iJRASET
International Journal For Research in Applied Science and Engineering Technology

# INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

**Volume:** 5 **Issue:** VI **Month of publication:** June 2017

**DOI:**

Volume 5 Issue VI, June 2017
ISSN: 2321-9653
International Journal for Research in Applied Science & Engineering Technology (IJRASET)

# Molecular Characterization of Medicinal Plant – Cassia Fistula L by Matk Gene

Hari Prasath. B[1], Chandra Mohan. A[2], Divya. S. R[3]
*M.Sc Student[1], Guide[2], Assistant Professor[3], PG Research, Dept of Biochemistry and Chemistry, Jaya College of Arts and Science, Affiliated to University of Madras.*

***Abstract-The studies on taxonomy, ethnobotany and antimicrobial potential are imperative for plants with traditional medicinal value. Two important reasons for plant barcoding is to identity unknown specimens to known species and to share it with the community resource of sequences to establish its taxonomical clarification. MaturaseK gene (matK) of chloroplast is highly conserved in plants which is associated in Group II intron RNA splicing. This reputed gene product matK is the only maturase present in chloroplasts making its presence unique. DNA barcoding by plastid coding matK markers involves sequencing of a standard region of DNA as a tool for angiosperm identification. In this study, leaf samples of Cassia fistula L were collected from Chennai and Polymerase chain reaction (PCR) was performed to amplify the matK gene and molecular characterization was performed.***
***Keywords-DNA Barcoding, matK, PCR and NCBI- KY978892.***

## I. INTRODUCTION

### *A. Traditional Herbal Medicine*

By definition, 'traditional' use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as 'traditional herbal medicines' the pharmacological treatment of disease began long ago with the use of herbs [17]. Methods of folk healing throughout the world commonly used herbs as part of their tradition.

### *B. Dna Barcoding*

In effect, barcoding in its modern form was popularized in a paper by [9], who proposed to use the mitochondrial gene CO1 as the standard barcode for all animals. This was readily adopted by the scientific community, and assessments have since shown that CO1 can be used to distinguish over 90% of species in most animal groups [14]. In recent years the barcoding movement has grown substantially, and worldwide efforts coordinated by CBOL (the Consortium for the Barcode of Life) are now being put into retrieving barcode sequences from all organisms [2].

DNA barcoding has been applied to a broad range of subjects, including taxonomic studies of "cryptic" taxa or species complexes, e.g. skipper butterflies [1]. Barcoding has also been used in ecological studies to survey animal diets through the analysis of plant remains in feces and in identifying smoked fish products sold under ambiguous product names [18].

### *C. Megakaryocyte-associated tyrosine kinase (matK)*

The rapidly evolving and highly variable gene maturase K [10] has been recommended as a locus for DNA barcoding by the Consortium for the Barcode of Life (CBOL) Plant Working Group [11]. Amplification and sequencing of the matK barcoding region is difficult due to high sequence variability in the primer binding sites [12]. Currently, there are three popular matK primer pairs available to amplify approximately the same region of the gene: 390F and 1326R [19] and [3], XF and 5R [8] and 1R_KIM and 3F_KIM ([11], [13] and [15]) used these three primer pairs to amplify DNA barcodes from 296 shrub and tree species. Disclosing evolutionary descents of different plant species with matK gene could be supreme for constructing a systematic phylogenetic tree. Comparing a molecular sequence data is indispensable to acquire knowledge of biodiversity and to provide insights into the selective force that occurred during evolution of different species [5] and [20].

## II. MATERIALS AND METHODS

### *A. Sample Collection*

Fresh, disease free healthy leaves of the Indian medicinal plant Cassia fistula L. were collected from the city of Chennai during December 2016. The collected plant was authenticated by Plant Anatomy Research Centre (**PARC/2016/3323**). The samples were transferred to the laboratory within 24 hours of collection, washed and stored till further use.

*B. Genomic Dna Isolation [7]*

Fresh-leaf tissue (0.5 g) was ground in a 1.5-mL centrifuge tube with a mortar and pestle and 4mL of preheated, freshly prepared CTAB extraction buffer (0.1 M Tris-Cl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, CTAB (3%, w/v), b-mercaptoethanol (0.2%, v/v), PVP (2% w/v) was immediately added to the tube. The tubes were incubated at 65°C for 60min, with inversion during incubation. An equal volume of chloroform: isoamyl alcohol (24:1, v/v) was added and then the tubes were inverted 8-10 times. The tubes were centrifuged at 10,000 rpm for 15 min. The supernatant was transferred to a new centrifuge tube. An equal volume of absolute ice-cold isopropanol was added. The tubes were centrifuged at 10,000 rpm for 10 min. The supernatant was discarded and the pellet was washed with 70% (v/v) ethanol. The pellet was air-dried at room temperature and then dissolved in 50 µL TE buffer. The DNA samples were stored at -20°C until further use.

*C. Quantitative Determination Of Dna By Spectrophotomeric Method*

A solution of nucleic acids strongly absorbs UV with an absorbance maximum of 260nm and proteins at 280nm which is linearly related with the concentration of DNA and RNA and the amount of contamination in the solution. The intense absorption is primarily due to the presence of aromatic rings in the purine, pyrimidine. The concentration of nucleic acid in a solution can be calculated if one knows the value of A260 of the solution.

A solution of double-stranded DNA at a concentration of 50ug/ml in a 1cm quartz cuvette will give A260 reading of 1.A solution of single-stranded DNA/RNA that has A260 of 1 in a cuvette with a 1cm path length has a concentration of 40ug/ml. Proteins are usually the major contaminants in nucleic acids extract and these have absorption maximum at 280nm. The ration of absorbance at 260 and 280nm hence provides a clear idea about the extent of contamination in the preparation. A ratio between 1.7 and 1.9 is indicative of fairly pure DNA preparation. But values less than 1.8 signify the presence of proteins as impurities. The values greater than 1.8 signify the presence of organic solvent in the DNA preparations. A ratio of 1.8 determines the pure DNA preparation.

The spectrophotometer and the UV lamp was switched on. The wavelength was set at 260nm and 280nm. The instrument is set at zero absorbance with T.E buffer or sterile water as blank. 5 or 7ul of the sample is taken in a quartz cuvette and made up to 3ml with TE buffer or sterile water. Absorbance of the solution with the sample was read. The concentration of DNA in the sample was calculated using the given formula:

Concentration of dsDNA = A260 * 50µg * dilution factor

Purity of the DNA = A260 : A280 ratio = A260 / A280

= 1.83; pure DNA

= 1.7 – 1.9; fairly pure DNA (acceptable ratio for PCR)

= less than 1.8; presence of proteins.

= greater than 1.8; presence of organic solvent

*D. Polymerase Chain Reaction*

PCR was carried out in Eppendorf Personnel Master Cycler (Germany). The PCR reaction constituents are the following

Optimized PCR condition: matK

| | | | |
|---|---|---|---|
| □ | Milli Q water | _ | 8.8µl |
| □ | 10x Buffer with 20mM Mgcl2 | _ | 2.0µl (1x) |
| □ | 2mM DNTP's | _ | 2.0µl (0.2µM) |
| □ | DNA | _ | 3.0 µl |
| □ | 3µM Forward Primer | _ | 2.0 (0.3µM) |
| □ | 3µM Reverse Primer | _ | 2.0µl (0.3µM) |
| □ | Taq polymerase (5U/µl) | _ | 0.2µl (1Unit) |

The Total Volume of the reaction is 20µl. The concentration of DNA was varied from 0.5 µl to 1 µl for optimization. The Whole reaction setup was carried out at 4°C.

*E. Pcr Programme*

| | | |
|---|---|---|
| Step 1 | Initial denaturation | -94°C for 3minutes |

Volume 5 Issue VI, June 2017
ISSN: 2321-9653
International Journal for Research in Applied Science & Engineering Technology (IJRASET)

| | | |
|---|---|---|
| Step 2 | Denaturation | -94$^{0}$C for 45 seconds |
| Step 3 | Primer annealing | -47$^{0}$C for 1minute |
| Step 4 | Extension | -72$^{0}$C for 1minute 20 seconds |
| Step 5 | Go to step 2 repeat 35 times | |
| Step 6 | final extensions | -72$^{0}$C 7 minutes |
| Step 7 | Hold | - 4$^{0}$C |

*F. Gel Electrophoresis Of Pcr Products*

DNA quality was assessed on a 0.8% Agarose Gel (in Tris Acetate EDTA buffer) electrophoresis at 50 Volts. DNA was stained with ethidium bromide visualized on a UV transilluminator.

*G. Dna Sequencing And Blast*

The PCR products were subjected to sequencing by Sanger method in an AB Sequencer. The result obtained was analysed using BLAST.

## III. RESULTS AND DISCUSSION

DNA barcoding is a method of recognizing an organism based on sequence data from one to several gene regions. Barcoding has several applications and has been used for ecological surveys [6], enigmatic taxon identification, and authentication of medicinal plant samples [21]. A number of chloroplast gene regions are characteristically used as plant barcodes, with maturase K (matK) considered core barcodes.

An ideal DNA barcode should be routinely retrievable with a single primer pair and amenable to bidirectional sequencing [4]. matK gene is one of the most rapidly evolving plastid coding regions consistently illustrating high levels of discrimination among angiosperm species. Studies have proved wholeness of matK primers assorting from successful identities to erratic resurgence with no impact on its constant usage [4]. Total genomic DNA was isolated from the collected plant samples and its purity (1.83) was found using a Spectrophotometer. Amplicons obtained after PCR were of 900bp visualized on a 0.8% agarose gel as shown in (Fig.1). Majority of Indian tribes still adhere to herbal medicines for treating infections and various outrageous diseases. This knowledge of potential use of medicinal plants are in a verge of extinction as they are of oral forms and also most of them are not taxonomically identified [16].

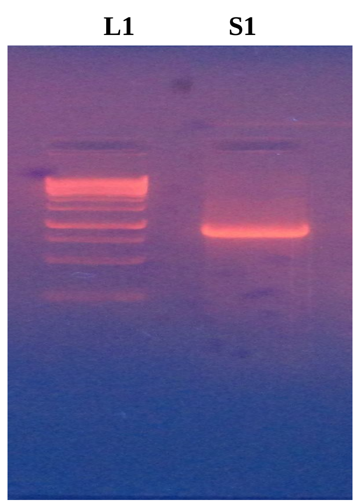



Fig. 1: PCR matK
Lane 1: 1 KB Ladder
Lane 2: S1 900bp
Agarose gel (0.8 %) for genomic DNA of Plants

*A. Dna Quantification By Spectrophotometric Method Dna Quantification*

The isolated DNA was quantified by spectrophotometer at two different wavelengths 260 nm and 280 nm. A260/A280 ratio gives

Volume 5 Issue VI, June 2017
ISSN: 2321-9653
International Journal for Research in Applied Science & Engineering Technology (IJRASET)

purity of the DNA as proteins absorb at 280 nm due to tyrosine and tryptophan residues. The ratios were in the range of 1.83 which indicated a pure DNA. DNA concentrations are given in (Table. 1)

Table 1: Concentration Blank and Sample

| Sample | OD at 260nm | OD at 280nm | Concentration (ng/µl) | Purity |
|---|---|---|---|---|
| Blank | 0.000 | 0.000 | -- | -- |
| Sample | 0.198 | 0.108 | 9900 | 1.83 |

In plants, several candidate DNA barcode regions have been proposed. Among them, the matK gene has been accepted as an important candidate barcode by many researchers. In addition, the CBOL (Consortium for the Barcode of Life) Plant Working Group [2] recommended the matK region as a plant barcode. The chloroplast gene matK is about 900 bp in length and is located within the trnK intron. It encodes a maturase-like protein that is involved in group II intron splicing. The gene exhibits a high rate of substitutions and is thus emerging as an important gene for the study of plant systematic and evolution.

The PCR products were subjected to sequencing by Sanger method in the AB Sequencer.The obtained sequence from the purified PCR product was compared with nucleotide database and was found to have maximum identity to Cassia fistula L, a weed found mostly on in around Chennai and in agricultural farms. Sequence was submitted to NCBI Genbank and its accession number generated KY978892. The sequence so obtained was about 900 bp and when analysed using NCBI-BLAST Tool and the sample was found to be 100% congeneric to *Cassia fistula* L. FASTA sequence thus obtained with Graphical Representation, Tabular representation and Alignment representation Fig. 2- Fig. 5. FASTA Sequence 1 (matK) >BRT_CF_matK

AAAGTCTTTGATAAGGATTTTCCGTCCACCCTATGGTTCTTCAAGGACCCTTTCATTCATTATGTTAGATATCAAGGAA
AATCCATTCTGGCTTCAAAGAATACGCCCTTTTTGATGAATAAATGGAAATACTATCTTATCCATTTATGGCAATGTC
ATTTTTATGTTTGGTCTCAACCAGGAAAGATCCATATAAACCAATTATCCGAGCATTCATTTTACTTTTTGGGCTATTT
TTCAAATGTGCGGCTAAATCCTTCAGTGGTACGGAGTCAAATGCTGGAAAATTCATTTCTAATTGAAAATGTTATGAA
AAAGCTTGATACAATAATTCCAATTATTCCACTAATTAGATCATTGGCTAAAGCGAAATTTTGTAATGTATTAGGGCA
TCCCATTAGTAAGCCGGTCTGGGCCGATTCATCCGATTTGGATATTATTGACCGATTTTTGCGGAGATGCAGAAATCT
TTCTCATTATTACAATGGATCCTCAACAAAAAGGAGTTTGTATCGAATCAAATATATACTTCGGCTTTCTTGTATTAAA
ACTTTGG

Fig. 2 FASTA Sequence

Graphical Representation

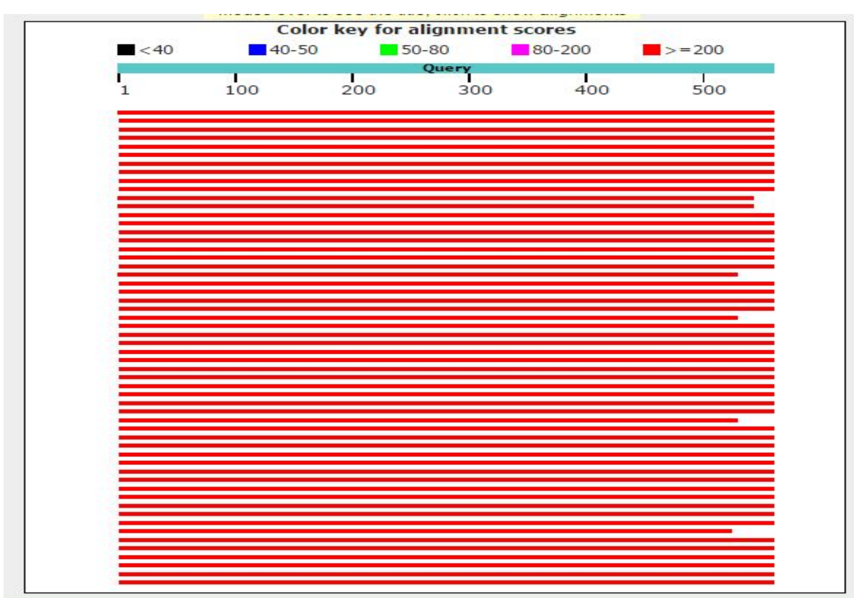



Fig. 3 Graphical Representation

**International Journal for Research in Applied Science & Engineering Technology (IJRASET)**

Tabular representation

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

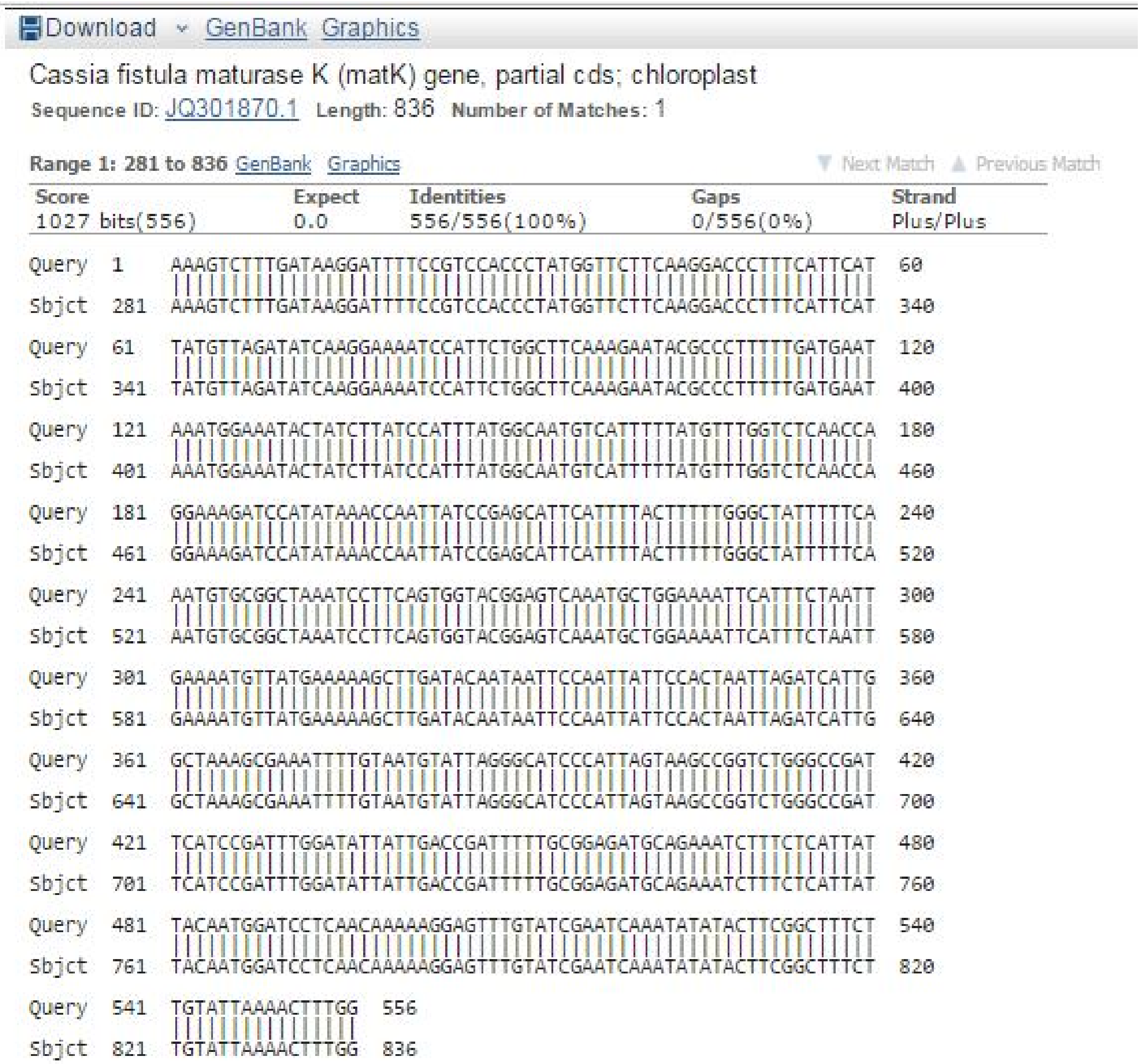
| Description | Max score | Total score | Query cover | E value | Ident | Accession |
|---|---|---|---|---|---|---|
| Cassia fistula maturase K (matK) gene, partial cds; chloroplast | 1027 | 1027 | 100% | 0.0 | 100% | JQ301870.1 |
| Cassia javanica chloroplast matK gene sequence | 1026 | 1026 | 99% | 0.0 | 100% | LC080887.1 |
| Cassia javanica maturase K (matK) gene, partial cds; chloroplast | 1026 | 1026 | 99% | 0.0 | 100% | JQ301871.1 |
| Bauhinia bracteata voucher USF:Maxwell s.n. tRNA-Lys (trnK) gene, intron; and maturase K (matK) gene, complete cds; chloroplast | 1026 | 1026 | 99% | 0.0 | 100% | JN881434.1 |
| Cassia javanica voucher Fougere-Danezan 6 (MT) tRNA-Lys (trnK) gene, partial sequence; and maturase K (matK) gene, complete cds; chloroplast | 1020 | 1020 | 99% | 0.0 | 99% | EU361910.1 |
| Cassia javanica var. indochinensis voucher M dePompert 48 ASU maturase K (matK) gene, complete cds; chloroplast | 1014 | 1014 | 99% | 0.0 | 99% | JQ619983.1 |
| Cassia grandis voucher Smith 2061 (MY) tRNA-Lys (trnK) gene, partial sequence; and maturase K (matK) gene, complete cds; chloroplast | 1014 | 1014 | 99% | 0.0 | 99% | EU361909.1 |
| Senna alata maturase K (matK) gene, partial cds; chloroplast | 1003 | 1003 | 99% | 0.0 | 99% | JQ301868.1 |
| Vouacapoua macropetala voucher Breteler 13793 (WAG) tRNA-Lys (trnK) gene, partial sequence; and maturase K (matK) gene, complete cds; chloroplast | 998 | 998 | 99% | 0.0 | 99% | EU362063.1 |
| Batesia floribunda voucher Grenand 3032 (CAY) tRNA-Lys (trnK) gene, partial sequence; and maturase K (matK) gene, complete cds; chloroplast | 992 | 992 | 99% | 0.0 | 99% | EU361869.1 |
| Cassia fistula voucher ATREE101 maturase K (matK) gene, partial cds; chloroplast | 989 | 989 | 96% | 0.0 | 99% | KJ638429.1 |
| Cassia fistula voucher ATREE102 maturase K (matK) gene, partial cds; chloroplast | 983 | 983 | 96% | 0.0 | 99% | KJ638430.1 |
| Senna sophera maturase K (matK) gene, partial cds; chloroplast | 981 | 981 | 99% | 0.0 | 99% | JQ301875.1 |
| Andira aubletii voucher NH200006 maturase K (matK) gene, partial cds; chloroplast | 981 | 981 | 99% | 0.0 | 99% | FJ037922.1 |
| Senna candolleana tRNA-Lys (trnK) gene, intron; and maturase-like protein (matK) gene, complete cds; chloroplast | 981 | 981 | 99% | 0.0 | 99% | AY386848.2 |
| Senna uniflora maturase K (matK) gene, partial cds; chloroplast | 976 | 976 | 99% | 0.0 | 98% | JQ301887.1 |
| Senna lindheimeriana voucher M F Wojciechowski 1275 (ASU) maturase K (matK) gene, complete cds; chloroplast | 976 | 976 | 99% | 0.0 | 98% | JQ619950.1 |
| Senna bauhinioides maturase-like protein (matK) gene, complete cds; chloroplast | 976 | 976 | 99% | 0.0 | 98% | EU025911.1 |
| Senna tora chloroplast, complete genome | 970 | 970 | 99% | 0.0 | 98% | KR136271.1 |
| Cassia fistula voucher Hosam00042 maturase K (matK) gene, partial cds; chloroplast | 970 | 970 | 94% | 0.0 | 100% | JX495682.1 |
| Chamaecrista absus maturase K (matK) gene, partial cds; chloroplast | 970 | 970 | 99% | 0.0 | 98% | JQ301883.1 |
| Senna montana maturase K (matK) gene, partial cds; chloroplast | 970 | 970 | 99% | 0.0 | 98% | JQ301874.1 |
| Senna auriculata maturase K (matK) gene, partial cds; chloroplast | 970 | 970 | 99% | 0.0 | 98% | JQ301878.1 |
| Senna sulfurea maturase K (matK) gene, partial cds; chloroplast | 970 | 970 | 99% | 0.0 | 98% | JQ301873.1 |
| Cassia javanica subsp. nodosa voucher Hosam00046 maturase K (matK) gene, partial cds; chloroplast | 968 | 968 | 94% | 0.0 | 100% | JX495683.1 |
| Senna tora maturase K (matK) gene, partial cds; chloroplast | 965 | 965 | 99% | 0.0 | 98% | JQ301877.1 |

Fig. 4 Tabular representation

Volume 5 Issue VI, June 2017
ISSN: 2321-9653



Alignment representation



Download GenBank Graphics

Cassia fistula maturase K (matK) gene, partial cds; chloroplast

Sequence ID: JQ301870.1 Length: 836 Number of Matches: 1

Range 1: 281 to 836 GenBank Graphics — Next Match — Previous Match

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 1027 bits(556) | 0.0 | 556/556(100%) | 0/556(0%) | Plus/Plus |

```
Query  1    AAAGTCTTTGATAAGGATTTTCCGTCCACCCTATGGTTCTTCAAGGACCCTTTCATTCAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  281  AAAGTCTTTGATAAGGATTTTCCGTCCACCCTATGGTTCTTCAAGGACCCTTTCATTCAT  340

Query  61   TATGTTAGATATCAAGGAAAATCCATTCTGGCTTCAAAGAATACGCCCTTTTTGATGAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  341  TATGTTAGATATCAAGGAAAATCCATTCTGGCTTCAAAGAATACGCCCTTTTTGATGAAT  400

Query  121  AAATGGAAATACTATCTTATCCATTTATGGCAATGTCATTTTTATGTTTGGTCTCAACCA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  401  AAATGGAAATACTATCTTATCCATTTATGGCAATGTCATTTTTATGTTTGGTCTCAACCA  460

Query  181  GGAAAGATCCATATAAACCAATTATCCGAGCATTCATTTTACTTTTTGGGCTATTTTTCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  461  GGAAAGATCCATATAAACCAATTATCCGAGCATTCATTTTACTTTTTGGGCTATTTTTCA  520

Query  241  AATGTGCGGCTAAATCCTTCAGTGGTACGGAGTCAAATGCTGGAAAATTCATTTCTAATT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  521  AATGTGCGGCTAAATCCTTCAGTGGTACGGAGTCAAATGCTGGAAAATTCATTTCTAATT  580

Query  301  GAAAATGTTATGAAAAAGCTTGATACAATAATTCCAATTATTCCACTAATTAGATCATTG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  581  GAAAATGTTATGAAAAAGCTTGATACAATAATTCCAATTATTCCACTAATTAGATCATTG  640

Query  361  GCTAAAGCGAAATTTTGTAATGTATTAGGGCATCCCATTAGTAAGCCGGTCTGGGCCGAT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  641  GCTAAAGCGAAATTTTGTAATGTATTAGGGCATCCCATTAGTAAGCCGGTCTGGGCCGAT  700

Query  421  TCATCCGATTTGGATATTATTGACCGATTTTTGCGGAGATGCAGAAATCTTTCTCATTAT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  701  TCATCCGATTTGGATATTATTGACCGATTTTTGCGGAGATGCAGAAATCTTTCTCATTAT  760

Query  481  TACAATGGATCCTCAACAAAAAGGAGTTTGTATCGAATCAAATATATACTTCGGCTTTCT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  761  TACAATGGATCCTCAACAAAAAGGAGTTTGTATCGAATCAAATATATACTTCGGCTTTCT  820

Query  541  TGTATTAAAACTTTGG  556
            ||||||||||||||||
Sbjct  821  TGTATTAAAACTTTGG  836
```

Fig. 5 Alignment representation

## IV. CONCLUSION

It is clear that the DNA barcoding has great potential for enhancing ecological and evolutionary investigations if the right genetic markers are selected. In this study, molecular characterization of the collected plant was carefully studied and its evolutionary relationship was constructed. Sequence was submitted to NCBI Genbank and its accession number generated KY978892. Nucleotide BLAST (blastn) shows 100% congeneric to Cassia fistula L. FASTA sequence thus obtained.

## REFERENCES

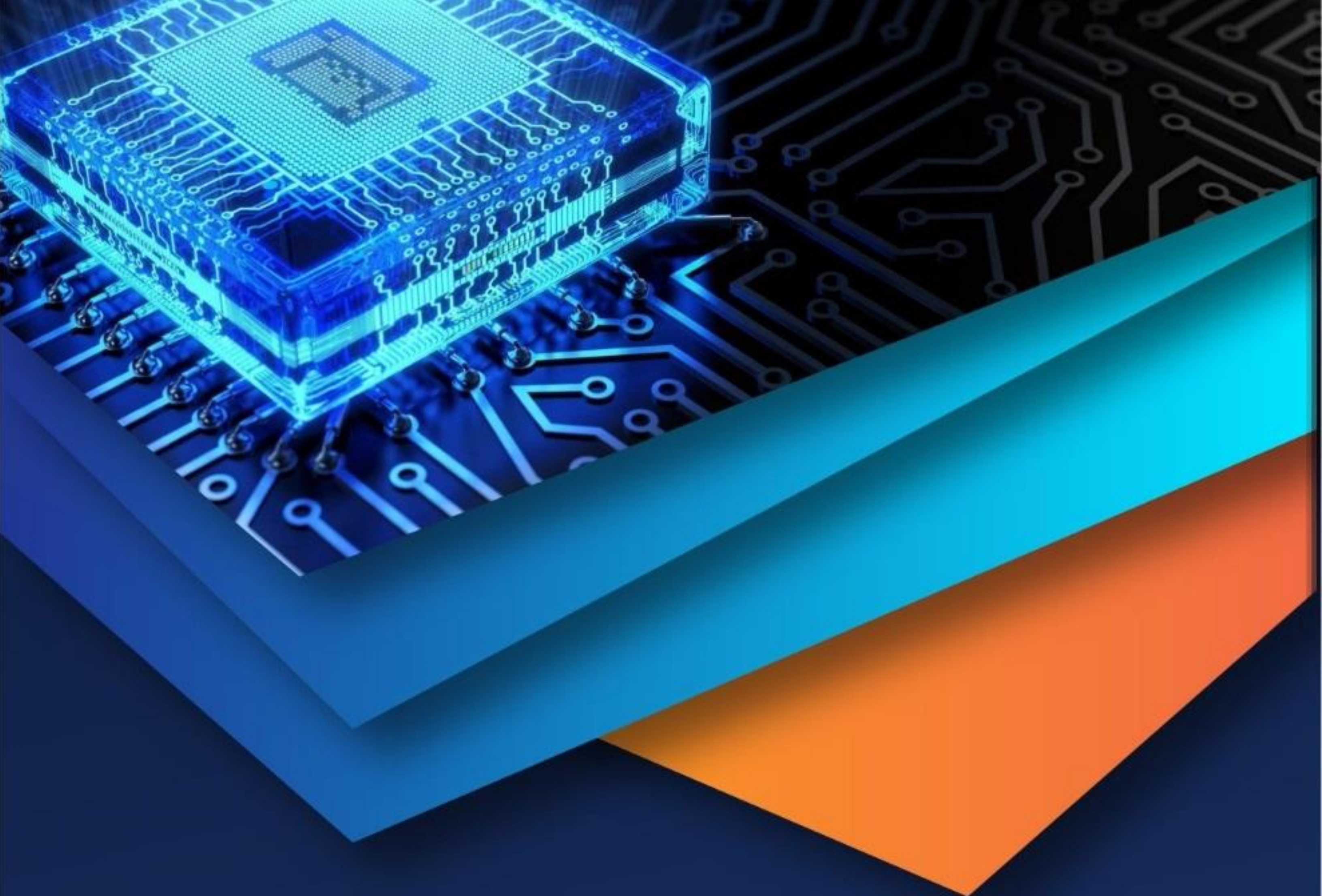
[1] Burns. JM, Janzen. DH, Hajibabaei. M, Hallwachs. W and Hebert. PDN (2007) DNA barcodes of closely related (but morphologically and ecologically distinct) species of skipper butterflies (Hesperiidae) can differ by only one to three nucleotides. J Lepid Soc 61:138–153.

[2] CBOL Plant Working Group. (2009). A DNA barcode for Land Plants. Pnas; 106 (31): 12794–12797.

[3] Cuénoud P., Savolainen V., Chatrou L. W., Powell M., Grayer R. J., Chase M. W. 2002. Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid rbcL, atpB, and matK DNA sequences. American Journal of Botany 89: 132–144. [PubMed]

Volume 5 Issue VI, June 2017
ISSN: 2321-9653
International Journal for Research in Applied Science & Engineering Technology (IJRASET)

[4] Daniel H. Janzen. (2009). "A DNA barcode for land plants". PNAS, vol. 106 _ no. 31.
[5] David Baum (2008). Reading a Phylogenetic Tree: The Meaning of Monophyletic Groups.
[6] Dick. C.W. and W.J. Kress. (2009). Dissecting tropical plant diversity with forest plots and a molecular toolkit. BioScience; 59: 745-755.
[7] DOYLE, J.J.; DOYLE J.L. Isolation of plant DNA from fresh tissue. Focus, v.12, p.13-15, 1990.
[8] Ford C. S., Ayres K. L., Toomey N., Haider N., Van Alphen Stahl J., Kelly L. J., Wikström N., et al. 2009. Selection of candidate coding DNA barcoding regions for use on land plants. Botanical Journal of the Linnean Society 159: 1–11.
[9] Hebert PDN, Cywinska A., Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proc Roy Soc Lond Ser B 270:313–321.
[10] Hilu K. W., Liang H. 1997. The matK gene: Sequence variation and application in plant systematics. American Journal of Botany 84: 830–839. [PubMed]
[11] Hollingsworth P. M., Forrest L. L., Spouge J. L., Hajibabaei M., Ratnasingham S., van der Bank M., Chase M. W., et al. 2009. A DNA barcode for land plants. Proceedings of the National Academy of Sciences, USA 106: 12794–12797.
[12] Hollingsworth P. M., Graham S. W., Little D. P. 2011. Choosing and using a plant DNA barcode. PLoS ONE 6: e1925.
[13] Jeanson M. L., Labat J. N., Little D. P. 2011. DNA barcoding: A new tool for palm taxonomists? Annals of Botany 108: 1445–1451. [PMC free article] [PubMed]
[14] Kerr. KCR, Stoeckle. MY, Dove. CJ, Weigt. LA, Francis. CM and Hebert. PDN (2007). Comprehensive DNA barcode coverage of North American birds. Mol Ecol Notes 7:535–543
[15] Kress W. J., Erickson D. L., Jones F. A., Swenson N. G., Perez R., Sanjur O., Bermingham E. 2009. Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. Proceedings of the National Academy of Sciences, USA 106: 18621–18626. [PMC free article] [PubMed]
[16] Lenin B.J and Venkat R.S. (2009). "Traditional Uses of Some Medicinal Plants by tribals of Gangaraju Madugula Mandal of Visakhapatnam District, Andhra Pradesh". Ethnobotanical Leaflets 13: 388-98.
[17] Schulz. V, Hänsel, R. and Tyler, V.E. (2001). Rational Phytotherapy. A Physician's Guide to Herbal Medicine, 4th Ed., Berlin, Springer-Verlag.
[18] Smith. PJ, McVeagh. SM *and* Steinke. D *(*2008). DNA barcoding for the identification of smoked fish products. J Fish Biol 72*:*464–71.
[19] Sun H., McLewin W., Fay M. F. 2001. Molecular phylogeny of Helleborus (Ranunculaceae), with an emphasis on the East Asian-Mediterranean disjunction. Taxon 50: 1001–1018.
[20] Tamura. K, Peterson. D, Peterson. N, Stecher. G, Nei. M and Kumar. S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, Mol Biol Evol. , 2011, vol. 28 (pg. 2731-2739).
[21] Xue. C.Y. and Li. D.Z. (2011). Use of DNA barcode sensu latoto identifies traditional Tibetan medicinal plant Gentianopsis paludosa (Gentianaceae). J. Sys. Evol; 49(3): 267-270.




10.22214/IJRASET

INDEX COPERNICUS

45.98

IMPACT FACTOR: 7.129

TOGETHER WE REACH THE GOAL

IMPACT FACTOR: 7.429


# INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY