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Molecular Identification and Phylogenetic Analysis of Some Selected Plants in the Home Gardens of Udupi and Dakshina Kannada Districts of Karnataka, India

Ramya Rai P. D.[1], Shobha[2]

[1]Department of Botany, Alva's College, Moodbidri-574227, Karnataka, India
Corresponding Author: *ramyamanoharrai[at]gmail.com*

[2]Department of Botany, University College, Mangaluru-575001, Karnataka, India

Abstract: ***Varieties of plants act as a sink for CO_2 thereby fixing carbon during photosynthesis and store excess carbon in the form of biomass. Vast forest areas present in India and its different states accumulate large amount of carbon as CO_2 from atmospheric air and play an important role for sequestering carbon. Thus, the goal of our study was to perform field survey to collect carbon sequestering home garden plants present in and around Dakshina Kannada, Karnataka and subject them for DNA barcoding technique to authenticate those medicinal plants. DNA barcoding methods have been shown to be an effective tool for species authentication targeting specific genes from genomic DNA which is widely used in molecular plant taxonomy. DNA samples were extracted from shortlisted 15 plants including trees, shrubs and herbs. Plants collected were Artocarpus camansi, Nerium indicum, Averrhoa bilimbi, Laurus nobilis, Morinda citrifolia, Caesalpinia pulcherrima, Barleria acanthoides, Sauropus androgynus, Crossandra infundibuliformis, Allamanda cathartica, Achyranthes aspera, Talinum fruticosum, Kalanchoe pinnata, Dieffenbachia seguine and Pandanus amaryllifolius, chosen based on phytosociological properties and biodiversity of plants. The selected plants were further taxonomically identified using MatK, ITS2 and Rbcl2 genes as best molecular markers for DNA barcoding purposes. The genomic DNA was isolated from plants, mentioned genes were amplified using PCR and subjected to Sanger sequencing. The sequences were screened in NCBI BLAST for identifying species and phylogenetic analysis was conducted using Clustal Omega. Our findings authenticated the plant species that play major role in purifying air around us. Hence, this barcoding technique behaves as genuine and simple method to authenticate plant species which had socioeconomic importance.***

Keywords: genomic DNA, PCR, Sanger sequencing, NCBI, phylogeny, species authentication

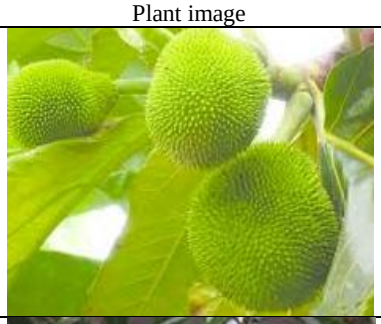
1. Introduction

Generally in terrestrial ecosystem, it is seen that carbon is sequestered in rocks, sediments, wetlands, forests, soils of forestland, grasslands and agricultural lands. Normally plants store carbon throughout their life in the form of live biomass. Once they die, this biomass becomes part of food chain and it enters the soil as soil carbon. In case of incineration of this biomass, carbon is re-emitted into atmosphere, where terrestrial vegetation and soil represents important source acting as sink for atmospheric carbon (Watson et al., 2001). This way trees behave as sink for CO2 by fixing carbon during photosynthesis process and storing excess carbon as their biomass. Forest ecosystem plays important role in carbon cycle globally by sequestering substantial amount of carbon dioxide present in the atmosphere (Vashum and Jay Kumar, 2012; Gorte, 2009; Chavan and Rasal, 2010). To through much knowledge on amount of carbon stored and sequestered by the common ornamental plants, field survey of shrubs, herbs and trees were done and 15 plants were selected by studying their physiological details. The traditional approaches available for identification of plants include organoleptic methods by sensing taste, sight, smell, touch; macroscopic and microscopic studies including shape, colour, texture etc wherein professional taxonomists are needed (Techen et al., 2014). Moreover, even the specialist will not be able to identify the specimens if it is damaged, dried or at immature stage of development. To overcome all these problems, there lies a necessity of novel approach to identify plant species in an effective and precise manner. Therefore, authentication of plants using DNA barcoding approach helps in overcoming these problems and acts as a rapid and exact identification method for plant species based on isolating genomic DNA from specimen of any organism. This will allow taxonomists to recognize species closely and quickly by retrieving information about them. Also, it allows non-experts to recognize species from damaged, dried or tiny material. There exists several potential barcoding candidates so far reported such as ITS2, matK, psbK-psbI, rbcL, trnH-psbA, etc. (Luo et al., 2010; Pang et al., 2010; Bhavana et al., 2021).

In our current study we have done field survey in and around Udupi and Dakshina Kannada Districts, Karnataka for common ornamental and having sociophysiological importance in this region. Based on the availability and biodiversity we collected 15 plants such as *Artocarpus camansi, Nerium indicum, Averrhoa bilimbi, Laurus nobilis, Morinda citrifolia, Caesalpinia pulcherrima, Barleria acanthoides, Sauropus androgynus, Crossandra infundibuliformis, Allamanda cathartica, Achyranthes aspera, Talinum fruticosum, Kalanchoe pinnata, Dieffenbachia seguine* and *Pandanus amaryllifolius*. **Table 1** shows the herb, shrub, tree varieties and their taxonomic details of 15 plants taken in our present research. Each of the plant collected is

known for growing in home gardens, to purify environment and balance the ecosystem. In the present study, we have isolated genomic DNA from listed plants and DNA was barcoded using nuclear, mitochondrial and chloroplast genes to authenticate the plant species.

Table 1: Showing the plant varieties and their details

Sl. No.	Type	Common name	Scientific name	Plant image
1	Tree	Bread fruit	*Artocarpus camansi*	
2	Tree	Karaveera	*Nerium indicum*	
3	Tree	Bimbuli	*Averrhoa bilimbi*	
4	Tree	Bay leaf	*Laurus nobilis*	
5	Tree	Noni fruit	*Morinda citrifolia*	

6	Shrubs	Caesalpinia	*Caesalpinia pulcherrima*	
7	Shrubs	barleria	*Barleria acanthoides*	
8	Shrubs	Vitamin soppu	*Sauropus androgynus*	
9	Shrubs	crossandra	*Crossandra infundibuliformis*	
10	Shrubs	allamanda	*Allamanda cathartica*	
11	Herbs	uttarani	*Achyranthes aspera*	

12	Herbs	Nelabasale	*Talinum fruticosum*	
13	Herbs	kalonche	*Kalanchoe pinnata*	
14	Herbs	Dumb cane	*Dieffenbachia seguine*	
15	Herbs	Biriyani/ pandan leaf	*Pandanus amaryllifolius*	

2. Materials and methods

Collection of Plant material:
The fresh and not so tender leaves of selected medicinal plants *Artocarpus camansi, Nerium indicum, Averrhoa bilimbi, Laurus nobilis, Morinda citrifolia, Caesalpinia pulcherrima, Barleria acanthoides, Sauropus androgynus, Crossandra infundibuliformis, Allamanda cathartica, Achyranthes aspera, Talinum fruticosum, Kalanchoe pinnata, Dieffenbachia seguine* and *Pandanus amaryllifolius* were collected in and aroundUdupi and Dakshina Kannada Districts, Karnataka selecting the socio important and ornamental garden plants that are involved in carbon sequestration and air purification. The leaves were washed with sterile water to remove the dirt and further processed for molecular studies.

Genomic DNA preparation:
Genomic DNA was isolated from above said fresh leaves by modified CTAB method (Cota et al., 2006). Approximately 0.1g of sample was grounded in 1ml of CTAB buffer using pestle and mortar followed by incubation at 60^0c for 30 mins. The content was further cooled to room temperature and equal volume of chloroform: isoamyl alcohol in 24: 1 ratio was added and mixed gently. This mixture was centrifuged at 10, 000 rpm for 10 min in refrigerated centrifuge and pellet was discarded transferring supernatant to another fresh tube to which equal volume of ice-cold isopropanol was added. Followed by centrifugation the supernatant was discarded and 500µl of 70% ethanol was added, pellet was retained and allowed to dry for 45 mins dissolving in 10µl TE buffer. This DNA was treated with RNAse and subjected to column purification to get rid of impurities. This isolated plant DNA was run on 1.5% agarose gel for quantification and compared with 1kb standard ladder.

PCR Amplification:
The genes such as ITS2, MatK and rbcL were amplified from isolated plant DNA using PCR method. Amplification reaction was performed in 25µl reaction mixture, by adding 40ng DNA, 10pM of each primer, 10pM dNTPs, 2X buffer and 0.5µl Taq DNA polymerases. Gene amplification was performed using thermal cycler program of 35 cycles: initial

denaturation step and final denaturation at 94°c followed by annealing at 60°c and extension at 72°C. The quality of PCR products was checked on 1 % agarose gel electrophoresis stained with ethidium bromide.

Table 2: Showing primers sequences used for gene amplification

Primer	Sequence	Annealing Temperature
Matk F	CCCRTYCATCTGGAAATCTTGGTT C	54^{0}C
MatkR	GCTRTRATAATGAGAAAGATTTCTGC	59^{0}C
ITS2-F	ATGCGATACTTGGTGTGAAT	53^{0}C
ITS2-R	GACGCTTCTCCAGACTACAAT	51^{0}C
Rbcl F	ATGTC CCACAAACAGAAAC	50^{0}C
Rbcl R	TCGCATGT CCTGCAGTAGC	56^{0}C

Sanger Sequencing:
Bidirectional sequencing of PCR products was carried in genetic analyzer-Applied Biosystems (Hitachi) 3130x1 Sequencer and processed in Finch TV. The electropherogram files in. AB1 format were converted to .pdf and fasta files using Sequence Scanner Software 2. The sequence data generated during this study were subjected to BLAST in nucleotide database GenBank (http://blast.ncbi.nlm.nih.gov/) to determine their most probably closely related taxa.

Construction of phylogenetic tree:
Phylogenetic inference incorporates computational approaches by implementing optimality criteria and methods such asparsimony and maximum likelihood (ML) based on Bayesian inference. All these explanation will depend upon implicit or explicit model describing the evolution of characters observed. And all the sequences obtained will be aligned using Clustal omega to understand the evolutionary relationship of unknown sequences comparing with other related species.

3. Results and discussion

Genomic DNA was isolated following CTAB method to check quality and quantity of DNA on agarose gel as shown in **figure 1**. Using this genomic DNA PCR was performed using ITS2, MatK and RbCl primers and obtained PCR product was subjected to gel purification to get rid of salt contamination and other impurities as shown in **figure 2**. Sequencing files were obtained in. AB1 format which were viewed using software Finch TV as electropherogram peaks of individual nucloetide in its position. The quality of the obtained sequences was observed through electropherogram peaks as seen in **figure 3**and **figure 4**. FASTA sequences for further analysis were obtained by converting. AB1 file in Seq Scanner 2.0. Using the BLAST server unknown sequences were identified at molecular level based on query coverage, percentage identity and e-value. **Figure 5**shows the percentage similarity between the predicted species as per BLAST analysis and phylogenetic analysis was done to find out the evolutionary relationship of our query sequence obtained from *Artocarpus camansi*. The BLAST values and phylogenetic tree obtained for *Nerium indicum, Averrhoa bilimbi* and *Laurus nobilis* from all three genes are shown in **figure 6, figure 7** and **figure 8** respectively. Similarly, the Phylogenetic trees and BLAST results of *Morinda citrifolia, Caesalpinia pulcherrima, Barleria acanthoides, Sauropus androgynus, Crossandra infundibuliformis* are shown in **figure 9, figure 10, figure 11, figure 12** and **figure 13.** The obtained results of *Allamanda cathartica, Achyranthes aspera, Talinum fruticosum, Kalanchoe pinnata, Dieffenbachia seguine* and *Pandanus amaryllifolius* using Clustal Omega are as shown in **figure 14, figure 15, figure 16, figure 17, figure 18 and figure 19** respectively. Each of the figure depicts BLAST values showing similarity percentage and their evolutionary relationship with closely and distantly related species.

DNA barcoding is been applicable in taxonomy field and is becoming very popular in recent era (Luo et al., 2010). This technique acts as useful tool for taxonomists due to its rapid and accurate method of species identification and behaves as an important parameter. Therefore, there is a need to entertain this rapid and simple DNA barcoding identification system to all the biologists to avoid falling burden on taxonomists (Chase et al., 2005). Currently the candidate barcodes available so far are ITS2 of nuclear ribosomal DNA, matK, psbA-trnH, rbcL, ycf5, rpoC1, petB etc in the chloroplast. The observations done so far has shown the potential of ITS2 being appropriate marker for taxonomic classification when compared to other markers. This ability of conventional amplification by incorporating universal primer pairs and thus generating unambiguous bidirectional DNA sequences with less editing acts as major criteria of finalizing ideal DNA barcode. Also, the ITS2 has good primer sites in the gene due to its short region and easy amplification and sequencing (Kress et al., 2005). Thus, the results obtained here demonstrate that the primers used provide promising universal DNA barcode to recognize 15 selected medicinal plants by providing high percentage of maximum identity for each sample. However, few plants have showed lesser percentage of identity around 95% and below than 95% were disallowed because of chances of ambiguous species identification (Kool et al., 2012). Here, maximum identity suggests measure of likeliness of identified species and each successful species identification is influenced by factors such as quality of DNA, suitable primer used and the quality of sequence (Mahadani and Ghosh, 2013). In many of the cases the successful rates of DNA sequencing is relatively low due to the presence of polymorphic DNA in multiple copies (Alvarez and Wendel, 2003) and also may be because of contamination by fungal or other parasitic species (Hollingsworth et al., 2011, Che et al., 2018). Thus, DNA barcoding stands as standardized technique to identify and differentiate between species in recent years (Dhananjaiah et al., 2020). In addition, the phylogenetic

analysis highlights the diversity present between our plants and other known species, which will help us to understand the evolutionary relationship of our plant species.

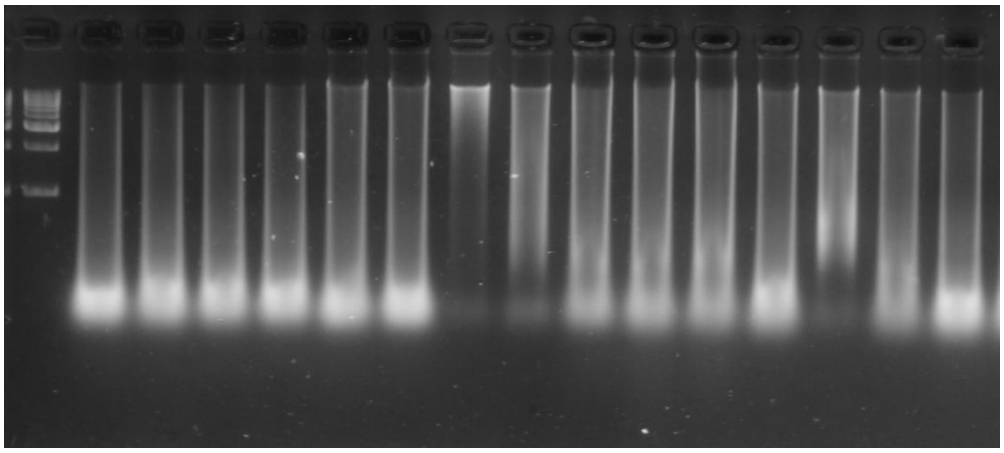


Figure 1: Genomic DNA of 15 plants compared with 1kb ladder

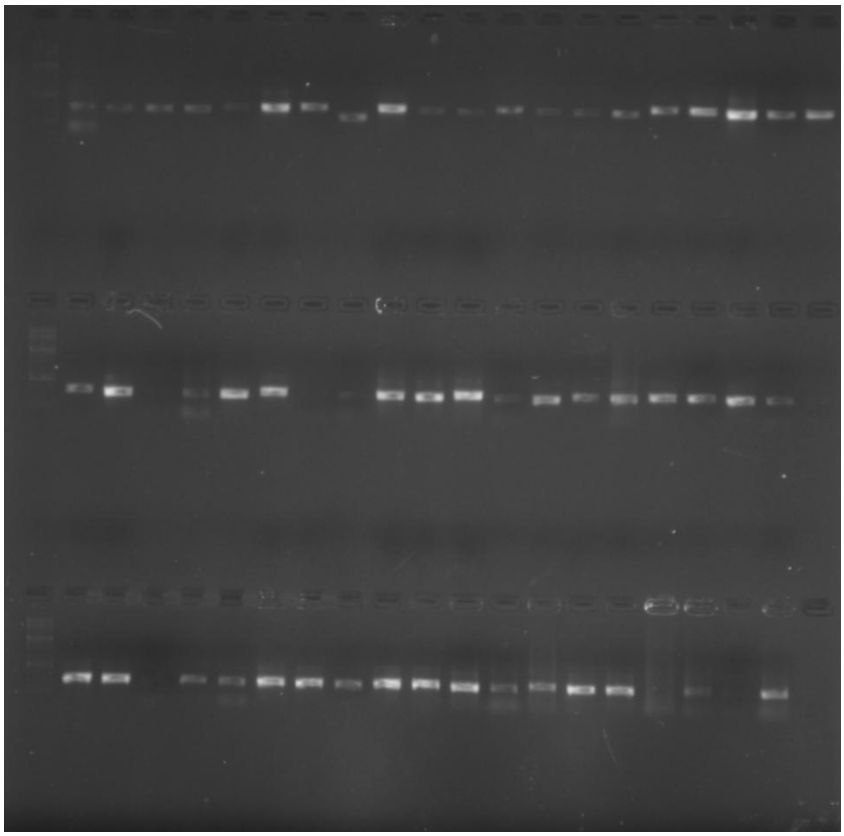


Figure 2: Amplified ITS2, MAtk and RbCl genes (~700bp) from plant DNA and compared with 100bp ladder

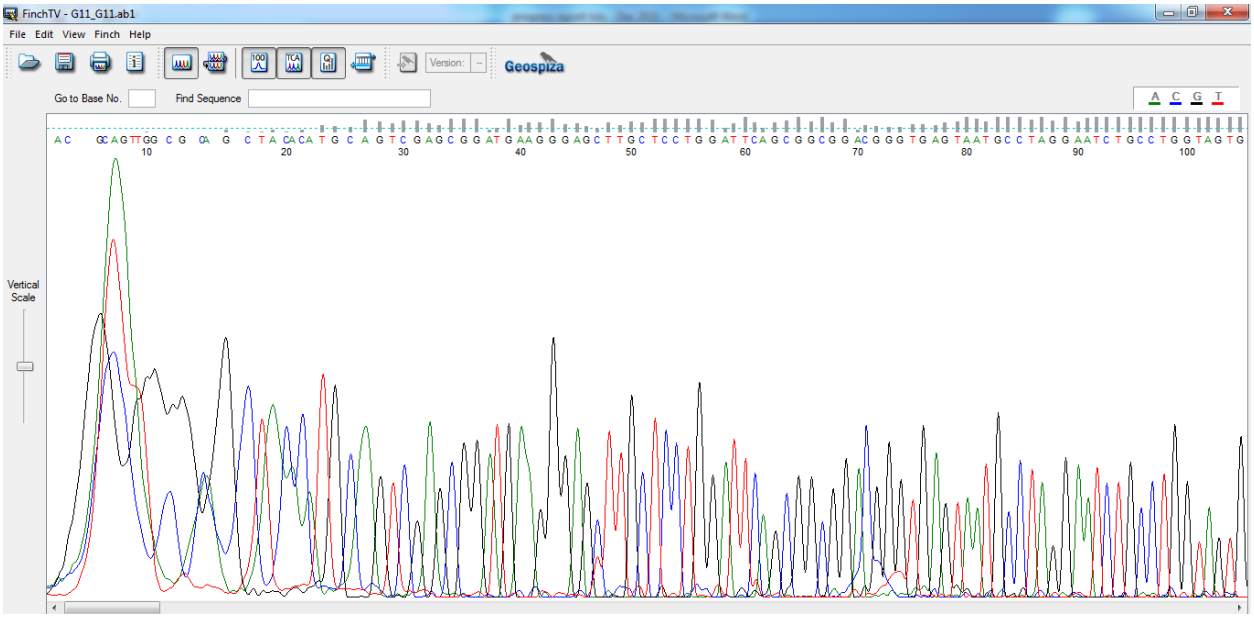


Figure 3: Representative Electropherogram peaks showing the nucleotide bases obtained from Sanger sequencing.

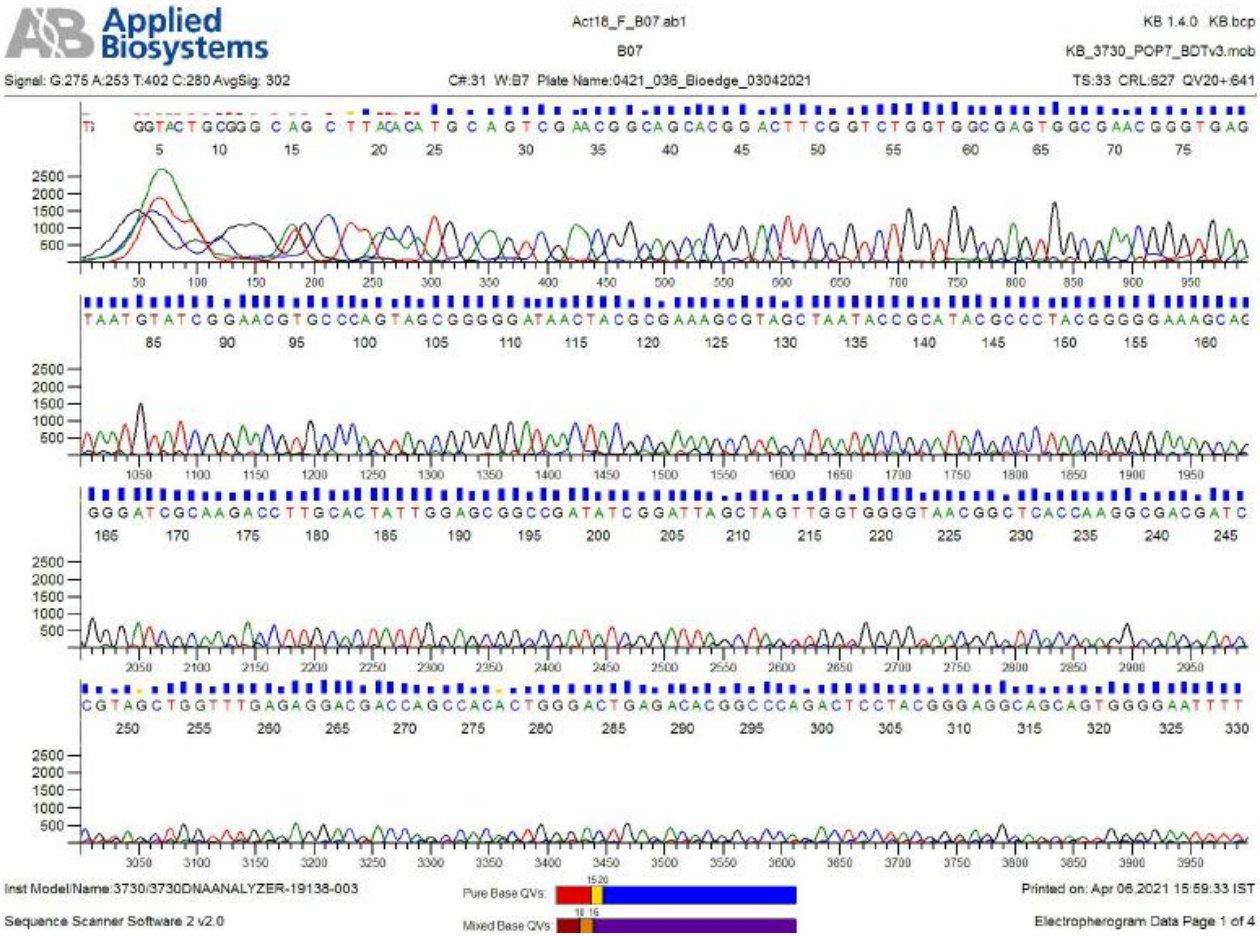


Figure 4: Representative pdf file showing overall view of the sequence as obtained from genetic analyzer.

Sl. No.	Description	Query (%)	E Value	% Identity	Accession No.	Gene
1	*Artocarpus camansi* voucher Artocarpus camansi JTG91 internal transcribed spacer 1, partial seq;5.8S ribo RNA gene,ITS2	99	0.0	96.75	MT020424.1	ITS2
2	*Artocarpus camansi* voucher DMB144 photosystem Q (B) protein (psbA) gene, partial cds; and psbA-trnH intergenic spacer, partial sequence; chloroplast	99	0.0	96.80	MN082750.1	RBCl2
3	*Artocarpus camansi* chloroplast, complete genome	98	0.0	99.99	NC_054247.1	MatK

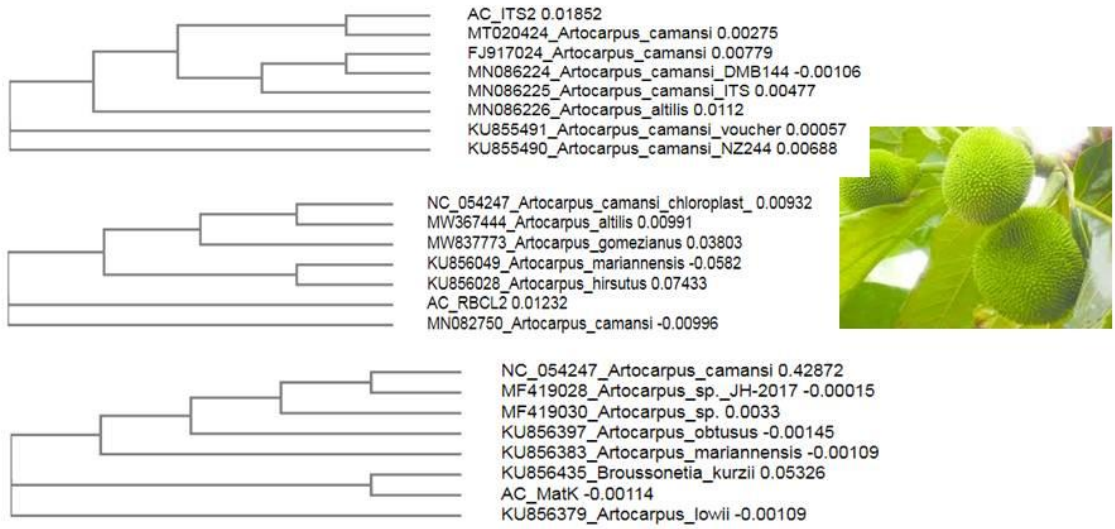


Figure 5: Showing predicted hits from NCBI BLAST and evolutionary relationship of plant *Artocarpus camansi*.

Sl. No.	Description	Query (%)	E Value	% Identity	Accession No.	Gene
1	*Nerium oleander* voucher 1301 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete	99	0.0	92.35	MG730486.1	ITS2
2	*Nerium oleander* isolate 1767 psbA-trnH intergenic spacer region, partial sequence	93	0.0	94.91	MH837747.1	RBCl2
3	*Nerium oleander* isolate MGGM_AGERI_167 maturase K (matk) gene, partial cds;chlr	99	0.0	96.82	KU556667.1	MatK

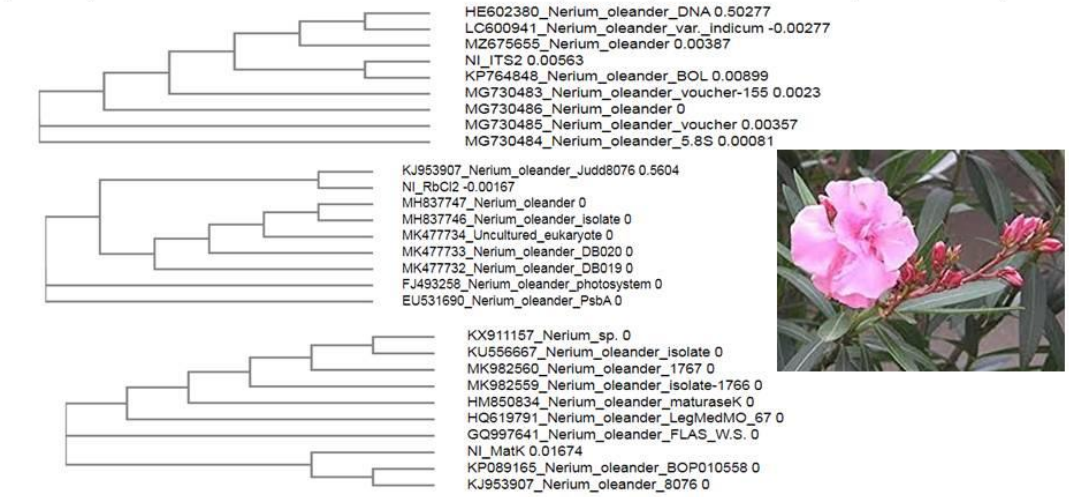


Figure 6: Showing predicted hits from NCBI BLAST and evolutionary relationship of plant *Nerium indicum*.

Sl. No.	Description	Query (%)	E Value	% Identity	Accession No.	Gene
1	*Averrhoa bilimbi* isolate AbITS-9 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene	98	0.0	97.14	KR905602.1	ITS2
2	*Averrhoa bilimbi* isolate cujiang_1 chloroplast, complete genome	97	0.0	97.51	MT522015.1	RBCl2
3	*Averrhoa bilimbi* isolate AbITS-9 internal transcribed spacer 1, partial sequence; 5.8S RNA complete sequence	99	0.0	97.53	KR905602.1	MatK

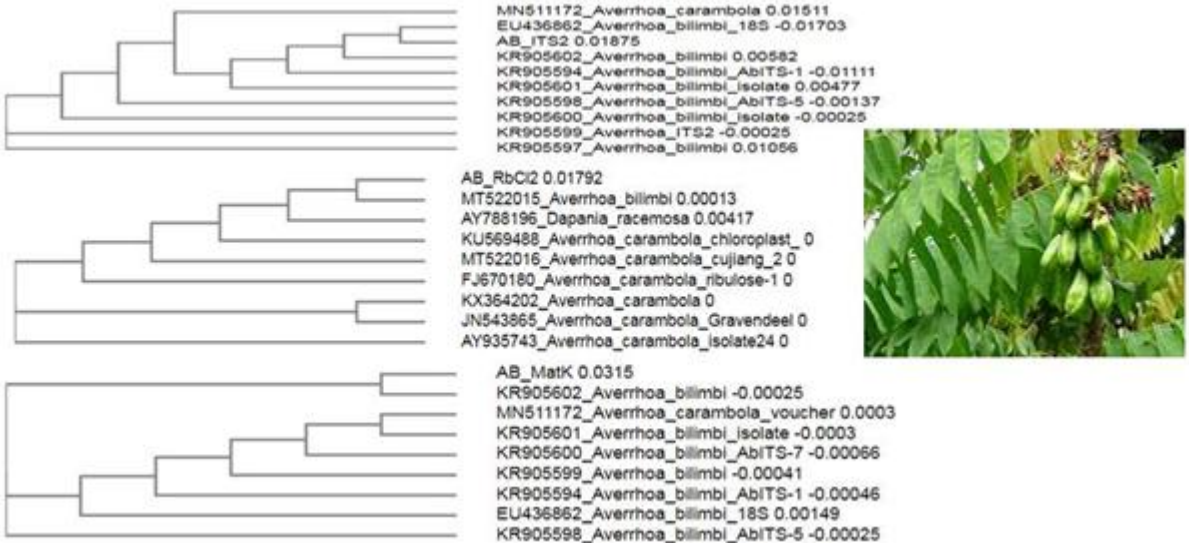


Figure 7: Showing predicted hits from NCBI BLAST and evolutionary relationship of plant *Averrhoa bilimbi*.

Sl. No.	Description	Query (%)	E Value	% Identity	Accession No.	Gene
1	*Laurus nobilis* isolate 7 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S	99	0.0	97.60	MT113320.1	ITS2
2	*Laurus nobilis* plastid, complete genome	98	0.0	95.36	KY085912.1	RBCl2
3	*Laurus nobilis* maturase (matK) gene, partial cds; chloroplast gene for chloroplast product	99	0.0	97.16	AF244407.1	MatK

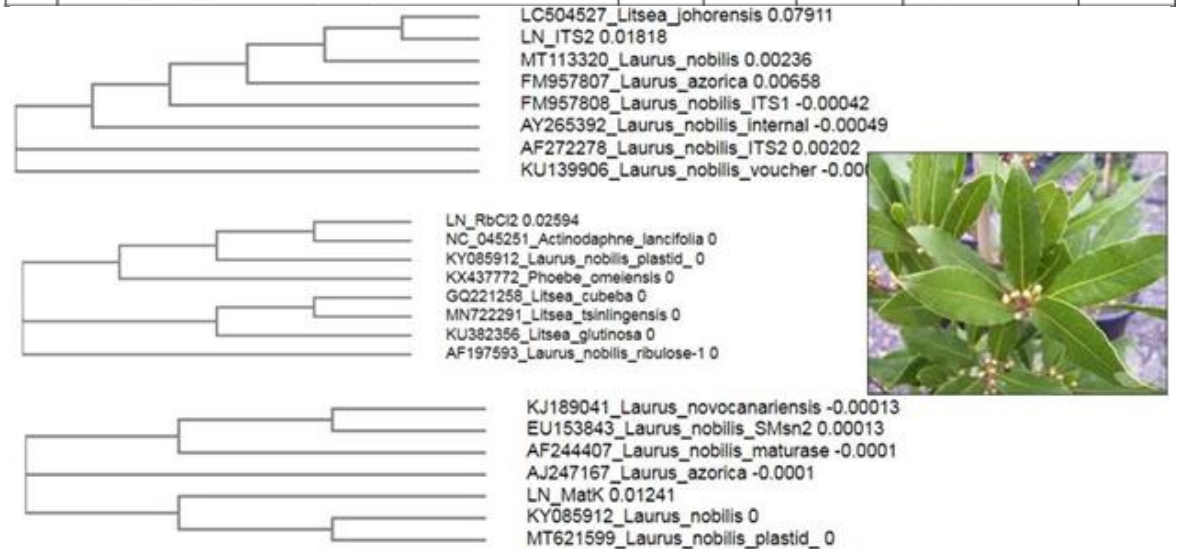


Figure 8: Showing predicted hits from NCBI BLAST and evolutionary relationship of plant *Laurus nobilis*.

Sl. No.	Description	Query (%)	E Value	% Identity	Accession No.	Gene
1	*Morinda citrifolia* genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 26S rRNA, partial sequence, country: Panama	97	0.0	96.48	AB608796.1	ITS2
2	*Morinda citrifolia* chloroplast, partial genome	99	0.0	97.53	KY378694.1	RBCl2
3	*Morinda citrifolia* Chiang Mai University, Faculty of Pharmacy: MOC-CM11032021 chloroplast matK gene for maturase	99	0.0	98.21	LC633827.1	MatK

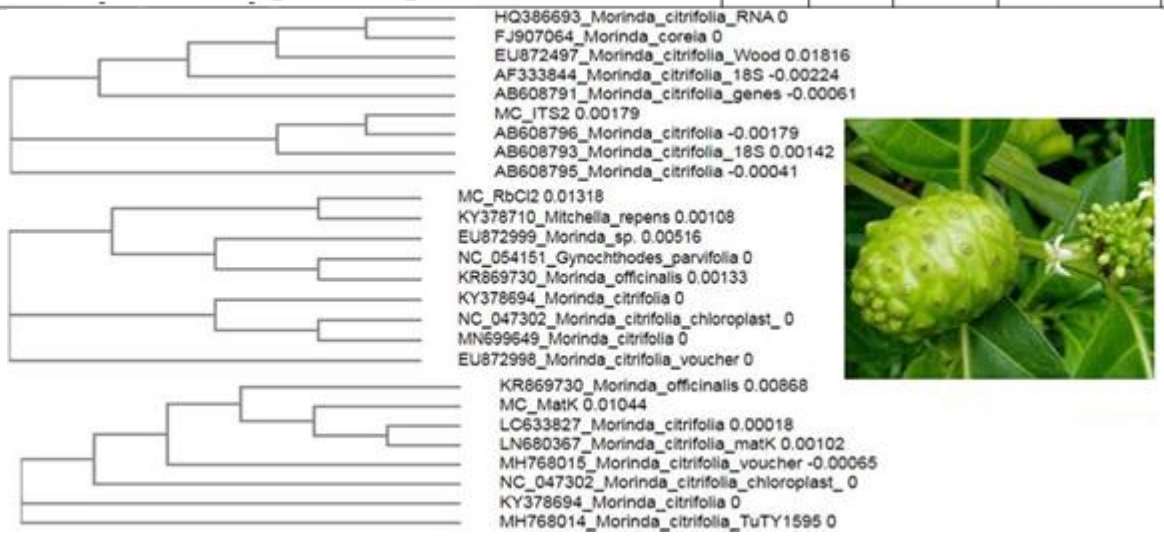


Figure 9: Showing predicted hits from NCBI BLAST and evolutionary relationship of plant *Morinda citrifolia*.

Sl. No.	Description	Query (%)	E Value	% Identity	Accession No.	Gene
1	*Caesalpinia pulcherrima* isolate AD038 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer	99	0.0	97.37	KX057841.1	ITS2
2	*Caesalpinia pulcherrima* voucher RNG:51664 tRNA-Leu (trnL) gene and trnL-trnF intergenic spacer, partial seq	93	0.0	96.98	KX268156.1	RBCl2
3	*Caesalpinia pulcherrima* voucher MO:Carlsen3202 maturase K (matK) gene, partial cds; chloroplast	99	0.0	99.15	OL689908.1	MatK

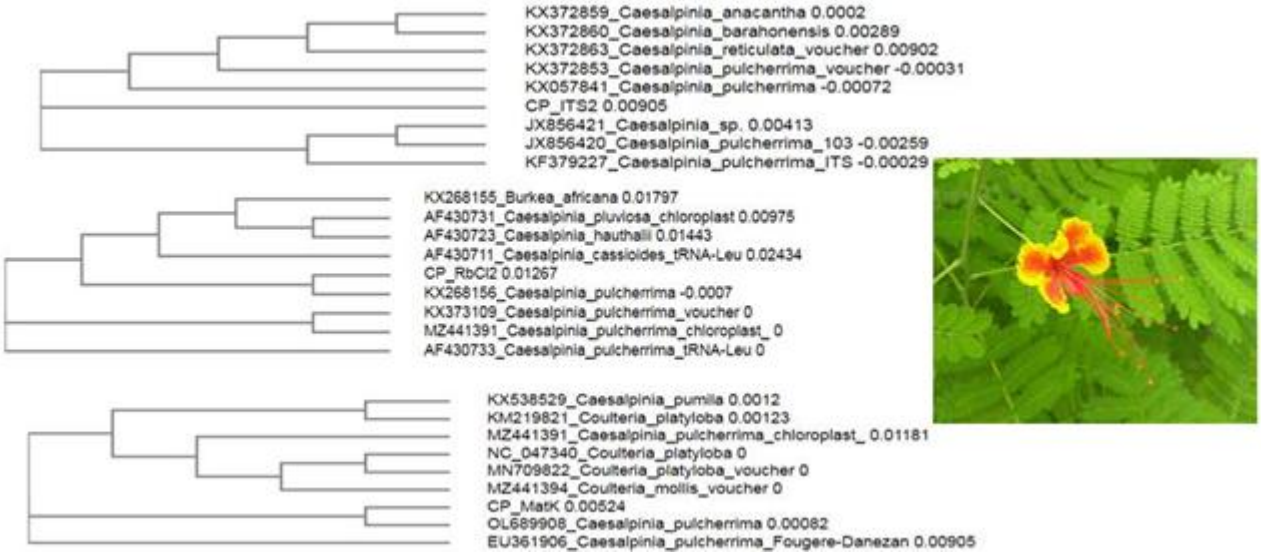


Figure 10: Showing predicted hits from NCBI BLAST and evolutionary relationship of plant *Caesalpinia pulcherrima*.

Sl. No.	Description	Query (%)	E Value	% Identity	Accession No.	Gene
1	*Barleria acanthoides* voucher Boulos et. al. 16678 (E) internal transcribed spacer 1,partial sequence; 5.8S ribosome	99	0.0	94.03	MK066127.1	ITS2
2	*Barleria acanthoides* var. gracilispina ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	99	0.0	96.28	KR737435.1	RBCl2
3	*Barleria acanthoides* var. gracilispina maturase K (matK) gene, partial cds; chloroplast	97	0.0	97.79	KR735076.1	MatK

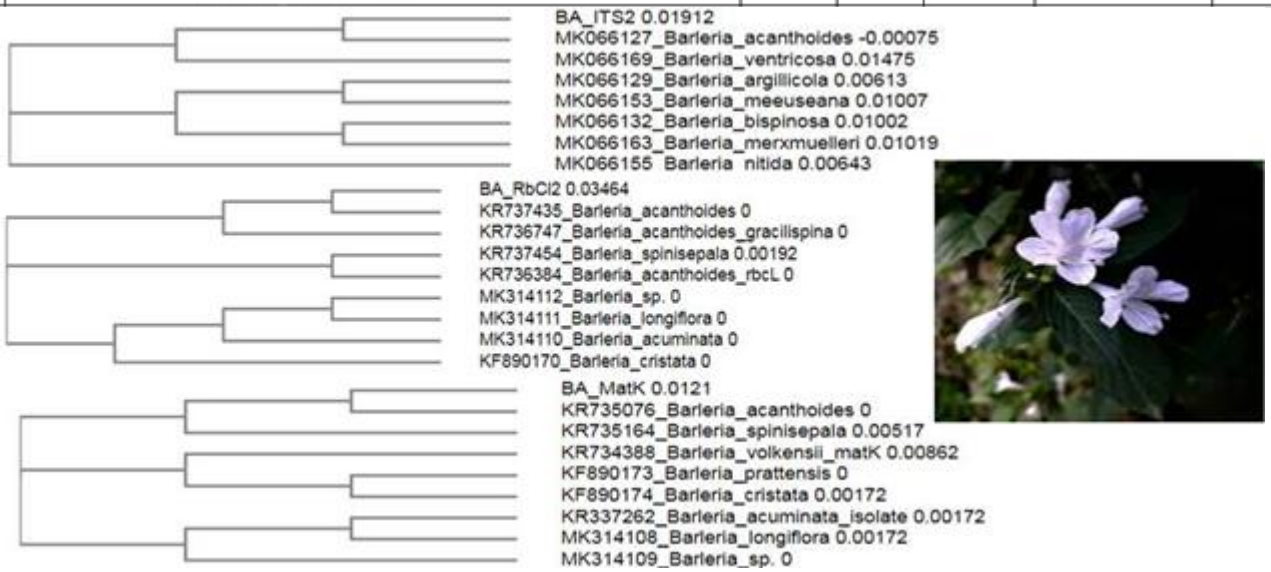


Figure 11: Showing predicted hits from NCBI BLAST and evolutionary relationship of plant *Barleria acanthoides*.

Sl. No.	Description	Query (%)	E Value	% Identity	Accession No.	Gene
1	*Sauropus androgynus* 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2	99	0.0	94.35	JQ670926.1	ITS2
2	*Sauropus androgynus* SS-553 chloroplast rbcL gene for ribulose-1,5 -bisphosphate carboxylase/oxygenase large	99	0.0	96.01	LC466956.1	RBCl2
3	*Sauropus androgynus* SS-553 chloroplast matK gene for maturase K, partial cds	99	0.0	98.80	LC467715.1	MatK

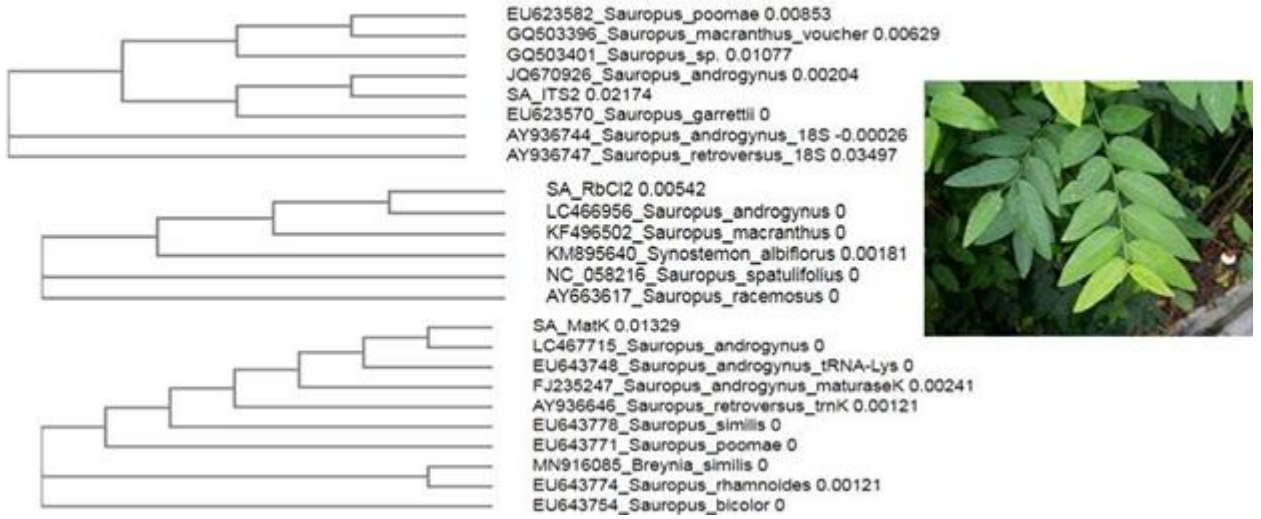


Figure 12: Showing predicted hits from NCBI BLAST and evolutionary relationship of plant *Sauropus androgynus*.

Sl. No.	Description	Query (%)	E Value	% Identity	Accession No.	Gene
1	*Crossandra infundibuliformis* internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence	99	0.0	97.70	JF346168.1	ITS2
2	*Crossandra infundibuliformis* voucher US:Ingram102 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	98	0.0	96.68	MH749098.1	RBCl2
3	*Crossandra infundibuliformis* voucher CCMB-26-84-SJ2 maturase K (matK) gene, partial cds; chloroplast	99	0.0	98.14	MN006714.1	MatK

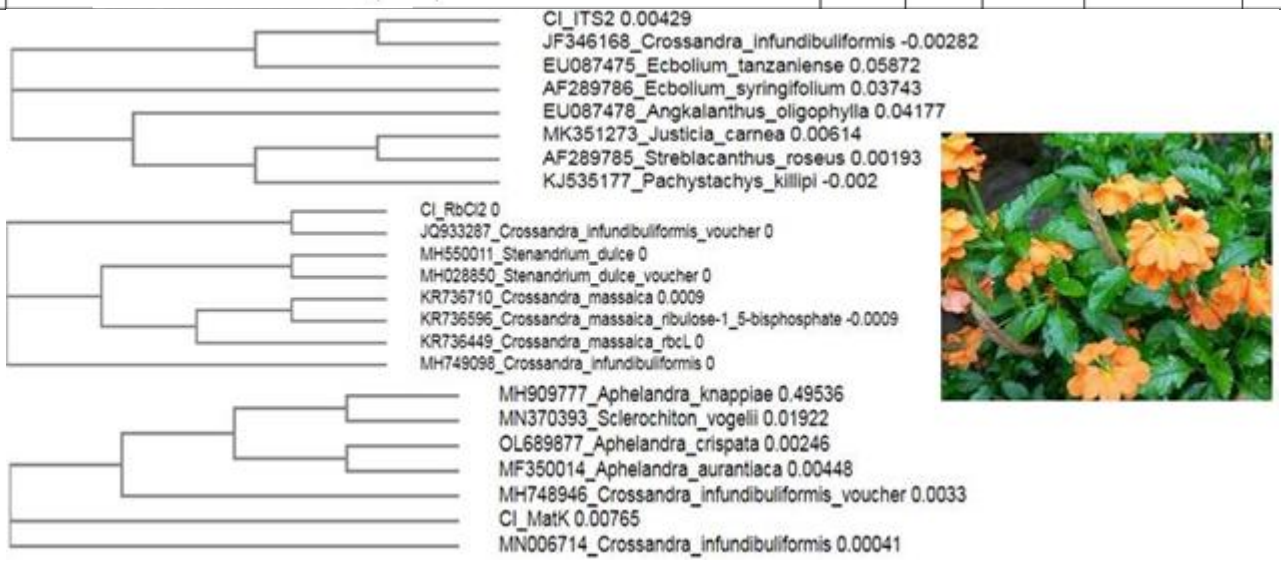


Figure 13: Showing predicted hits from NCBI BLAST and evolutionary relationship of plant *Crossandra infundibuliformis*.

Sl. No.	Description	Query (%)	E Value	% Identity	Accession No.	Gene
1	*Allamanda cathartica* voucher 1863 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer	99	0.0	97.92	MH566887.1	ITS2
2	*Allamanda cathartica* isolate Ou10 tRNA-Leu (trnL) gene and trnL -trnF intergenic spacer, partial sequence	93	0.0	97.15	MT078032.1	RBCl2
3	*Allamanda cathartica* voucher MO:Carlsen3534 maturase K (matK) gene, partial cds; chloroplast	98	0.0	97.73	OL689868.1	MatK

Figure 14: Showing predicted hits from NCBI BLAST and evolutionary relationship of plant *Allamanda cathartica*.

Sl. No.	Description	Query (%)	E Value	% Identity	Accession No.	Gene
1	*Achyranthes aspera* 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene	99	0.0	96.05	LT992995.1	ITS2
2	*Achyranthes aspera* voucher MSB92575(K) ndhF-rpl32 intergenic spacer region, partial sequence	99	4e-139	97.83	KY884457.1	RBCl2
3	*Achyranthes aspera* voucher MSB92575(K) maturase K (matK) gene, partial cds; chloroplast	98	0.0	96.96	KY941419.1	MatK

Figure 15: Showing predicted hits from NCBI BLAST and evolutionary relationship of plant *Achyranthes aspera*

Sl. No.	Description	Query (%)	E Value	% Identity	Accession No.	Gene
1	*Talinum fruticosum* voucher DMB60 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene	97	0.0	95.73	MK610436.1	ITS2
2	*Talinum fruticosum* voucher FR04499535 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	99	0.0	96.57	MT385745.1	RBCl2
3	*Talinum fruticosum* voucher DMB60 trnK gene, intron; and maturase K (matK) gene, partial cds; chloroplast	99	0.0	94.07	MK610255.1	MatK

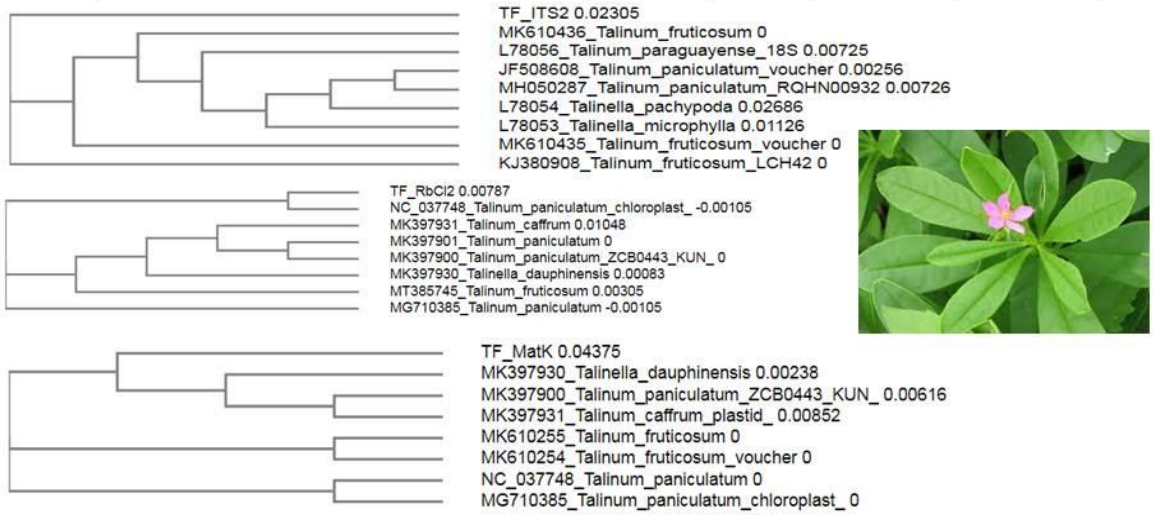


Figure 16: Showing predicted hits from NCBI BLAST and evolutionary relationship of plant *Talinum fruticosum*

Sl. No.	Description	Query (%)	E Value	% Identity	Accession No.	Gene
1	*Kalanchoe pinnata* 5.8S rRNA gene and ITS1 and ITS2, clone pHM28-1	99	0.0	95.31	AJ231323.1	ITS2
2	*Kalanchoe pinnata* voucher S.B. Davis 1290 (FLAS) ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	99	0.0	96.89	GU135277.1	RBCl2
3	*Kalanchoe pinnata* voucher S.B. Davis 1290 (FLAS) maturase K (matK) gene, partial cds; chloroplast	98	0.0	95.85	GU135118.1	MatK

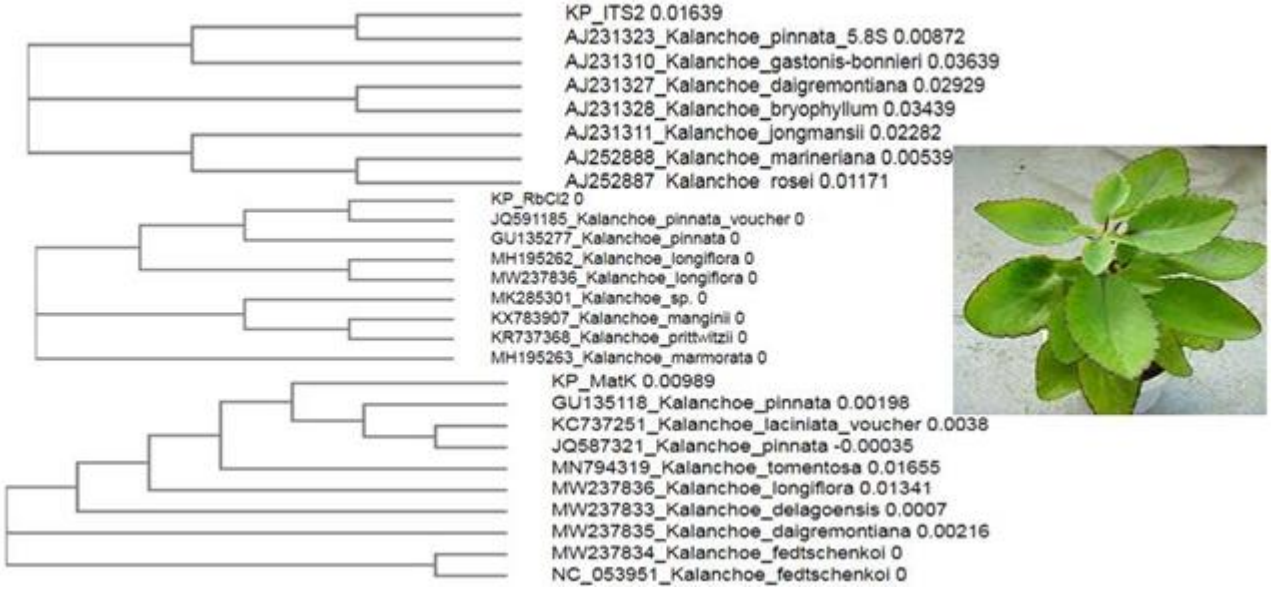


Figure 17: Showing predicted hits from NCBI BLAST and evolutionary relationship of plant *Kalanchoe pinnata*

Sl. No.	Description	Query (%)	E Value	% Identity	Accession No.	Gene
1	*Dieffenbachia seguine* chloroplast partial rpoB gene for RNA polymerase beta subunit, specimen voucher	99	0.0	99.27	FN668863.1	ITS2
2	*Dieffenbachia seguine* chloroplast partial rpoB gene for RNA polymerase beta subunit, specimen voucher MIB	99	0.0	93.27	FN668863.1	RBCl2
3	*Dieffenbachia seguine* chloroplast partial matK gene for maturase K, specimen voucher MIB:zpl:01669	97	0.0	97.91	FN668814.1	MatK

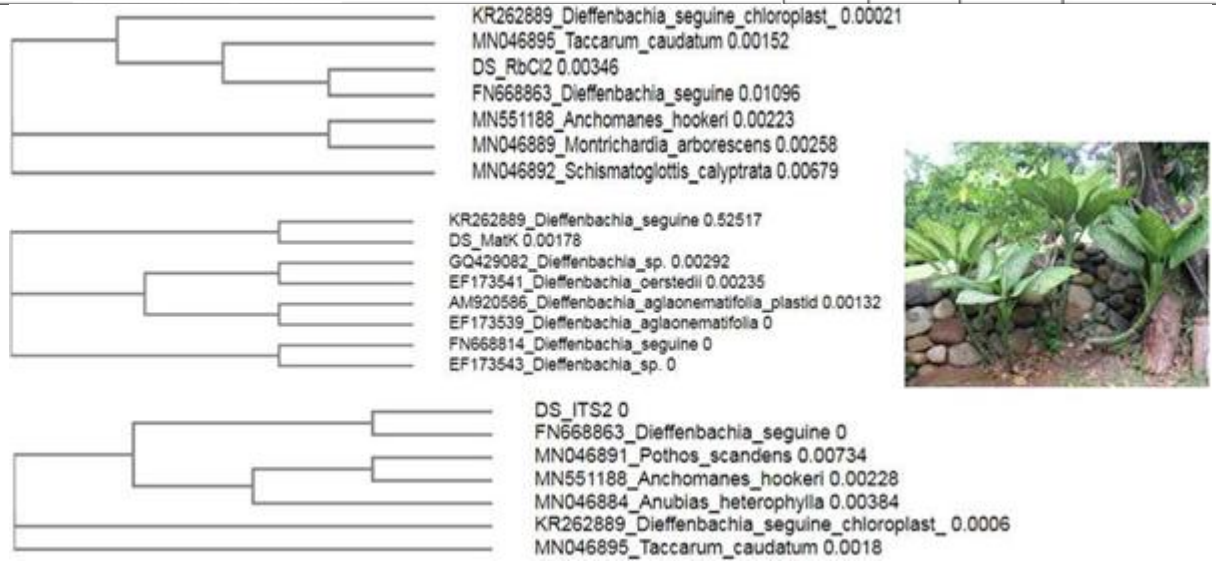


Figure 18: Showing predicted hits from NCBI BLAST and evolutionary relationship of plant *Dieffenbachia seguine*.

Sl. No.	Description	Query (%)	E Value	% Identity	Accession No.	Gene
1	*Pandanus amaryllifolius* isolate T0030 tRNA-Gln (trnQ) gene and trnQ-rps16 intergenic spacer, partial sequence	99	0.0	97.49	KJ681941.1	ITS2
2	*Pandanus amaryllifolius* isolate Pan_17a tRNA-Leu (trnL) gene, intron; chloroplast	99	0.0	94.24	JQ220392.1	RBCl2
3	*Pandanus amaryllifolius* isolate RBGK37355 maturase K (matK) gene, partial cds; chloroplast	99	0.0	97.75	JX286802.1	MatK

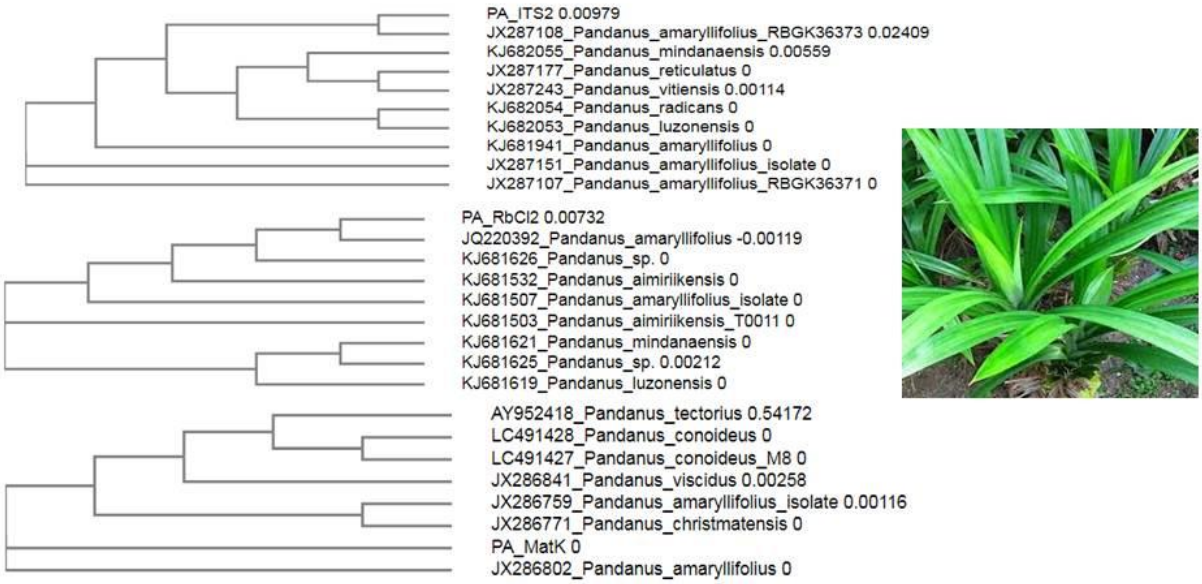


Figure 19: Showing predicted hits from NCBI BLAST and evolutionary relationship of plant *Pandanus amaryllifolius*.

4. Conclusion

Various technologies are under development to reduce the rate of increase of atmospheric concentration of carbon dioxide (CO2) from different sectors acts as important issue of the twenty-first century. Therefore, reducing the global energy use, develop low carbon fuel and finding out plants which sequester more carbon involves both abiotic and biotic technologies. Carbon sequestration thus implies transfer of atmospheric CO2 inside other long-lived global pools such as oceanic, biotic and geological strata reducing net rate of increase in atmospheric CO2. Carbon is being sequestered by the plant through photosynthesis and will be stored as biomass in different parts of the tree. To address this we have selected few ornamental and socioeconomic plants that are said to purify air in their premises. DNA barcoding acts as a reliable tool for authentication of any plant species with minimal help of core taxonomists. Our present study was to authenticate the plant species at molecular level using DNA barcoding technique. It is very much appreciable to get species level identity with maximum percentage and identify plants as *Artocarpus camansi, Nerium indicum, Averrhoa bilimbi, Laurus nobilis, Morinda citrifolia, Caesalpinia pulcherrima, Barleria acanthoides, Sauropus androgynus, Crossandra infundibuliformis, Allamanda cathartica, Achyranthes aspera, Talinum fruticosum, Kalanchoe pinnata, Dieffenbachia seguine* and *Pandanus amaryllifolius.* Therefore, our findings provide strong base for usage of these plant species as garden plants to keep surrounding air clean. The usage of many potential markers like ITS2, matK and rbcL can be implemented in future days for studying and identifying any plant species. Further studies towards determining exact carbon and carbon dioxide sequestration methods are in the process.

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