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Efficacy of rbcL gene as DNA bar-code to identify an unknown pteridophyte

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Abstract

In the present communication, an attempt has been made to examine the efficacy of rbcL gene for identification of an unknown pteridophyte. Through this study the rooted, distance neighbour joining algorithmic tree has shown that the unknown pteridophyte is closely related to *Selaginella kraussiana*.

Key words: rbcL gene, DNA bar-coding**,** *Selaginella***.**

INTRODUCTION

DNA bar-coding is a unique technique in which the species identification is performed by using DNA sequences from small fragment of the genome, with the aim of contributing to a wide range of ecological and conservation studies. In fact it is difficult to choose the right DNA sequence which can be used for the purpose. In recent years different plastid and nuclear genes and spacer regions have been used for bar-coding, e.g. atpF-atpH, matK , rbcl, rpoB, $rpoC_1$, psbK-psbL, trnH-psbA. H.D. Janzen (2009) recommended the two locus combination of rbcL + matK as barcodes for the land plants. According to Lahaye *et al* (2008) matK alone is very efficient in identifying species listed in convention of International Trade of Endangered Species (CITES) appendixes (mostly orchids). It has also been reported that a group of scientists were trying to identify the pteridophytic flora of Japan with the help of barcodes (Ebihara *et al* 2010).

This article deals with the identification of a mystery plant collected from Carolina Biological Supply (Burlington, NC) to understand the efficacy of rbcL gene as barcode.

MATERIAL AND METHODS

Morphological study:

The morphological study of the plant was done under light microscope. The photograph of the mystery plant was taken to compare with the related species after the molecular study.

Molecular studies:

DNA extraction was performed using DNeasy Plant Mini™ Kit (Qiagen, CA). The PCR amplification was conducted using standard DNA Polymerase (Biolare™ Taq Polymerase, Bioline) and rbcL primers in 25 µl reactions following the protocol of Kress & Erickson (2007). The PCR amplification of either locus for a generic pair was successful. DNA sequencing was done by 313016- capillary automated DNA Sequencer in The University of Chicago Cancer Research Center, USA. For viewing the chromatogram the software Finch TV was used and to get the reverse complements of the sequences the website www.bioinformatics.org/sms/rev_comp.html was used. The reverse complements were used for Gene bank BLAST. The alignment of eight plants (including the mystery plant) was done in Clustal W. The rooted, distance neighbour joining tree was obtained from the BLAST search.

RESULTS

Sequences of the rbcL gene from the mystery plant:

Madhu 2

>CATCATCCTTAGTGAAATCGAGTCCGCCACGGAGGCATTCGTAGACTGCTCTACCATAGT
TCTTAGCAGATAGACCCAGTTTAGGTTTTATGGTGCATCCCAGCAGGGGTCGACCGTATTT
GTTCAATTTATCCCTTTCAACCTGGATACCGTGAGGCGGGCCCTGAAAGGTCTTGGAATAA
GCGGGGGGAATCCGCAAATCCTCCAATCGCAATGCCCGTAAGGCCTTGAATCCGAAAACG
TTACCCACGATAGATGTGAACATGTTAGTAACGGAACCCTCTTCGAAGAGATCCAGGGGGT
AGGCCACGTAGGCTATATATTGGTCCTTTTCTCCAGCCACGGGTTCGATGTCATAGCATCG
CCCCTTGTAACGATCAAGATTAGTAAGCCCGTCGGTCCAGACCGTAGTCCATGTACCGGTG
GAGGACTCCGCGGCTACCGCGGCCCCTGCTTCCTCGGCGGGAACGCCGGGTTGCGGGGTC
ATTCGGAATGCTGCCAATATATCGGTATCCTTGGTTTCGTAGTCGGGGGTGTAGTAAGTTA
ATCTGTAATCTTTAACGCCAGCCTTGAATCCAACACTTGCTTTAGTCTCTGTTG<

Madhu 1

>ACAGATTAACTTACTACACCCCCGACTACGAAACCAAGGATACCGATATATTGGCAGCAT
TCCGAATGACCCCGCAACCCGGCGTTCCCGCCGAGGAAGCAGGGGCCGCGGTAGCCGCGG
AGTCCTCCACCGGTACATGGACTACGGTCTGGACCGACGGGCTTACTAATCTTGATCGTTA
CAAGGGGCGATGCTATGACATCGAACCCGTGGCTGGAGAAAAGGACCAATATATAGCCTA
CGTGGCCTACCCCCTGGATCTCTTCGAAGAGGGTTCCGTTACTAACATGTTCACATCTATCG
TGGGTAACGTTTTCGGATTCAAGGCCTTACGGGCATTGCGATTGGAGGATTTGCGGATTCC
CCCCGCTTATTCCAAGACCTTTCAGGGCCCGCCTCACGGTATCCAGGTTGAAAGGGATAAA
TTGAACAAATACGGTCGACCCCTGCTGGGATGCACCATAAAACCTAAACTGGGTCTATCTG
CTAAGAACTATGGTAGAGCAGTCTACGAATGCCTCCGTGGCGGACTCGATTTCACTAAGGA
TGATGAGAACGTAAATTCTCAGCCATTCATGCGTTGGCGAGATCGATTCTTATTTGTAGCA
GAAGA<

Reverse complements of the above sequences obtained with the help of http:// www.bioinformatics.org/sms/rev_comp.html:

>Mystery plant

CATCATCCTTAGTGAAATCGAGTCCGCCACGGAGGCATTCGTAGACTGCTCTACCATAGTTCTTAGC
AGATAGACCCAGTTTAGGTTTTATGGTGCATCCCAGCAGGGGTCGACCGTATTTGTTCAATTTATCC
CTTTCAACCTGGATACCGTGAGGCGGGCCCTGAAAGGTCTTGGAATAAGCGGGGGGAATCCGCAAA
TCCTCCAATCGCAATGCCCGTAAGGCCTTGAATCCGAAAACGTTACCCACGATAGATGTGAACATG
TTAGTAACGGAACCCTCTTCGAAGAGATCCAGGGGGTAGGCCACGTAGGCTATATATTGGTCCTTTT
CTCCAGCCACGGGTTCGATGTCATAGCATCGCCCCTTGTAACGATCAAGATTAGTAAGCCCGTCGGT
CCAGACCGTAGTCCATGTACCGGTGGAGGACTCCGCGGCTACCGCGGCCCCTGCTTCCTCGGCGGG
AACGCCGGGTTGCGGGGTCATTCGGAATGCTGCCAATATATCGGTATCCTTGGTTTCGTAGTCGGGG
GTGTAGTAAGTTAATCTGTAATCTTTAACGCCAGCCTTGAATCCAACACTTGCTTTAGTCTCTGTTG
reverse complement<

>mystery plant2

ACAGATTAACTTACTACACCCCCGACTACGAAACCAAGGATACCGATATATTGGCAGCATTCCGAA
TGACCCCGCAACCCGGCGTTCCCGCCGAGGAAGCAGGGGCCGCGGTAGCCGCGGAGTCCTCCACCG
GTACATGGACTACGGTCTGGACCGACGGGCTTACTAATCTTGATCGTTACAAGGGGCGATGCTATG
ACATCGAACCCGTGGCTGGAGAAAAGGACCAATATATAGCCTACGTGGCCTACCCCCTGGATCTCT
TCGAAGAGGGTTCCGTTACTAACATGTTCACATCTATCGTGGGTAACGTTTTCGGATTCAAGGCCTT
ACGGGCATTGCGATTGGAGGATTTGCGGATTCCCCCCGCTTATTCCAAGACCTTTCAGGGCCCGCCT
CACGGTATCCAGGTTGAAAGGGATAAATTGAACAAATACGGTCGACCCCTGCTGGGATGCACCATA
AAACCTAAACTGGGTCTATCTGCTAAGAACTATGGTAGAGCAGTCTACGAATGCCTCCGTGGCGGA
CTCGATTTCACTAAGGATGATGAGAACGTAAATTCTCAGCCATTCATGCGTTGGCGAGATCGATTCT
TATTTGTAGCAGAAGA reverse complement<

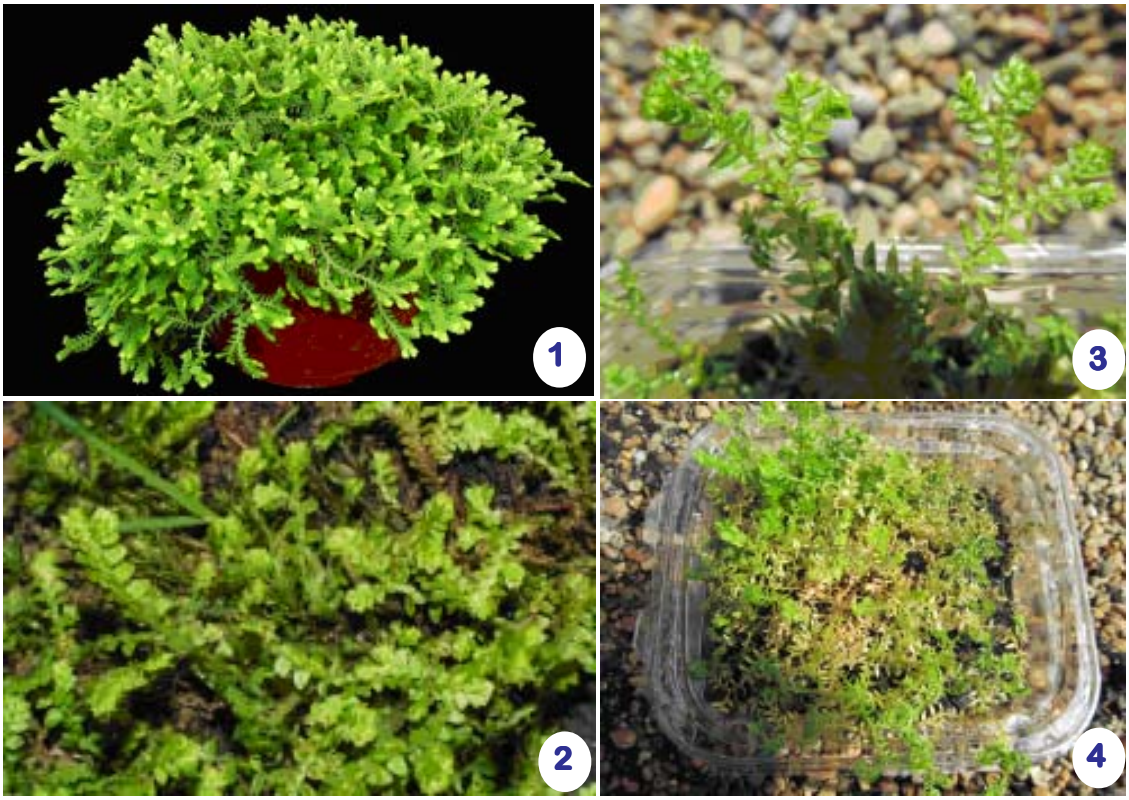

PLATE - I: Fig. 1. *Sellaginella kraussiana* **(**plantoftheweek.edu**); Fig. 2.** *Sellaginella apoda* (cas.vanderbitt.edu); **Figs. 3 & 4.** Mystery plant (taken by author)

CLUSTAL W (1.81) Multiple Sequence Alignments with the highest and lowest scored plants obtained from blast analysis:

Sequence type explicitly set to DNA

Sequence format is Pearson

Sequence 1: mystery_plant2ACAGATTAACTTACTA 116 bp

Sequence 2: Selaginella_apoda 1360 bp

Sequence 3: Selaginella_kraussiana_CTTACTA 1225 bp

Sequence 4: Selaginella_remotifolia_TTACAA 1031 bp

Sequence 5: Selaginella_articulata_TACGAGA 1204 bp

Sequence 6: Selaginella_lepidophylla_AGTGT 1284 bp

Sequence 7: C.deodara 1508 bp

Sequence 8: Pinus_nigra_TAGTGTCGGATTCAAAGC 1366 bp

Sequence 9: Picea_abies 553 bp

Rooted, distance Neighbour joining algorithmic tree is presented in Fig. 5.

DISCUSSION

From the BLAST result it was almost clear that the plant belonged to Trachaeophyta (root, cellular organism, Eukaryota, Viridiplantae, Streptophytina, Embryophyta); *Selaginella* (Lycophyta, Isoetopsida, Selaginellales, Selaginellaceae). But the BLAST scores were highest for *Selaginella*

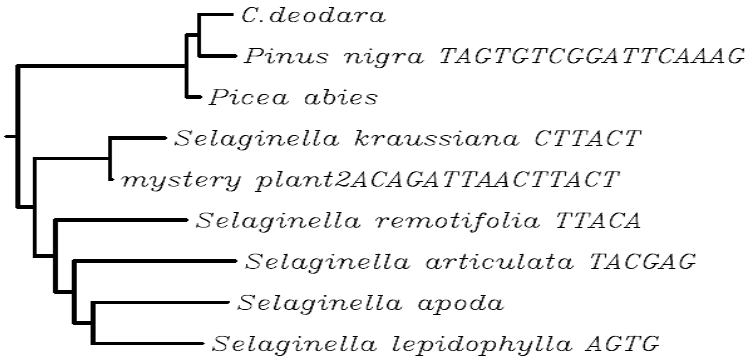

Fig.. 5. Rooted, distance Neighbour joining algorithmic tree

apoda (1092) [Fig. 2] and *Selaginella kraussiana* (1005) [Fig. 1] with maximum identity of 99% and 100% respectively. From the Clustal W alignment and the phylogenetic tree of rooted, distance neighbor joining algorithm [Fig. 5] it was confirmed that on the basis of rbcL sequences the mystery plant was more related to *S. kraussiana* than to *S.apoda*. The leaves of *S. kraussiana* and mystery plant [Figs. 3 & 4] are lanceolate whereas the leaves of *S. apoda* are ovoid. Through this study the efficacy of rbcL gene as a barcode is recognized.

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