5 min at 72°C. PCR products were subsequently visualized on a 1.5% agarose gel. Purified products were sequenced, using the same conditions as the PCR. 18S sequences of *Sesuvium portulacastrum* was obtained using both primers and sequencing was carried out on ABI Sequencer (Chromous Biotech, Bangalore) .

Sequence alignment and phylogenetic tree:

All 18S gene sequences of mangrove were retrieved from NCBI genbank and aligned with 18S region of *Sesuvium portulacastrum* using Clustal computer program with Gap Open Penalty 15 and Gap Extension Penalty 6.66. [12]

Results and Discussion:

GC percentage:

The length of 18S regions and GC % of were calculated by using online bioinformatics tools. The length of 18S gene found to be 1638 base pairs (bp) where as GC% found 51.2 The number of nucleotide found to be 414 A's, 385 C's, 473 G's, 426T's, and 0 N's.

Gene Bank Sequence submission

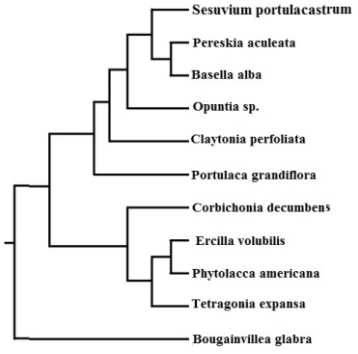
The nuclear 18S rDNA sequence was submitted to NCBI genbank and the Accession Number is Kc146551.

Sequence alignment and phylogenetic tree

Molecular characterization can play a role in uncovering the history, and estimating the diversity, distinctiveness and population structure.

Many studies have reported the use of different molecular markers for establishing phylogenetic relationship between mangroves. Molecular phylogenetic analysis of mangroves for evolution studies of vivipary and salt secretion has been studied by analysing and sequencing the 18S rRNA, *rbcL*, and matR genes of large and representative samples across mangroves.[13]

Fig. No. 1. Phylogenetic Tree



Phylogeny is an evolutionary tree that diagrammatically shows how species are related to each other Phylogenetic tree infer that *Sesuvium portulacastrum* is closely related to Pereskia aculeate and Basella alba Along with these two species *Sesuvium portulacastrum* forms monophyletic group with other closely related species such as Opuntia sp, *Claytonia perforliata, Portulaca grandiflora.* Whereas *Corbichonia decumbens, Ercilla volubilis, Phytolacca americana, Tetragonia expansa* are closely related species forming another monophyletic group. *Bougainvillea glabra* is distantly related to *Sesuvium portulacastrum.*

The studies is congruent with the work done to understand the inter- and infrafamilial phylogenetic relationships in the order the Caryophyllales , using nuclear 18 S DNA and plastid rbcL, matK, atpB gene.[14]

Thus in order to classify and trace out evolutionary path among related species, it is important to investigate them at the molecular level for better understanding of evolution of plants.

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References:

1. Ryder SD. Clinical assessment of liver disease. Medicine 2006;35(suppl.):1-4
2. Wu J, Xiao Q, Xu J, Li MY, Pana JY, Yang M. Natural products from the mangrove flora: source and bioactivities. Nat Prod Rep 2008;25:955-61.
3. Sundaram Ravikumar . Hepatoprotective and antioxidant properties of Rhizopora muronata Mangrove plant in CCl4 intoxicated rats. J Expt .C . Med 2012 ;4(1):66-72.
4. Chang HT. Analysis of the mangrove flora in the world (Collection of Chang Hungta) 1995,202–211 (Zhong- shan Univ Press, Guangzhou).
5. Templeton AR. Translocation as conservation tool. In: Biodiversity in mangrove landscapes, theory and practice (ed. Szaro R. I. N) (Oxford University Press). 1993
6. Bandaranayake WM. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wet Ecol Manag 2002;10:421-2.
7. Magwa ML, Gundidaza M . Chemical composition and biological activities of essential oils from the leaves of Sesuvium portulacasrum .J Ethnopharmacology. 2006, 103 ,85-89.
8. Lokhande V, Gor B, Desai N, Nikam T, Suprasanna P. Sesuvium portulacastrum, a plant for drought, salt stress, sand fixation, food and phytoremediation. A review. Agronomy for Sustainable Development, Springer Verlag/EDP Sciences/INRA, 2013, 33 (2), 329-348.
9. Meyer A., Todt C., Mikkelsen N. T. & Lieb B "Fast evolving 18S rRNA sequences from Solenogastres (Mollusca) resist standard PCR amplification and give new insights into mollusk substitution rate heterogeneity". BMC Evolutionary Biology . 2010,10: 70.
10. Sahu S., Kathiresan K., Molecular markers: an intricate tool for new insights In mangrove genetics. 2012, 3(4) 847 -863
11. Saini A ,Gopala Krishna, T., Sreenivasulu Reddy, K. And Jawali N., The BamHI site in the Internal Transcribed Spacer Region of Mungbean ribosomal RNA gene is partially methylated. Euphytica . 2010,114 (1): pp 55-59
12. Higgins D., Thompson J., Gibson T., Thompson J.D., Higgins D.G., Gibson T.J. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research.1994, 22: 4673-4680.
13. Shi S. et al. Molecular phylogenetic analysis of mangroves: independent evolutionary origins of vivipary and salt secretion. MolecularPhylogenetics and Evolution. 2006, 40:298-304.
14. Philippe C, Vincent S, Lars W., Martyn P,Rene´ G, Mark C..Molecular phylogenetics of Caryophyllales Based on nuclear 18s rdna and plastid rbcl,atpb, and matk dna sequences. American Journal of Botany . 2002,89(1): 132–144
15. Chen F, Venkatesalu V, Kuo D and Shea P. Evaluation of Antioxidant Polyphenols from Selected Mangrove Plants of India, Asian Journal of Chemistry, 2008, 20(2) 1311-1322
16. J Wu, Xiao Q, Xu J, Min-Yi Li, Pan J and Yang M, Natural products from true mangrove flora: source, chemistry and bioactivities, Natural products Report.2008, 25,955-981
17. Ravikumar S, Jacob Inbaneson S, Suganthi P, Gnanadesigan M. In vitro antiplasmodial activity of ethanolic extracts of mangrove plants from South East coast of India against chloroquine-sensitive Plasmodium falciparum. Parasitol Res 2010;108:873-8
18. Ravikumar S, Gnanadesigan M, Jacob Inbaneson S, Kalaiarasi A. Hepatoprotective and antioxidant properties of Suaeda maritima (L.) Dumort ethanolic extract on concanavalin-A induced heaptotoxicity in rats. Indian J Exp Biol 2011;49:455-60.
19. Waugh R & Powell W. [1992] Using RAPD markers for crop improvement. Trends Biotech. 10,186-191.
20. Emmarold E M, Sinclair HM, & Mark B. [2001] Use of random amplified polymorphic DNA (RAPD) markers to reveal genetic diversity within and between populations of cashew (Anacardium occidentale L.). J. Hortic. Sci. Biotech. 76:375-383.