iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 5 **Issue:** VII **Month of publication:** July 2017

DOI:

www.ijraset.com

Call: 08813907089 | E-mail ID: ijraset@gmail.com

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor:6.887
Volume 5 Issue VII, July 2017- Available at www.ijraset.com

DNA Baracoding with Ribulose-1, 5-Bisphosphate Carboxylase Oxygenase in Indian Medicinal Plant-Cassia Fistula L

Rajavelu. N[1], Dhanarajan. M.S[2], Chandra mohan. A[3]
Research Scholar[1], Guide[2], Professor[3], PG Research, Department of Biotechnology and Biochemistry,
Jaya College of Arts and Science, Affiliated to University of Madras.

Abstract: ***To develop a barcode for the medicinally important plant Cassia fistula L. To collect leaf samples of the Indian medicinal plant Cassia fistula L and to isolate total genomic DNA from the collected sample then the qualitatively analyse the DNA using Agarose gel electrophoresis and the quantitate DNA yield using UV Spectrophotometry was done. The aim of study is to amplify rbcL gene using Polymerase Chain Reaction. In this current study, the leaf samples of Cassia fistula L were collected from Chennai and the PCR amplicons were analysed using Agarose gel electrophoresis, purify the amplicons and understand the sequence of DNA by Sanger method then the sequence alignment using BLAST (NCBI) tool.***
Keywords: DNA Barcoding, RuBisCo, PCR and Cassia fistula L

I. INTRODUCTION

A. DNA Barcoding

Taxonomy, the science of classifying living things according to shared features, has always been a part of human society. Classical taxonomy falls short in this race to catalog biological diversity before it disappears. The International Barcode of Life (iBOL) organizes collaborators from more than 150 countries to participate in a variety of "campaigns" to census diversity among plant barcoding relies on short, highly variably regions of the genome. With thousands of copies per cell, mitochondrial and chloroplast sequences are readily amplified by polymerase chain reaction (PCR), even from very small or degraded specimens. A region of the chloroplast gene rbcL—RuBisCo large subunit—is used for barcoding plants. The most abundant protein on earth, RuBisCo (Ribulose-1, 5-bisphosphate carboxylase oxygenase) catalyzes the first step of carbon fixation.

DNA is extracted from the tissue sample, and the barcode portion of the rbcL, COI, or ITS gene is amplified by PCR. The amplified sequence (amplicon) is submitted for sequencing in one or both directions. The sequencing results are then used to search a DNA database. A close match quickly identifies a species that is already represented in the database. However, some barcodes will be entirely new, and identification may rely on placing the unknown species in a phylogenetic tree with near relatives. Novel DNA barcodes can be submitted to GenBank® (www.ncbi.nlm.nih.gov).

In effect, barcoding in its modern form was popularized in a paper by [5], 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. 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].

A single barcoding locus combining these two traits has not been found, and a combination of two or more, probably plastid, loci will almost certainly be required to approach the level of species discrimination and universality that CO1 confers for animals [7]. 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 [14]. The main aim of DNA barcoding is to establish a shared community resource of DNA sequences that can be used for organismal identification and taxonomic clarification. This approach was successfully pioneered in animals using a portion of the cytochrome oxidase 1 (CO1) mitochondrial gene. In plants, establishing a standardized DNA barcoding system has been more challenging. DNA barcoding offers a possible solution to this problem and could greatly improve our understanding of fern gametophytes and their biology. Recently, unknown fern gametophytes were shown to be identifiable, often to species level, by using plastid DNA sequences [10], [13] and [3], suggesting that this DNA-based identification tool has the potential to be applied to large-scale ecological surveys [9]. The DNA barcoding approach has also been useful in distinguishing among fern species in the

horticultural trade [12] and in Chinese herbal medicine [15] and[11], two areas where species names are frequently confused. Despite these promising applications, ferns, with their critical phylogenetic position as sister to seed plants, have largely been neglected in choosing the standardized barcode for all land plants [8] and [6].

B. Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase (RBCL)

Ribulose-1,5-bisphosphate carboxylase/oxygenase, commonly known by the abbreviations RuBisCO, RuBPCase, or RuBPco, is an enzyme involved in the first major step of carbon fixation, a process by which atmospheric carbon dioxide is converted by plants and other photosynthetic organisms to energy-rich molecules such as glucose. In chemical terms, it catalyzes the carboxylation of ribulose-1,5-bisphosphate (also known as RuBP). It is probably the most abundant enzyme on Earth. Among plastid regions, rbcL is the best characterized gene. Improvements in primer design make it easily retrievable across land plants and it is well suited for recovery of high-quality bidirectional sequences. Although not the most variable region, it is a frequent component of the best performing multi-locus combinations for species discrimination.

II. MATERIALS AND METHODS

A. Sample Collection

Fresh, disease free leaves of the Indian medicinal plant Cassia fistula L. were collected from the city of Chennai during December 2016. The samples were transfreed to the laboratory within 24 hours of collection, washed and stored till further use.

B. Genomic DNA Isolation [4]

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. Qualitative Analysis of Genomic DNA by Agarose Gel Electrophoresis

For the majority of DNA samples, electrophoretic separation is carried out in agarose gels. This is because DNA molecules and their fragments are considerably larger than proteins; therefore larger size agarose gels are required. Under an electric field, any given fragment of DNA should move towards the anode with the same mobility. This is due to the charge per unit length owing to the phosphate groups. Separation on agarose gels is achieved because of resistance to their movement caused by the gel matrix. Thus the largest molecules will have difficulty moving, whereas the smallest molecules will be relatively unhindered. Consequently the mobility of DNA molecules during gel electrophoresis will depend on size. Gel concentrations must be chosen based on the molecules to be separated such as for plasmid molecules – 1%; genomic DNA – 0.8% and RNA – 1.5%, mitochondrial DNA – 0.8% and amplified samples at 1.5%. 0.8 % agarose gel was prepared with 1X TAE buffer and stained with 2µl of ethidium bromide. Samples were loaded with loading dye (2µl of loading dye is used). Electrophoresis of DNA fragments was performed at 50volts. Visualization of DNA fragments was done in the UV trans-illuminator.

D. 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 I n the solution 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

= 1.8; 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

E. Polymerase Chain Reaction

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

Optimized PCR condition: rbcL

- Milli Q water _ 7.8µl
- 10x Buffer with 20mM Mgcl2 _ 2.0µl (1x)
- 2mM DNTP's _ 2.0µl (0.2µM)
- DNA _ 4.0 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.

PCR PROGRAMME

Step 1	Initial denaturation	-94°C for 3minutes
Step 2	Denaturation	-94^{0}C for 45 seconds
Step 3	Primer annealing	-51^{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 1.5% 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 a AB Sequencer. The result obtained was analysed using BLAST.

III. RESULTS AND DISCUSSION

Total genomic DNA isolated showed a purity of 1.83. Upon PCR amplification using 100ng of this DNA, an amplicon of size ~900bp was observed in the agarose gel, when compared with a 1KB DNA ladder. The gel was cut and purified followed by DNA sequencing. The sequence so obtained was about 1050 bp and when analysed using NCBI-BLAST Tool and the sample was found to be 100% congeneric to *Cassia fistula* L. The procedure was repeated for amplifying the rbcL marker gene. Upon PCR amplification using 100ng of the DNA, an amplicon of size ~1200bp was observed in the agarose gel, when compared with a 1KB DNA ladder. The gel was cut and purified followed by DNA sequencing. The sequence so obtained was about 770 bp and when analysed using NCBI-BLAST Tool and the sample was found to be 100% congeneric to *Cassia fistula* L

ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor:6.887
Volume 5 Issue VII, July 2017- Available at www.ijraset.com

A. Genomic DNA

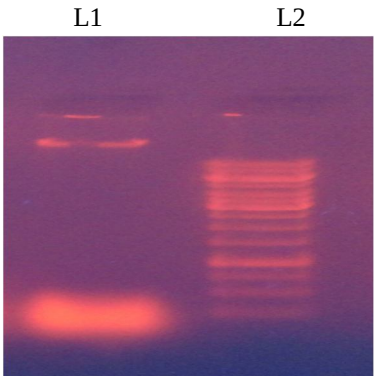


Fig. 1 Agarose gel (0.8 %) for genomic DNA of Plants
Lane 1: Genomic DNA
Lane 2: 1 Kb Ladder

B. Dna Quantiifcation by Spectrophotometric Method

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

C. Polymerase Chain Reaction

1) PCR RBCL:

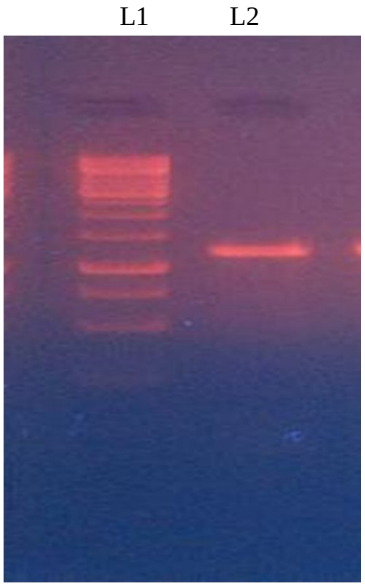


Fig. 2 PCR- rbcl
Lane 1: 1 KB Ladder
Lane 2: S1 ~1200bp

In plants, several DNA barcode the regions have been proposed. Among them, the matK and rbcl 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. 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 (Table. 2) and Alignment representation Fig. 3- Fig. 5.

D. Sequence 1 (RBCL)

>*BRT_CF_rbcL*GTTGGGTTCAAAGCTGGTGTTAAAGATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGA
TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGTGCCGCGGTAGCTGC
TGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTACCAGTCTTGATCGTTACAAAGGACGATGCTAC
CACATCGAGCCCGTTGCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGT
TCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATT
TGCGAATCCCTATTTCTTATATTAAAACTTTCCAAGGTCCGCCTCACGGCATCCAAGTTGAGAGAGATAAATTGAACA
AGTACGGCCGTCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTATCCGCTAAGAATTACGGTAGAGCTGTTT
ATGAATGTCTCCGCGGTGGACTTGATTTTACCAAAGATGATGAGAATGTGAATTCCCAACCATTTATGCGTTGGAGAG
ACCGTTTCTGCTTTTGTGCCGAAGCACTTTATAAAGCACAGGCCGAAACAGGTGAAATCAAAGGGCATTACTTGAAT
GCTACGGCAGGTACATGCGAAGAAATGATCAAAAGAGCTGTATTTGCCCGAGAATTGGGCGTTCCTATCGTAATGCA

Fig. 3 FASTA Sequence

E. Graphical Representation

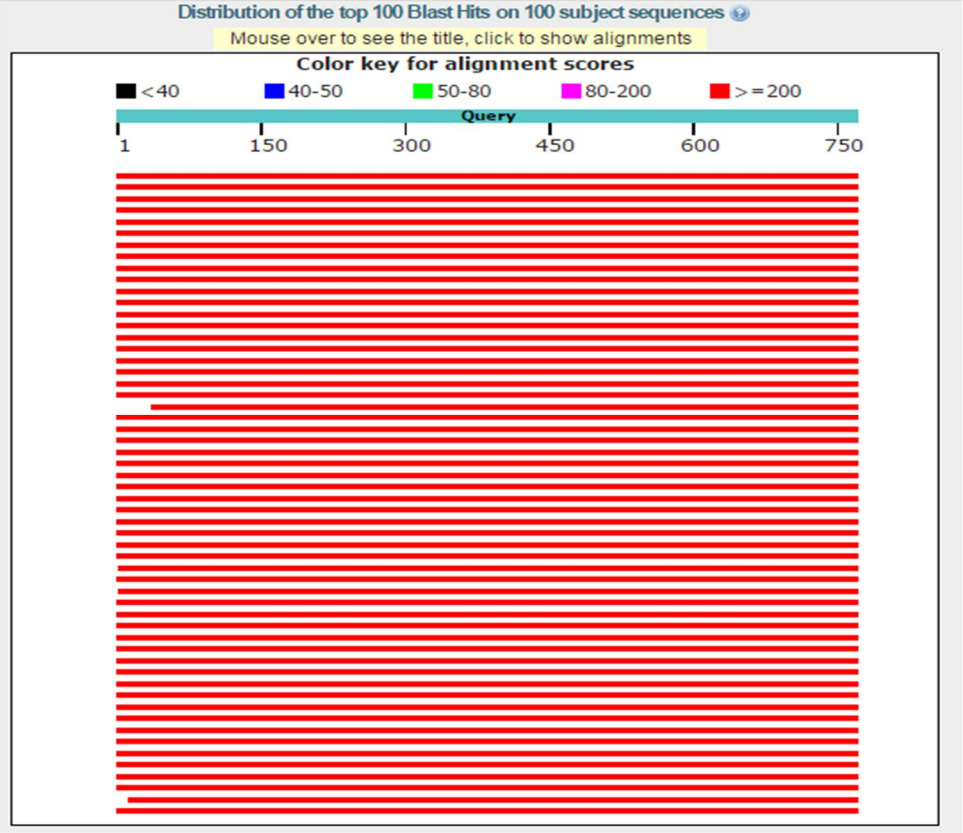


Fig. 4 Graphical Representation

Table. 2 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 ribulose 1,5-bisphosphate carboxylase-oxygenase large subunit (rbcL) gene, choloroplast gene encoding chloroplast protein, partial cds	1423	1423	100%	0.0	100%	U74195.1
Cassia grandis partial rbcL gene for ribulose bisphosphate carboxylase large chain, specimen voucher Smith 2061 (MT)	1406	1406	100%	0.0	99%	AM234244.1
Haematoxylum brasiletto chloroplast, complete genome	1373	1373	100%	0.0	99%	KJ468097.1
Erythrophleum ivorense ribulose 1,5-bisphosphate carboxylase-oxygenase large subunit (rbcL) gene, choloroplast gene encoding chloroplast protein, partial cds	1367	1367	100%	0.0	99%	U74205.1
Mezoneuron cucullatum chloroplast, complete genome	1362	1362	100%	0.0	99%	KU569489.1
Senna tora chloroplast, complete genome	1362	1362	100%	0.0	99%	KR136271.1
Senna tora voucher JKTM-1-000064 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1362	1362	100%	0.0	99%	KP058311.1
Sclerolobium sp. Klitgaard 687 partial rbcL gene for ribulose bisphosphate carboxylase large chain, specimen voucher Klitgaard B. B. 687 (K)	1362	1362	100%	0.0	99%	AM234242.1
Caesalpinia calycina partial rbcL gene for ribulose bisphosphate carboxylase large chain, specimen voucher Lewis 1885 (K)	1362	1362	100%	0.0	99%	AM234236.1
Parkinsonia microphylla voucher Hawkins 127 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1362	1362	100%	0.0	99%	AY904404.1
Jacqueshuberia loretensis voucher Rimachi 9050 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1362	1362	100%	0.0	99%	AY904391.1
Balsamocarpon brevifolium isolate 54 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1362	1362	100%	0.0	99%	AY308524.1
D.regia chloroplast rbcL gene	1362	1362	100%	0.0	99%	Z70156.1
C.senna chloroplast rbcL gene	1362	1362	100%	0.0	99%	Z70155.1
C.pulcherrima chloroplast rbcL gene	1362	1362	100%	0.0	99%	Z70153.1
C.didymobotrya chloroplast rbcL gene	1362	1362	100%	0.0	99%	Z70154.1
Tachigali paniculata ribulose 1,5-bisphosphate carboxylase-oxygenase large subunit (rbcL) gene, choloroplast gene encoding chloroplast protein, partial cds	1362	1362	100%	0.0	99%	U74240.1
Cercidium andicola voucher Hughes 2313 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1358	1358	100%	0.0	98%	AY904410.1
Caesalpinia eriostachys voucher Hughes 1832 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1358	1358	100%	0.0	98%	AY904381.1
Libidibia coriaria chloroplast, complete genome	1356	1356	100%	0.0	98%	KJ468095.1
Cassia sp. SH-2010 chloroplast gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds, isolate: A123	1356	1356	95%	0.0	100%	AB586310.1
Parkinsonia africana voucher Kolberg sn ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1356	1356	100%	0.0	98%	AY904411.1
S.japonica chloroplast rbcL gene	1356	1356	100%	0.0	98%	Z70142.1
Xanthocercis zambesiaca ribulose 1,5-bisphosphate carboxylase-oxygenase large subunit (rbcL) gene, choloroplast gene encoding chloroplast protein, partial cds	1356	1356	100%	0.0	98%	U74189.1
Pentaclethra macrophylla partial rbcL gene for ribulose bisphosphate carboxylase large chain, specimen voucher DeWilde 11496 (WAG)	1351	1351	100%	0.0	98%	AM234250.1
Caesalpinia velutina voucher Hughes 255 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1351	1351	100%	0.0	98%	AY904382.1

F. Alignment Representation

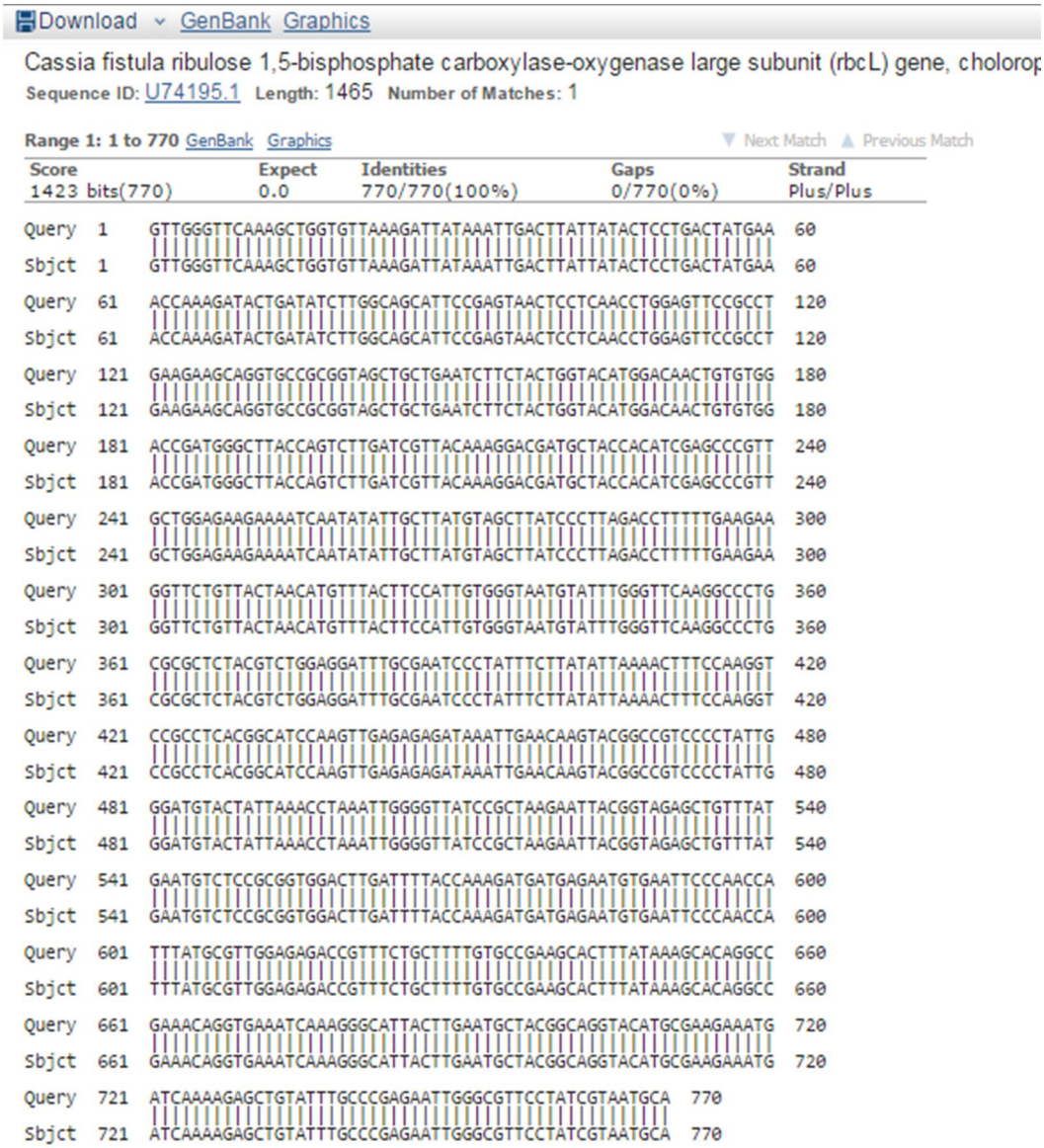


Fig. 5 Alignment representation

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. It is clear that the DNA barcoding has great potential for the identification of the plant specimen.

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] de Groot GA, During HJ, Maas JW, Schneider H, Vogel JC, et al. (2011) Use of rbcL and trnL-F as a two-locus DNA barcode for identification of NW-European ferns: an ecological perspective. PLoS ONE 6: e16371.GA de GrootHJ DuringJW MaasH. SchneiderJC Vogel2011Use of rbcL and trnL-F as a two-locus DNA barcode for identification of NW-European ferns: an ecological perspective.PLoS ONE6e16371
[4] DOYLE, J.J.; DOYLE J.L. Isolation of plant DNA from fresh tissue. Focus, v.12, p.13-15, 1990.
[5] Hebert PDN, Cywinska A., Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proc Roy Soc Lond Ser B 270:313–321.

[6] Hollingsworth ML, Andra Clark A, Forrest LL, Richardson J, Pennington RT, et al. (2009) Selecting barcoding loci for plants: evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants. Mol Ecol Resour 9: 439–457.ML HollingsworthA. Andra ClarkLL ForrestJ. RichardsonRT Pennington2009Selecting barcoding loci for plants: evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants.Mol Ecol Resour9439457.

[7] 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].

[8] Lahaye R, van der Bank M, Bogarin D, Warner J, Pupulin F, et al. (2008) DNA barcoding the floras of biodiversity hotspots. Proc Nat Acad Sci USA 105: 2923–2928.R. LahayeM. van der BankD. BogarinJ. WarnerF. Pupulin2008DNA barcoding the floras of biodiversity hotspots.Proc Nat Acad Sci USA10529232928.

[9] Li FW, Kuo LY, Huang YM, Chiou WL, Wang CN (2010) Tissue-direct PCR, a rapid and extraction-free method for barcoding of ferns. Mol Ecol Resour 10: 92–95.FW LiLY KuoYM HuangWL ChiouCN Wang2010Tissue-direct PCR, a rapid and extraction-free method for barcoding of ferns.Mol Ecol Resour109295.

[10] Li FW, Tan BC, Buchbender V, Moran RC, Rouhan G, et al. (2009) Identifying a mysterious aquatic fern gametophyte. Pl Syst Evol 281: 77–86.FW LiBC TanV. BuchbenderRC MoranG. Rouhan2009Identifying a mysterious aquatic fern gametophyte.Pl Syst Evol2817786.

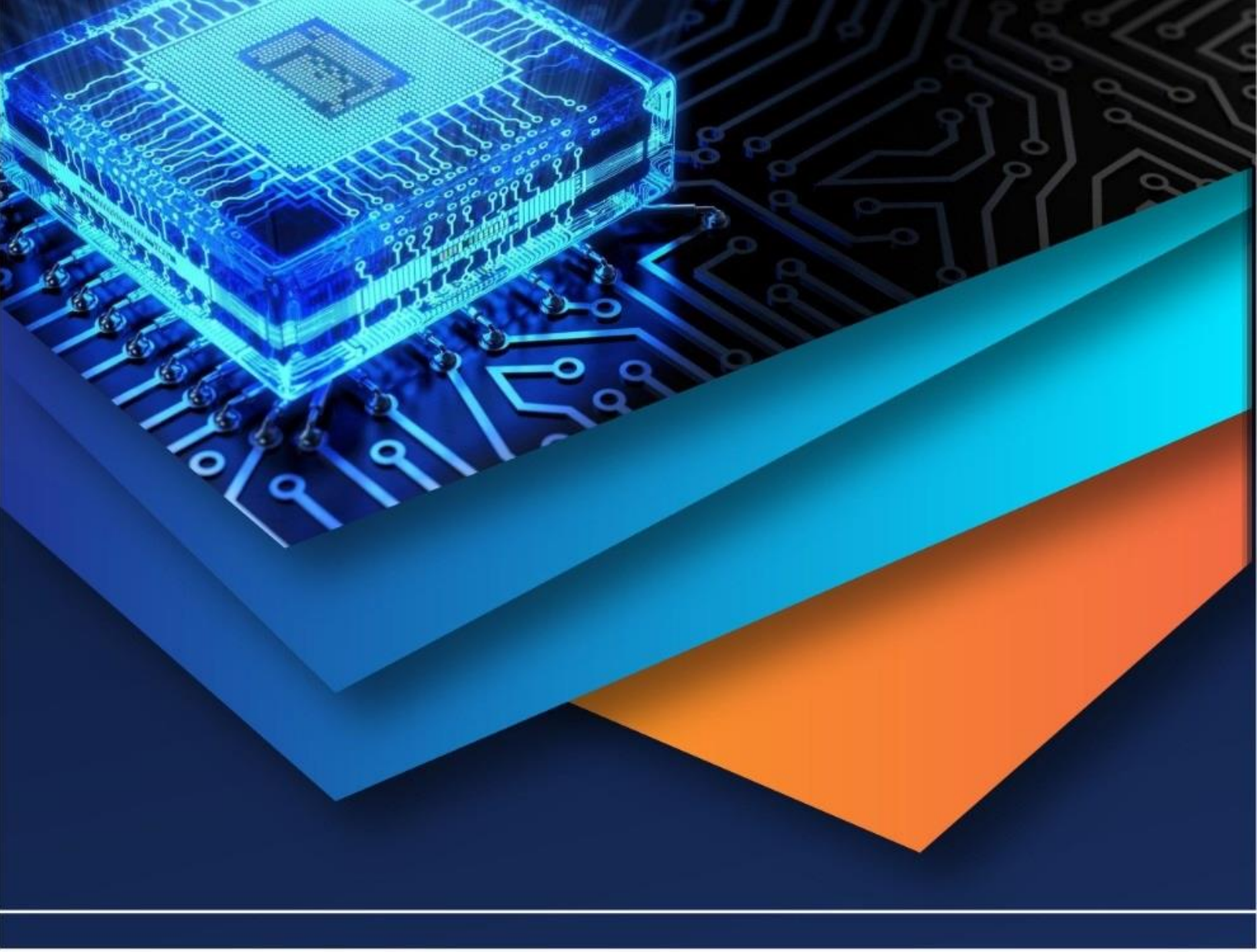
[11] Ma XY, Xie CX, Liu C, Song JY, Yao H, et al. (2010) Species identification of medicinal pteridophytes by a DNA barcode marker, the chloroplast psbA-trnH intergenic region. Biol Pharm Bull 33: 1919–1924.XY MaCX XieC. LiuJY SongH. Yao2010Species identification of medicinal pteridophytes by a DNA barcode marker, the chloroplast psbA-trnH intergenic region.Biol Pharm Bull3319191924.

[12] Pryer KM, Schuettpelz E, Huiet L, Grusz AL, Rothfels CJ, et al. (2010) DNA barcoding exposes a case of mistaken identity in the fern horticultural trade. Mol Ecol Resour 10: 979–985.KM PryerE. SchuettpelzL. HuietAL GruszCJ Rothfels2010DNA barcoding exposes a case of mistaken identity in the fern horticultural trade.Mol Ecol Resour10979985.

[13] Schneider H, Schuettpelz E (2006) Identifying fern gametophytes using DNA sequences. Mol Ecol Notes 6: 989–991.H. SchneiderE. Schuettpelz2006Identifying fern gametophytes using DNA sequences.Mol Ecol Notes6989991.

[14] Valentini A, Miquel C, Nawaz MA, Bellemain E, Coissac E, Pompanon F, Gielly L, Cruaud C, Nascetti G, Wincker P, Swenson JE, Taberlet P: New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the trnL approach. Mol Ecol Resour. 2009, 24 (2): 110-117.

[15] Zhao ZL, Leng CH, Wang ZT (2007) Identification of Dryopteris crassirhizoma and the adulterant species based on cpDNA rbcL and translated amino acid sequences. Planta Med 73: 1230–1233.ZL ZhaoCH LengZT Wang2007Identification of Dryopteris crassirhizoma and the adulterant species based on cpDNA rbcL and translated amino acid sequences.Planta Med7312301233.



10.22214/IJRASET

45.98

IMPACT FACTOR: 7.129

IMPACT FACTOR: 7.429

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY