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Original Article

Use of *rbcl* gene sequence of plastid region as a tool in taxonomic authentication and phylogeny of Medicinal Plants

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ABSTRACT

The Western Ghats of India are known to be a major biological hotspot that supports the plant diversity and endemism. The *Euphorbia fusiformis* representative species of Euphorbiaceae is famous for its use as medicinal herbs. The tuber and latex is used in various ayurvedic medicines. Taxonomic identification of tubers and powders is becoming a great challenge as per classical plant taxonomy. The present study deals with generation of *rbcl* gene sequences from *Euphorbia fusiformis* and its phylogenetic relationship with other species of the genus *Euphorbia.*

Keywords: DNA sequencing, Medicinal herb, *rbcl* gene, Western Ghats.

INTRODUCTION

Euphorbia fusiformis (Synonym : *Euphorbia aqualis*, Euphorbiaceae) is a rare medicinal plant found in Andhra Pradesh, Bihar, Goa, Gujarat, Karnataka, Maharashtra, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal states of India. In Gujarat state, it is found in Dangs, Rajpippala and Chota Udaipur regions. In Maharashtra it is found in Western Ghats at Khandala, Lonawala, Panchagani, Mahabaleshwar, Bhimashankar, Karjat and Konkan region. [1-7].

Euphorbia fusiformis has one of the largest tuberous root stocks in this genus. The species epithet refers to its fusiform root. Locally it is called "Ban-Muli" by the tribal people. It is said to be of medicinal value, its latex being used as an antidote for snake and scorpion bites. The tuber pulp is used as a cure for arthritic pains in some regions of the Himalaya [8].The ethnobotanical value of this plant is due to its action as a remedy for several diseases like rheumatism, gout, paralysis and arthritis [9], liver disorders and diarrhea [10].The tuberous roots of this plant are being used by *Bhagats* (tribal physicians) of Dangs forest for the treatment of various abdominal disorders, especially for tumors of abdomen, and urinary stones. However, after extensive literature search, it is realized that only few pharmacological studies have been carried out on this plant pertaining to its anti-inflammatory [11] and antibacterial activities [12, 13].

In traditional medicines plant material is used either in tuberous or powdered stage making taxonomic identification difficult. Misidentified collections could lead to introduction of unsuitable or unwanted plant species in medicinal preparations. In order to avoid this problem proper identification of plant species of these powders or tubers is essential. Species identification on the basis of DNA sequences has been done for some time.

In present investigation we have generated ribulose-15-bisphosphate carboxylase large subunit (*rbcl*) sequence for *Euphorbia fusiformis.* Its molecular phylogenetic relationships with other species is also studied.

MATERIALS AND METHODS

Sampling: Fresh samples of tubers of *Euphorbia fusiformis* were collected from Lonawala, District Pune of Western Ghats of Maharashtra (Figure 1 and 2) in August 2012. These plants were identified and authenticated using herbarium collection at Department of Botany, DST-FIST School of Life Science, SRTM University, Nanded (MS), India

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and Department of Botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Fresh tubers were washed thoroughly under running tap water followed by sterile distilled water and dried under shade. The shade dried material was ground into coarse powder using mechanical grinder. This coarse powder was sieved by 1 mm pore size sieve. The powder was stored in airtight containers at room temperature till further molecular biological work.

***DNA extraction and quantification*:** DNA Extraction was carried out using HiPurA Plant Genomic DNA Miniprep Purification Spin kit (Himedia, MB507). Concentration of DNA was determined using UV-1800 spectrophotometer (Schimadzu Corporation A11454806498). The DNA was stored at -20^{0}C till further use.

***PCR amplification*:** The DNA isolated from plant was subjected to polymerase chain reaction (PCR) amplification using Biometra thermal cycler (T-Personal 48). *rbcl*a F (Forward) 'ATGTCACCACAAACAGAGACTAAAGC3' and *rbcl*a R (Reverse) 5'GTAAAATCAAGTCCACCRCG 3' primers were used for amplification. The PCR reaction mix contained 2.5µl of 10X buffer, 1µl of each primer, 2.5µl of 2.5mM of each dNTP, 2.5 Units of Taq DNA polymerase and 1µl Template DNA and 8.5µl nuclease free water. The PCR amplification cycle consisted of, a cycle of 5 min at 94°C; 35 cycles of 1min at 94°C, 1 min at 50°C, 2 min at 72°C; and additionally 1 cycle of 7 min at 72°C.

***Gel electrophoresis*:** Gel electrophoresis of the amplified product was performed using 1.0% agarose (Seakem, 50004L) to analyze the size of amplified PCR product. The size obtained was approx. 600bp for *rbcl* region (Figure 3).

***DNA sequencing*:** The PCR product was purified using AxyPrep PCR Clean up kit (Axygen, AP-PCR-50). Itwasfurther sequenced using Applied Biosystems 3730xl DNA Analyzer USA and chromatogram was obtained. For sequencing of PCR product *rbcl*a F - 5' ATGTCACCACAAACAGAGACTAAAGC 3' sequencing primer was used.

***Bioinformatics analysis*:** The DNA sequence was submitted to NCBI Gene Bank (http://www.ncbi.nlm.nih.gov/). NCBI's web-based BLAST algorithm [14] using the default settings were then used to identify the query sequences. The BLAST results were used to find out evolutionary relationship of *Euphorbia fusiformis*. Altogether 20 species including sample were used to generate Maximum Likelihood phylogenetic tree (Figure 4). Phylogenetic and molecular evolutionary analyses were conducted using *MEGA* version 6 [15].

RESULT AND DISCUSSION

Fresh plant material of *Euphorbia fusiformis* were identified and authenticated using herbarium collection at Department of Botany, DST-FIST School of Life Science, SRTM. University, Nanded (MS), India and Department of Botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. We confirmed the identity of the current species using molecular phylogenetic methods. Model Test in MEGA 6 suggested that models JC (BIC= 1389.073, AIC = 1136.538, ln L -531.063 explained the nucleotide patterns in the *rbcl* gene sequences. A consensus phylogenetic tree (Figure 4) that compared known sequences of *Euphorbia fusiformis* from the current collection, suggested that the specimens in our collection were closely related to genus *Euphorbia*.

Conclusion

The molecular phylogeny of *Euphorbia fusiformis* was determined by analyzing ribulose-15-bisphosphate carboxylase large subunit (*rbcl*) gene sequence. On the basis of position of sequence of the given plant samples in the phylogenetic tree, the sample showed closest similarity with *E. abyssinica*. On the basis of morphology and phylogeny the given plant is of *Euphorbia fusiformis* belonging to family Euphorbiaceae. Present work will assist in molecular identification of other *Euphorbia* species from Western Ghats and establishing their molecular relationships. It will also help in further ethano-pharmacologicial studies pertaining to properly identified plant species.

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Figure 1: Rosette habit of *Euphorbia fusiformis.*

Figure 2: Tuber of *Euphorbia fusiformis.*

Figure 3: Amplification of *rbcl* plastid region in *Euphorbia fusiformis.*

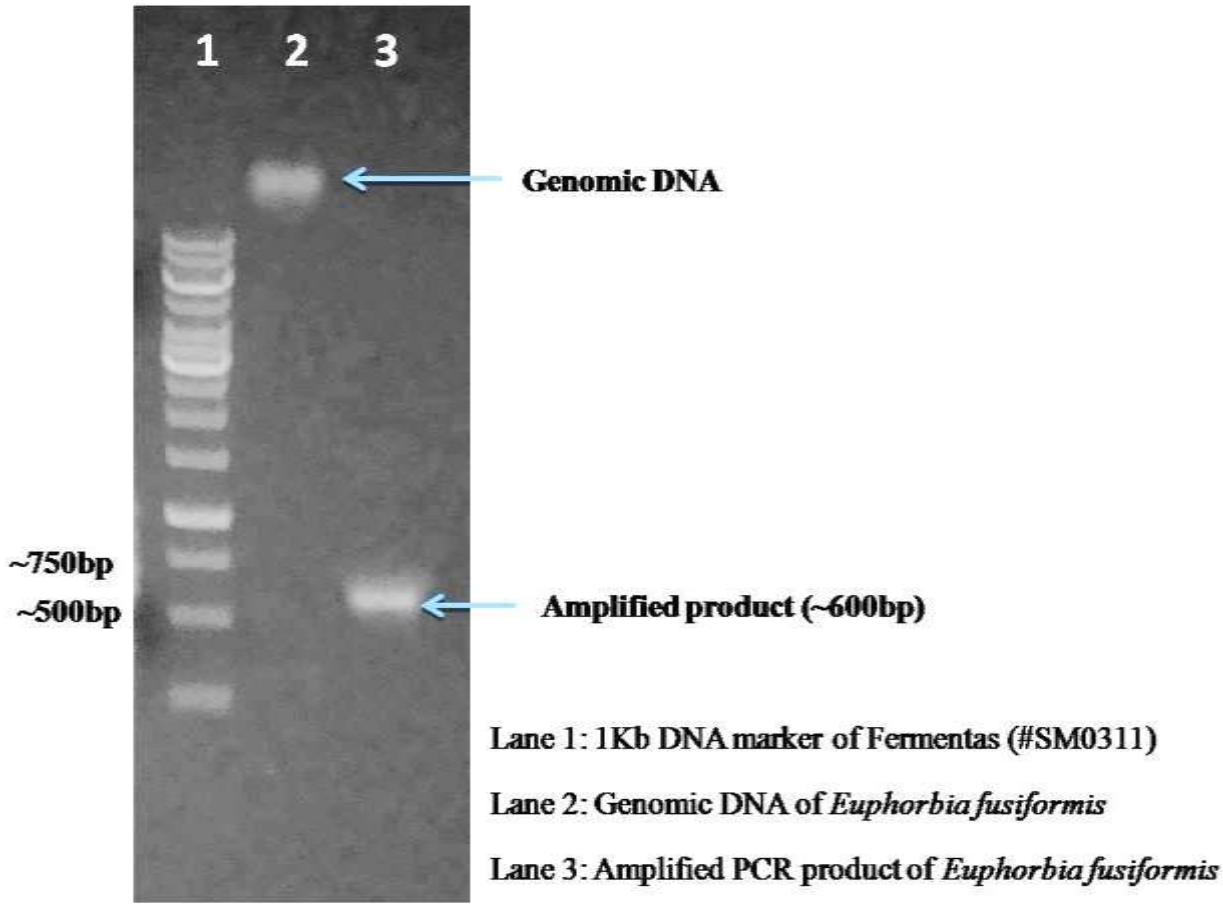
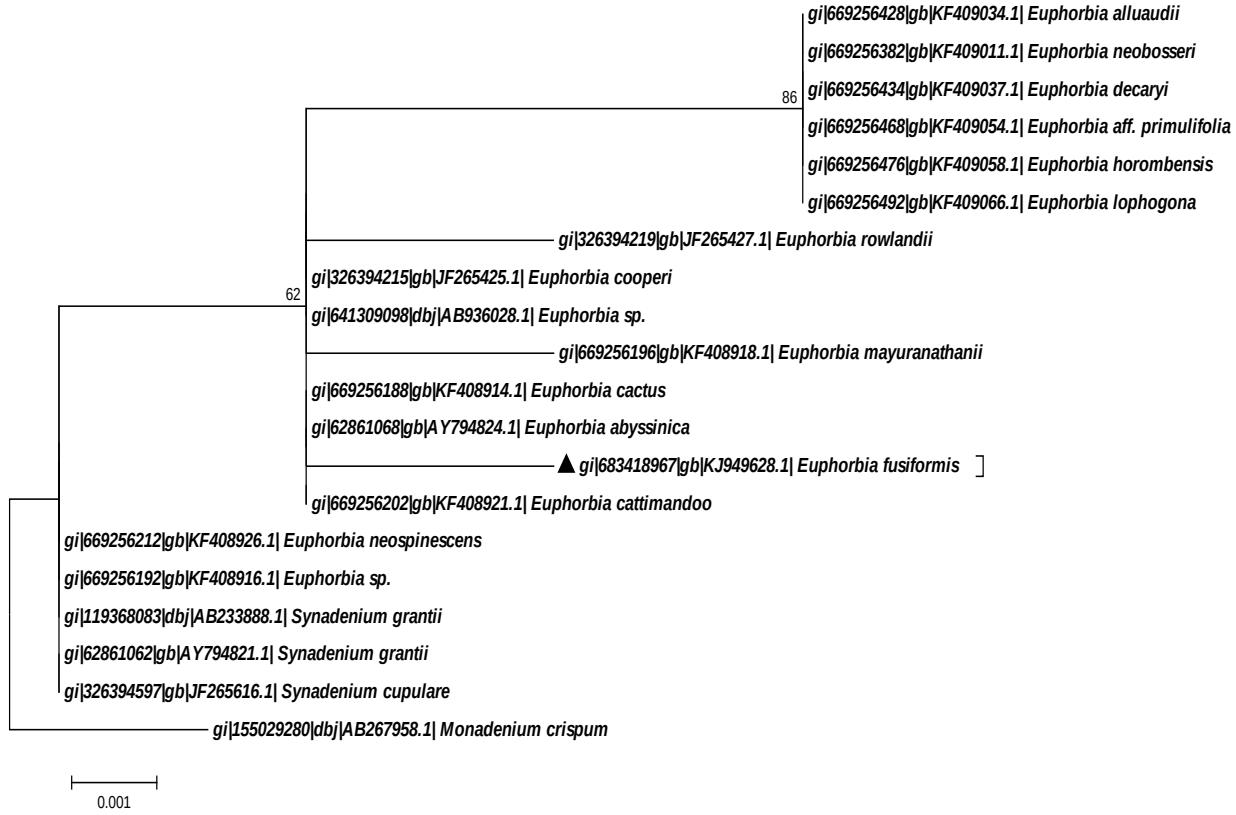


Figure 4: Molecular Phylogenetic analysis by Maximum Likelihood method

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [16]. The tree with the highest log likelihood (-527.4165) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 20 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 344 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [15].



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