WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 11, 1075-1083. Research Article ISSN 2277– 7105

USE OF DNA SEQUENCING AS ADVANCED TOOL IN TAXONOMIC AUTHENTICATION AND PHYLOGENY OF GEOPHYTES OF MEDICINAL IMPORTANCE.

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Article Received on
17 Aug 2015,

Revised on 10 Sept 2015,
Accepted on 04 Oct 2015,

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ABSTRACT

Curcuma pseudomontana J. Graham belongs to the family Zingiberaceae, commonly known as Hill Turmeric. It is an endemic geophyte to the Western and Eastern Ghats, of peninsular India. *C. pseudomontana* rhizome is beneficial against leprosy, dysentery, cardiac diseases. The Savara, Bagata, Valmiki tribes of Andhra Pradesh use tuber extracts to cure jaundice and Bagata tribes use this plant for Diabetes. Taxonomic identification of rhizomes and crude powders of traditional medicinal plants is becoming a great challenge as per classical plant taxonomy. The accuracy of authentication has limitations because of the amounts of samples, the stability of chemical constituents, the variable sources and the chemical complexity DNA sequencing, are useful for the authentication and standardization of medicinal plant species The present study deals with generation of rbcl gene sequences from *Curcuma pseudomontana* and its phylogenetic relationship with other species of the genus Curcuma.

KEYWORDS: DNA sequencing, Hill turmeric, Geophyte, Western Ghats

INTRODUCTION

Curcuma pseudomontana is an endemic geophyte to the Western and Eastern Ghats, of peninsular India. It is distributed widely in peninsular and extra peninsular parts of India; Palakkad, Kottayam, Idukki, Wayanad, Malappuram, Kannur, Thiruvananthapuram, Kozhikkode districts of Kerala, Kodagu district of Karnataka, Thane, Raigad, Pune, Ratnagiri districts of Maharashtra.[1-9]

Curcuma pseudomontana J. Graham belongs to the family Zingiberaceae, commonly known as Hill Turmeric. This species is a rhizomatous herbaceous perennial, which is found in

usually moist shady places on the fringes of wet forests or grasslands, in riparian areas, at moderately high altitude along the western side of Scientific classification of plant Kingdom Plantae Super division Spermatophyta Division Magnoliophyta Class Monocotyledonae Order Zingiberales Family Zingiberaceae Genus Curcuma Species *C pseudomontana* J.Graham 29 the Western Ghats.[10]

This species was reported to be common and abundant in the Western Ghats in the 1950s, however, the population has shrunk due to alarming rates of habitat loss in the region and overharvesting for the medical trade. The Western Ghats population is noted to have declined by more than 30% over 10 years, due to habitat loss and over collection.[11] The species has also been rated as Vulnerable in Ahdhra Pradesh (Eastern Ghats).[12] and in the Western Ghats (Karnataka, Kerala). *Curcuma pseudomontana* is therefore currently rated as Vulnerable based on criterion.

C. pseudomontana has, small root stock, bearing small almond like or subglobose tubers at the ends of the fibres (but no sessile tubers); tubers pure white inside and it is edible. Leaves are uniformly green, reaching 2ft or more long (including the petiole), 4-6' broad, lanceolate oblong acuminate, tapering to the base, petioles 8-15 in long. Flowers are bright yellow appearing with the bracts, 2 or 3 in each bract, in autumnal central narrowly oblong spikes 2-5 by 1-1 ¾ inch; peduncles 3-4in long embraced by leaf- sheaths; flowering bract 1 ¼- 1 ¾ by 5/8– 7/8 inch., obovate- lanceolate, the lowest with purple edges only. The inflorescence of *C. pseudomontana* is lateral in the early part of the rainy season and terminal later in the season. The colour of the coma is variable within the species.[10] Flowering starts from the month of June and ends in the month September.

Curcuma pseudomontana J. Graham known as Tavaksheera (Ayurveda) Kachura (Hindi), Raan halada, shindalavana or shindalavani (Marathi), Kattu manjal (Tamil), Kattu manjal (Malayalam). The Savara, Bagata, Valmiki tribes of Andhra Pradesh use tuber extracts to cure jaundice and Bagata tribes use this plant for Diabetes.[13] *C. pseudomontana* rhizome is beneficial against leprosy, dysentery, cardiac diseases.[14] Jatapu and Kaya tribes apply warm tuber paste to treat body swellings. Khand tribes apply the tuber paste on the head for cooling effect, crushed and boiled rhizome is edible.[15] Women of Jatapu and Savara tribes eat boiled tubers to increase lactation.[10] The Kukus-Mukus eat fresh tubers as a blood purifier.[16] Rhizome past used to apply to wounds and cuts.[17] The Savara, Bagata, Valmiki tribes of 30

Andhra Pradesh use tuber extracts to cure jaundice and Bagata tribes use this plant for Diabetes.[18] The tubers are also edible.[19]

The regional substitutes and adulterants have little medicinal properties commonly. Inevitably, the confusion may compromise the genuine resources and therapeutic effect of this Traditional Chinese Medicine (TCM); even imperil the safety of consumers.[20] The traditional methods are mainly based on the slight difference of morphological characters and analysis of compounds by high performance liquid chromatography (HPLC) fingerprints. The accuracy of authentication has limitations because of the amounts of samples, the stability of chemical constituents, the variable sources and the chemical complexity.

However, DNA can be extracted from fresh or dried organic tissue of the plant materials and is not restricted by the form of the samples. DNA markers are reliable for informative polymorphisms as the genetic composition is unique for each species. DNA sequencing, are useful for the authentication and standardization of medicinal plant species.[21] It also has been successfully used as a genetic marker for molecular authentication and identification of several medicinal plants and fungi, such as *Panax ginseng*.[22-23] Dendrobium Species.[24,25,26] *Euphorbia pekinensis*.[27] Bupleurum species.[28] *Boletus edulis*.[29]

In present investigation we have generated ribulose-15-bisphosphate carboxylase large subunit (rbcl) sequence for *Curcuma pseudomontana*. Its molecular phylogenetic relationships with other species are also studied. This can help in authentication of plant species for use in medical formulations in therapeutic uses.

Fig.1: Habit of *Curcuma pseudomontana*

Fig.2: Rhizome of *Curcuma pseudomontana*

MATERIALS AND METHODS

Sampling: Fresh samples of rhizomes of *Curcuma pseudomontana* were collected from Khandala district: Pune, region of Western Ghats of Maharashtra (Fig.1 and 2). The plants were identified and authenticated using herbarium collection at Botany research laboratory, DST-FIST School of Life Science, SRTM University, Nanded (MS) and Department of Botany, Dr.Babasaheb Ambedkar Marathwada University, Aurangabad (MS). Fresh rhizomes were washed thoroughly under running tap water followed by sterile distilled water and dried under shade. The material was ground into coarse powder using mechanical grinder. 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). rbcla F (Forward) 'ATGTCACCACAAACAGAGACTAAAGC3' and rbcla 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). It was further sequenced using Applied Biosystems 3730xl DNA Analyzer USA and chromatogram was obtained. For sequencing of PCR product rbcla F-5' ATGTCACCACAAACAGAGACTAAAGC 3' sequencing primer was used.

Bioinformatics analysis: The DNA sequence was submitted to NCBI GenBank (http://www.ncbi.nlm.nih.gov/). NCBI's web-based BLAST algorithm.[30] using the default settings were then used to identify the query sequences. The BLAST results were used to find out evolutionary relationship of *Curcume pseudomontana*. Altogether 10 to 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.[31]

RESULT AND DISCUSSION

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 *Curcuma pseudomontana* from the current collection, suggested that the specimens in our collection were closely related to genus Curcuma.

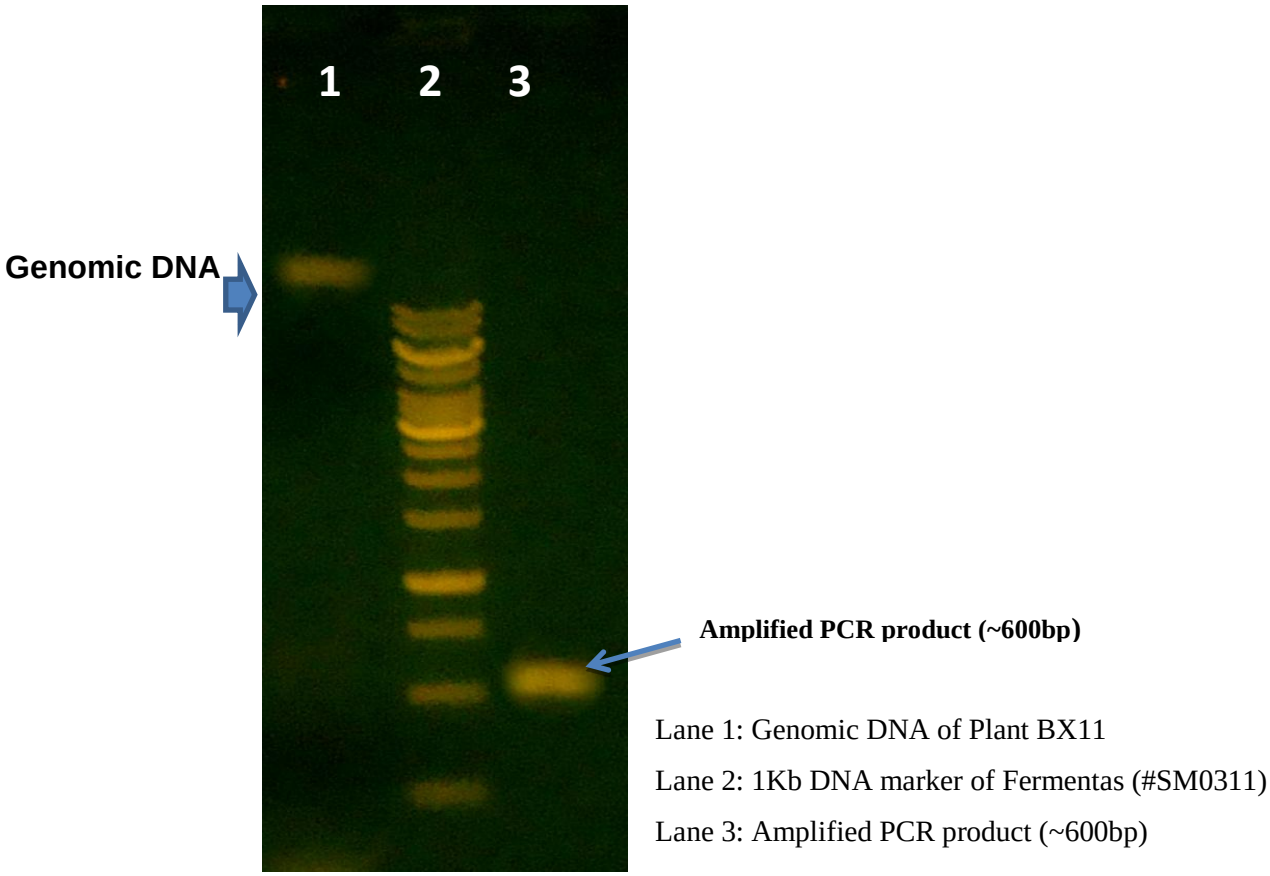

Figure 3: Amplification of rbcl region in *Curcuma pseudomontana*

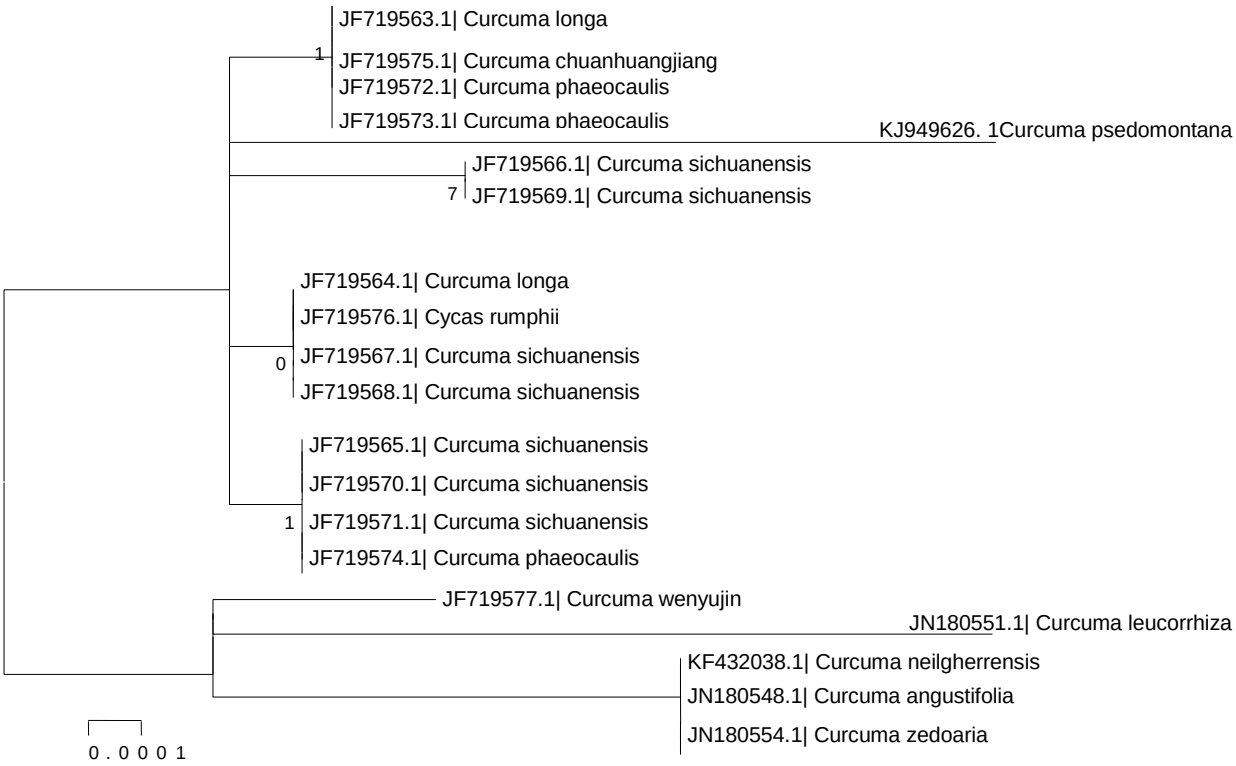

Figure 4: Phylogenetic tree for *Curcuma pseudomontana* using NCBI data

CONCLUSION

The molecular phylogeny of *Curcuma pseudomontana* 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 *Curcuma aromatica*. On the basis of morphology and phylogeny the given plant is of *Curcuma pseudomontana* belonging to family Zingiberaceae. Present work will assist in molecular identification of other Curcuma species from Western Ghats and establishing their molecular relationships. It will help in authentication of plant species of geophytes which are used in medical formulations in therapeutic uses. It will also help in further ethano-pharmacologicial studies of geophytes pertaining to properly identified plant species.

ACKNOWLEDGMENTS

The authors sincerely acknowledge the valuable support provided by Department of Biotechnology, Government Institute of Science, Aurangabad; Principal, Elphinstone College, Mumbai and Department of Botany, Elphinstone College, Mumbai.

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