BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, September 2024. Vol. **21**(3), p. 935-945

Different DNA Barcoding Techniques in Forensic Botany: A Review

Nisruti Anuja[1], Naga Jogayya Kothakota[2]*, Sheerin Bashar[1] and Pravallika Vataparthi[2]

[1]School of Forensic Science, Centurion University of Technology and Management, Bhubaneswar, Odisha, India.
[2]School of Forensic Science, Centurion University of Technology and Management, Vizianagaram, Andhra Pradesh, India.

http://dx.doi.org/10.13005/bbra/3275

(Received: 13 June 2024; accepted: 30 September 2024)

After a through literature review it was found that significant supporting evidences can be obtained through forensic botany in the course of criminal investigations. Nevertheless, this field of inquiry remains underutilized, as its most prevalent use is restricted to the identification of specific and suspected illicit plants. Plant evidences gathered at the crime scene could be a crucial piece in gathering information such as the suspect's route tracing, establishing links between crime scenes and suspects, examining alibis, identification of a narcotic plant and identifying rare or endangered species, determination of geographic locations of plant varieties etc. Conventional morphological attributes prove inadequate for the identification and species-level differentiation of numerous plant materials in instances where botanical specimens are degraded and devoid of physical characteristics. Because of this reason there is a need to rely on molecular techniques where identification will be solely based on analysis of the nucleotide sequences of the genome of the plants. Several studies have demonstrated the successful use of chloroplast DNA and its various barocode regions for taxon/species level identification of the different botanic evidences. Coding and non-coding regions of plastosome like rbcL, matK, trnH-psbA, trnL-trnF and their multiple combinations have helped in identifying Santalum species, Paphiopedilum species, Aloe species, woody plants and medicinal herbaceous plants. The technique of DNA barcoding is efficient, rapid, and precise when it comes to identifying plant species by examining the base sequences found in the genome (chloroplast, mitochondria or nuclear genome). In this review article we have collected manuscripts on application of DNA barcoding using chloroplast DNA barcode regions for species identification in plants.

Keywords: Aloe species; Chloroplast DNA; DNA Barcoding; Santalum album; Species Identification

A chloroplast is a cell organelle mostly found in plants and algae. It is photosynthetic as it contains huge amount of chlorophyll (green coloured pigments). The chloroplast consists of three membrane systems: outer membrane, inner membrane, and thylakoid system, where photosynthesis occurs. The matrix of the chloroplast, called as 'stroma', contains cpDNA (Plastosome), chemicals, enzymes and ions. Multiple copies of cpDNA molecules are found within individual chloroplasts. Structurally, cpDNA is a double stranded, closed circular in shape with

*Corresponding author E-mail: naga.kothakota@cutm.ac.in

1,20,000 to 1,70,000 base pairs long[1]. As compared to mitochondrial DNA (mtDNA), chloroplast DNA(cpDNA) is much larger in size. Its size can varies from species to species as well with high degree of variations. That's why cpDNA genomics can be considered as an important evidence in forensic identification of plant species.

A number of repetitive DNA sequences are also found in the cpDNA that repeats themselves more than once throughout the DNA. One of such repetitive sequences is known as 'Inverted Repeats' or IR sequence. These sequences are conserved within the species and vary in their positions as well within the chloroplast genome. As these 'conserved sequences' vary in their positions as well as base sequences from species to species they can be greatly employed in interspecies and intraspecies identification due to high degree of variability. It also helps in establishing phylogenetic relationships among different plant species.

Chloroplast microsatellites are also widely distributed throughout the chloroplast genome that helps in understanding the genetic diversity among the plant species. These microsatellites are the tandem repeats of nucleotides known as 'Simple Sequence Repeats' or SSR. SSRs are commonly found in the nuclear and organellar genomes of eukaryotic organisms[3,4,5,6]. These markers are highly regarded as excellent molecular markers for investigating plant genetics[4,7]. As these chloroplast microsatellites have advantages of high polymorphism and multi-allelic loci, these are greatly exploited of being used as reliable DNA Barcodes and thus can be employed in DNA Barcoding techniques for species identification.

More than 420,000 plant species are present worldwide, as indicated by the 2022 International Union for Conservation of Nature (IUCN) Red List data. However, only a portion of these species can be identified using conventional taxonomy[7] based on phenotypical characteristics. But the use of conventional taxonomy for identifying plant species holds a lot of challenges as this method rely on morphological characteristics for identification which is susceptible to environmental influences. It is imperative to incorporate molecular methods, such as Plant DNA barcoding, to address the difficulties presented by these constraints. DNA barcoding offers an efficient and expeditious approach for the identification of organisms, plants or any animal[8,9]. This technique utilizes DNA Barcodes, a standardized nucleotide sequences consisting of 400-800 base pairs that are unique thus useful in identifying organisms following amplification, sequencing, and comparison with a reference database containing the relevant sequences from different species[10].

In forensic investigations, DNA barcoding is an invaluable technology that enables the link between biological specimens and crime incidents. Specifically, the analysis of botanical evidence found at crime scenes, such as in instances involving the movement of a deceased body, monitoring the trajectory of a suspect, or recognizing illicit pharmaceutical plants, can have a crucial impact on the resolution of criminal investigations. Due to anthropogenic activities , illegal trading or smuggling of rare plant species it becomes crucial to conserve them to prevent their extinction from the natural habitat. For conservation, it is crucial to accurately identify those plant varieties and this can be achieved through plant DNA barcoding technique. Therefore, it also plays an important role in protecting and conserving rare endangered and threatened plant species. DNA barcoding is also known to be very efficient method employed in the authentication and traceability of food, particularly in processed consumables and nutritional supplements[11,12]. Among other innovative analytical techniques, the US Food and Drug Administration (FDA) promotes the use of DNA-based technology for the quality assessment of herbal products too[13]. The field of forensic science is considerably impacted by the application of plant DNA barcoding in forensic botany, which improves the accuracy and efficiency of investigations[14].

Chloroplast genome has a wide applicability in various fields ranging from biodiversity assessment, authentication of medicinal plants and food items, improves plant breeding and crop varieties to curbing wildlife crimes involving plants as illustrated below in Figure 1:

Importance of plant DNA barcoding system using cpDNA

The crime scene typically contains a variety of evidences, including not only human blood, hairs, and sperm, but also plant and animal-derived evidences. The examination of

both non-human and human DNA is becoming increasingly important in crime investigations. In the past, human identification has been crucial in establishing evidence, but today, non-human DNA analysis stands out.

Significant supporting evidence can be obtained through forensic botany in the course of criminal investigations. Nevertheless, this field of inquiry remains underutilized, as its most prevalent use is restricted to the identification of specific and suspected illicit plants. Plant evidences gathered at the crime scene could be a crucial piece in gathering information such as the suspect's route tracing, establishing links between crime scenes and suspects, examining alibis, identification of a narcotic plant and identifying rare or endangered species, determination of geographic locations of plant varieties etc. Conventional morphological attributes prove inadequate for the identification and species-level differentiation of numerous plant materials in instances where botanical specimens are degraded and devoid of physical characteristics, therefore an accurate and reliable molecular identification system is very much essential for such purpose. At present, several biomarker models that are already in existence serve as potential sources of DNA fingerprinting generators (RAPDs, AFLPs, SSRs, SNPs, and SCARs). These models generate unique identifiers for each organism[15] and can distinguish between organisms of different taxonomic levels, such as genera, or even distinguish distinct varieties within the same species.

Multigene Approach To Identify Botanic Evidences Using Chloroplast Non-coding Barcode Regions

By utilizing DNA sequencing, a universal barcode system, and other biomolecular techniques that are routinely employed in forensic investigations, Ferri et al. (2009)[16] conducted a study using multigene barcoding approach, where two chloroplast non-coding barcode regions trnL-trnF and psbA-trnH were used to identify and discriminate the botanic evidences. In order to assess the comparative discriminatory capability of the psbA-trnH and trnL-trnF loci, 63 chosen plants from the local flora were subjected to DNA extraction, invitro PCR amplification and sequence analysis by aligning the sequences utilizing the Jalview Java alignment editor in conjunction with Clustal W version 1.8. It was found that the selected barcode regions were successfully amplified for most of the plant species as it has successfully resolved monophyletic species in 60% of cases, with higher taxonomic identification achieved for remaining samples thus, proving the reliability of using the multi-marker approach for species identification. The utilization of multigene barcoding in forensic investigations to identify species has demonstrated remarkable efficacy and carries substantial ramifications for the field of forensic botany.

Identification Of *Papaver somniferum* Using Chloroplast DNA Barcode Regions

In an attempt to individualize *Papaver somniferum,* nine cpDNA barcode regions was utilized for forensic intelligence purposes by Graham & Houston (2022)[17] on "Evaluation of chloroplast DNA barcoding markers to individualize *Papaver somniferum* for forensic intelligence purposes". The nine cpDNA barcode regions which were considered are ndhF-rpl32, petA-psbJ, rpl32-trnL, rps16-trnQ, trnE-trnT, trnH-psbA, trnL-trnF, rpl16 intron, and psbE-petL for the screening of inter-species and intra-species variations and individualization in *P.somniferum* plant. For this purpose of research, 10 *P.somniferum* seed samples were collected from different vendors and then sequenced and compared with the published reference genomes from the NCBI GenBank database. Opium poppy (*Papaver somniferum L.*) is a plant of forensic significance owing to the milky latex contained within its capsules, which is utilized for both illicit and medicinal purposes. Morphine, codeine, and thebaine, alkaloids present in this latex, are utilized for their analgesic properties and/or in the synthesis of additional opioids. All these has led to their over exploitation, over harvesting and illicit trade. In order to curb these, use of morphological and chemical identification methods have not proved beneficial, therefore exploitation of the selected plant genome using the chloroplast DNA barcoding markers proved beneficial in accurately identifying the species of interest.

Researchers have found that out of 9 cpDNA barcode markers considered, *petA-psbJ* and *trnH-psbA* regions hold potential for interspecies and intraspecies individualization of P.*somniferum.*

Identification Of Different Aloe Species Based on Specific cpDNA Barcodes

Das & Joshi (2023)[18] conducted a research on 'Development of specific barcodes for identification of *Aloe* species based on chloroplast DNA barcoding', where they have developed specific barcodes for five different species of *Aloe* based on SNP (Single Nucleotide Polymorphism) analysis of selected barcode sequence for the purpose of species specific identification and also a DNA QR (rapid response) code was generated for each sequence in correspondence. This research assessed the two chloroplast DNA identifiers *rbcL* and *matK* in order to establish a foundational framework for the identification of species and the conservation of germplasm of *Aloe* species because *matK* and *rbcL*, two variable-coding genes found in chloroplasts, are among the most thoroughly researched in angiosperms; they have been suggested as a universal barcode for terrestrial plants. Then, after obtaining the sequences 'Maximum Parsimony' approach was made to find out the genetic or evolutionary relatedness of the aloe sequences. 49 Aloe accessions were classified into four main clades based on the findings of the *matK* phylogenetic analysis. In the same manner, the 29 accessions of Aloe were classified into two clades according to the *rbcL* phylogeny. Therefore, the feasibility of creating a chloroplast-based barcode for the identification and discrimination of closely related plant species as well as their conservation was demonstrated in this study.

Molecular Approach Of cpDNA Barcoding For Identification Of Endangered And Endemic Orchids of India

Similarly, Srivastava & Manjunath (2020)[19] utilized the molecular method of DNA Barcoding for identification of endangered and endemic orchids of India, In this study, 62 samples representing 35 species and 7 genera were gathered in total. MEGA-X software was utilized to compute evolutionary divergences and the barcoding gap in order to identify the most appropriate barcoding region from the ITS, matK, rbcL, and trnH-psbA loci and utilizing BLAST analysis, the barcoding locus with the highest species resolution was identified. Out of 133 barcode sequences generated from the collected sample, 46 novel sequences were identified new to the GenBank database. After reviewing all the selected four candidate barcode markers using Distance, BLAST and tree building methods, it was concluded by the authors that ITS is the best single-locus barcode region to be considered for the accurate identification of the diverse orchid species.

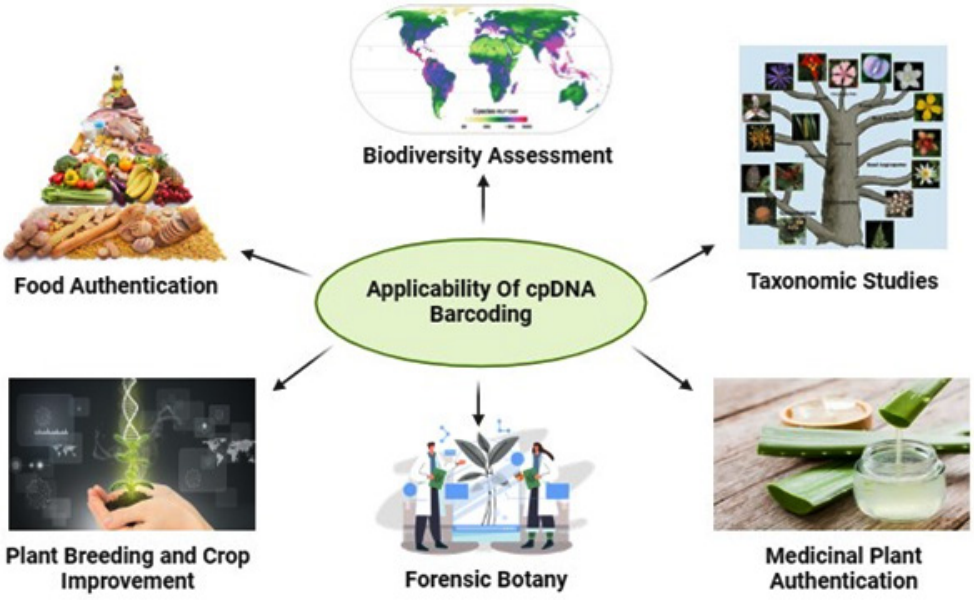

Fig. 1. Application Of Chloroplast DNA Barcodes In Various Fields

Utilization Of Chloroplast Genome For Plant Traceability and Phylogeny To Curb Crimes Related To It

In a review conducted by Freitas et al. (2018)[20] on utilization of the chloroplast genome for the traceability and phylogeny of plants via analysis of chloroplast DNA sequences demonstrated that the chloroplast genome is regarded as a potentially useful instrument for plant classification and differentiation, to ultimately curb the crimes related to food adulteration, illicit drug trafficking etc. In light of the growing concern for food safety, the identification of gene flow from genetically modified plants, and the development of more effective investigative tools for law enforcement, numerous studies have established the feasibility of employing cpDNA for these objectives.

There has been a growing concern regarding food safety, as well as the authenticity, quality, and legitimacy of food. This is largely due to the assessment of bioterrorism risks and the increasing implications of outbreaks of foodborne diseases[21,22]. Genetic traceability through PCR assay and Molecular marker techniques is a

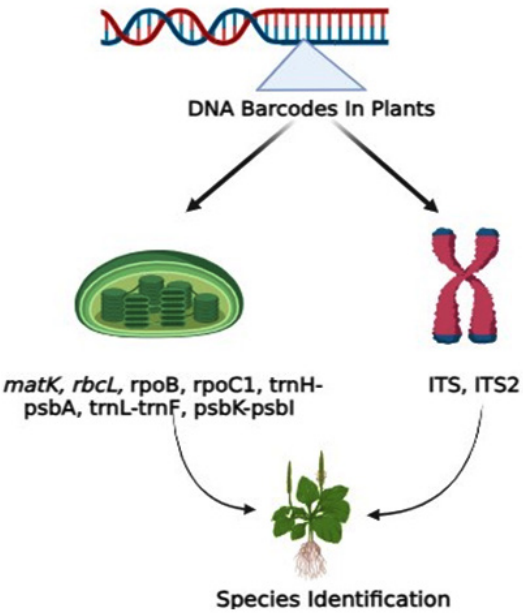

Fig. 2. Schematic representation of DNA Barcodes

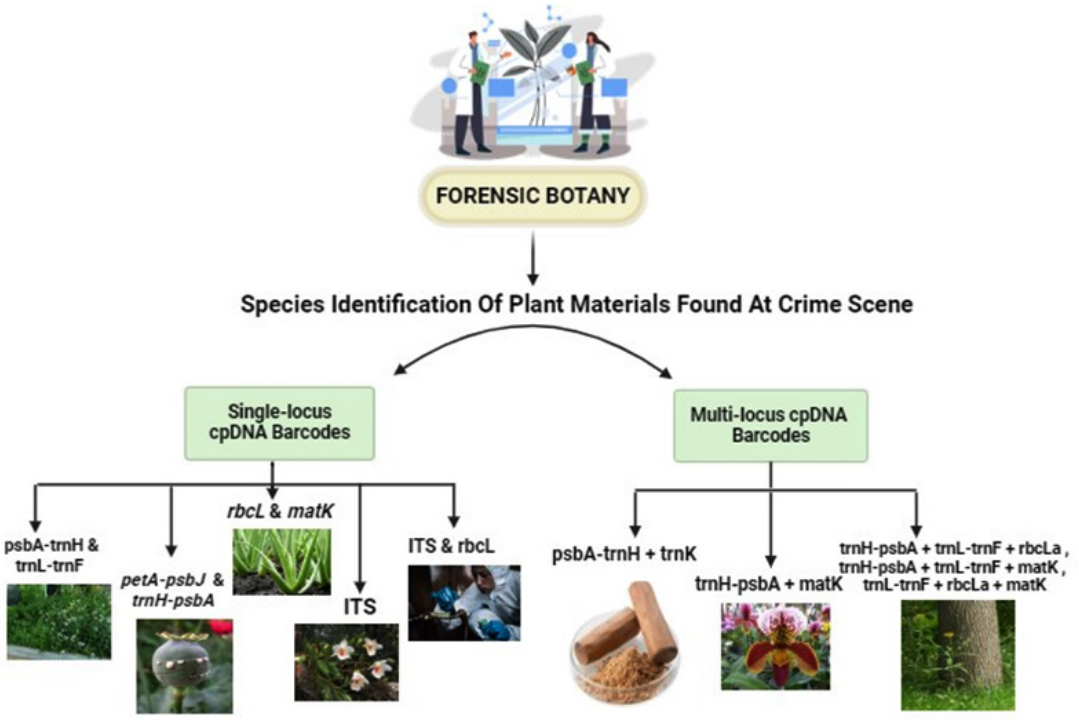

Fig. 3. Ideal cpDNA single-locus and multi-locus barcodes used in Forensic botany that have successfully identified the mentioned items.

method for ascertaining the genetic composition of plant products and including information about their origin, materials, or constituents used in the product[21]. These methodologies enable the verification and examination of the authenticity of the plant variety utilized in both product design and multiple stages of processing[23]. Likewise, tracing the routes of drug trafficking of illicit drugs could assist law enforcement in elucidating and combating drug-related offenses as it can provide information on geographical origin of the drugs from where it has came from[24]. Traceability of plants, such as *Cannabis sativa* and *Papaver somniferum* may also provide genetic information distinguishing plants grown for medicinal use from those grown for illicit use. In an experiment conducted on the two abovesaid species by Freitas et al. recently provided evidence of the utility of chloroplast genome sequences in forensic application where they differentiated various cultivars of C. sativa based on cpDNA, demonstrating that this cpDNA-based method is a viable and replicable instrument for tracing this species. The implementation of cpDNA markers derived from plants could provide an alternative method for such purpose for more precisely tracing the origin of seized plants and drugs.

Applicability Of Chloroplast Genome For Identification Of Plants From Trace Evidences

Chloroplast gene has also played a significant role in identifying the plants from trace evidences collected from crime scene as demonstrated by Bever & Camino[25], in their work on 'Identification Of Plants From Trace Evidence'. As the application of molecular systematic botany in forensic science is expanding, DNA-based techniques have been utilized to locate suspects at the site of the crime and to identify strains of marijuana in criminal cases[26,27]. Most botanical evidences are recovered in the form of mixtures as trace evidences and identifying each component plant from the mixture using traditional methods of identification based on macroscopic features is cumbersome and sometimes less effective also. Therefore, using genomic information based on DNA isolation is crucial. Investigation was conducted on two different type of botanical mixtures, one made up of previously identified eight plant specimens (Mixture 1) and another mixture made up of dust obtained from a piece of mock evidentiary clothing of unknown origin (Mixture 2). DNA was extracted from each of the collected samples from each type of the mixtures and was quantitate using AGE(Agarose Gel Electrophoresis). Then, was subjected to PCR amplification. For the amplification of DNA of eight plant samples from the mixture 1, rbcL gene encoded in chloroplast genome and a plant specific molecular marker was considered. For the amplification of extracted DNA from the dust samples molecular markers rbcL and ITS regions were considered. Phylogenetic analyses and BLAST were employed to ascertain the botanical affinity of the sequences in comparison to other sequences that were accessible in Genbank.

The botanical components of a mixture can be used for many aspects of criminal investigations as they can be used to infer the geographical location, habitat information as well as ecological data and can be used to identify potential matches or exclusion among the evidences collected. In the above study it was conferred that all the eight plant samples were successfully identified from the mixture to their known origin and also the primers ITS and *rbcL* effectively extracted sequences from the evidentiary dust and identified the trace botanical specimens to their genus level and species level as well.

It was discovered, although single-locus barcodes were utilized in identifying plant species they are not sufficient to identify every plant species, thus necessitates the use of multi-locus DNA barcode system. The CBOL [The Consortium for the Barcode of Life] Plant Working Group reports that the most frequently used marker combinations for identifying unidentified samples consist of the following sequences: matK, rbcL, rpoB, rpoC1, atpF-atpH, psbK-psbI, and trnH-psbA[28] as demonstrated in figure 2. We have reviewed some studies regarding this as mentioned below:

Regulation Of Illegal Timber Trade Using cpDNA Approach

In order to monitor and regulate the illegal timber trade across the globe chloroplast DNA (cpDNA) has also played a significant role in identifying the smuggled wood varieties. Jiao et al. (2018)[29] conducted a research on *Santalum album* and its adulterants in order to establish species level identification of the five selected

species of *Santalum* using cpDNA barcoding technique. *Santalum L*. is widely recognized for its fragrant heartwood extracted oil which diffused in to scent perfumes and incense sticks. Generally referred to as 'Sandalwood', it has high market value due to the increasing demand of its products and the detrimental consequences of escalating sandalwood product demand include the excessive and illicit logging of wild *Santalum* populations, which imperils their ecological distribution in numerous areas. cpDNA proved to be effective in curbing this. In general, wood identification through wood anatomy is the prevailing and customary approach. Nevertheless, this method is rather inefficient, particularly when it comes to identifying wood at the species level, due to the fact that taxonomically closely related species frequently possess comparable wood structures[30,31] so, as a complementary to it, DNA barcoding method was adopted by the researchers in this study based on four candidate chloroplast DNA (cpDNA) barcodes, i.e. matK, psbA-trnH, trnK and trnL, along with their combinations to discriminate between five Santalum species and to identify them. As mentioned by Yu et al. (2017)[32] it is required to achieve a retrieval success rate of higher than 70% in order to develop DNA barcode method for wood specimens. And the current study showed that recovery success rate was the highest for trnK (95.9%), followed by matK (91.8%), trnL (89.8%) and psbA-trnH (73.5%), which suggested that the above selected barcode markers have the potential to be employed in the wood forensics. After analyzing the inter-specific and intra-specific variation among the selected species of *Santalum L*. using Neighborjoining (NJ) and TaxonDNA methods, it was concluded that the combination of *psbA-trnH + trnK* demonstrated the highest level of efficacy in terms of discrimination ability and recovery rate (100%) and also using the suggested cpDNA barcodes, six unvouchered wood specimens were retrieved and accurately identified at the species level.

Use Of DNA Barcode Markers For the Identification Of Two *Paphiopedilum* Species

Thi Hai et al. (2023)[33] made an attempt to identify two *Paphiopedilum* species, which are over-exploited and illegally traded for their commercial values, i.e *Paphiopedilum hangianum* and *Paphiopedilum emersonii* based on DNA barcode markers. For such study, four chloroplast DNA(cpDNA) sequence markers were used *matK, rbcL, rpoC1* and *trnH-psbA*, along with their various combinations. Basically, *P.hangianum* and *P.emersonii* are morphologically very similar that creates confusion in their identification and also distinguishing identical species within this genus becomes a more challenging task when the plants are juvenile or have not obtained their complete flowers. Thus, chloroplast DNA barcodes helped in individualization of the selected orchid species.

For such purpose of study, following DNA isolation, invitro amplification, sequencing using 'ABI PRISM 3100 Avant Genetic Analyzer' automatic nucleotide sequencer and phylogenetic study using MEGA X software, it was found that, *trnH-psbA* is identified as the sole marker with best differentiation ability and among the combination markers, only the *trnH-psbA + matK* complex was capable of differentiating between two closely related species according to the Vietnamese Paphiopedilum taxonomic system. Therefore, the DNA barcoding indicator successfully distinguished the two species with precision and speed, even when the plants were immature or devoid of flowers.

Identification Of Woody And Herbaceous Plants Using cpDNA Barcoding System

In a study conducted by Park et al. (2017)[34] on identification of 11woody plants and 21 herbaceous plants using DNA Barcoding system, 4 different chloroplast genome barcodes along with their multiple combinations were used. The barcode regions that are used are *trnH-psbA, rbcLa, trnL-trnF and matK* region and their combinations include mixture of 2 markers -*trnH-psbA+trnL-trnF, trnH-psbA+rbcLa, trnHpsbA+matK, trnL-trnF+rbcLa, trnL-trnF+matK and rbcLa+matK* and mixture of 3 markers -*trnH-psbA+trnL-trnF+rbcLa, trnH-psbA+trnL-trnF+matK, trnL-trnF+rbcLa+matK.*

Approximately 5mg of tissue samples from each of the selected plant specimens were collected for the research. All the collected tissue samples were then subjected to DNA extraction using manufacturer manual of DNeasy Plant Mini kit. Then the extracted DNA is amplified at the trnH-psbA, rbcLa, trnLtrnF and matK regions using the universal primers. Amplified PCR products were then confirmed by the electrophorese on

2% agarose gel. Utilizing an ABI 3130l genetic analyzer (Applied Biosystems, USA), the final products were arrayed or sequenced. In order to analyze the extremely similar sequences of the obtained base sequences, BLAST (Basic Local Alignment Search Tool) was utilized, which is located on the GenBank of NCBI (National Center for Biological Information).It has been found that, success rate of species identification using single markers *trnH-psbA, rbcLa, trnL-trnF and matK* were 84.4%, 71.9%, 65.6%, 62,5% respectively, out of which, *trnH-psbA and trnL-trnF* were able to identify all 11 woody plants but few herbaceous samples remain undetected. Among the marker combinations, 3-marker combinations showed higher identification success rate with an average of 91.4% than the 2-marker combinations. Thus, it has been concluded that using the 4 selected markers, species level identification of woody plant evidences collected from the crime scene was successfully conducted but some extra markers need to be considered also as the selected markers failed to identify some specimens among the selected herbaceous samples.

DISCUSSION

DNA barcoding, which was devised around two decades ago, has made a substantial contribution to the advancement of molecular systematics. DNA barcodes are standardized sequences, preferably unique, coding or non-coding, derived from the organelles or genome of the organism, which are employed for the purpose of identifying and classifying an organismal group into various taxa. The procedure entails several steps, namely DNA barcode amplification, sequencing, and comparison with a reference database comprising the pertinent sequences from various species. In animals, this strategy has proven to be remarkably effective, as the COI has emerged as a universal DNA barcode utilized for the identification of taxa. However, the implementation of a universal DNA barcode in plants has not yet been accomplished.

Numerous investigations have been conducted to characterize plant DNA barcodes derived from the chloroplast. These barcodes include *rbcL, trnH-psbA, matK, trnL-trnF, psbK-psbI, and atpF-atp*[35] as it is discussed in the current study. Although these single-locus barcodes shown successful implementation in plant identification but failed in many cases which necessitates the use of their various combinations or multi-locus DNA barcodes as suggested by the CBOL [The Consortium for the Barcode of Life] Plant Working Group. Nevertheless, this methodology failed to yield a universally applicable combination for all plants.

In real life settings, implication of plant DNA barcoding has immense importance as it can be helpful in botanical fingerprinting for verifying the authenticity of plant evidence as to find out whether a specific plant material is unique to an environment surrounding the crime area because it will help in linking suspects to the crime area. DNA barcoding can also be employed to identify plants that are used in the production of illicit drugs, such as cannabis and coca plants. This assists law enforcement in identifying the origin of the drug production and distribution networks. In instances of illegal plant harvesting or trafficking (e.g., endangered cactus, orchids or medicinal plants), DNA barcoding is frequently utilized to identify the species and establish illegal activity, a critical component of wildlife crime investigations[36].

It can be considered that, the advancements in scientific technologies like NGS (Next Generation Sequencing) or Massive Parallel Sequencing, Third generation sequencing, Super barcoding can now possibly be employed to analyse larger sections of the chloroplast genome in plants to achieve the accuracy in species identification. The simultaneous sequencing of numerous DNA identifiers across a large number of samples is facilitated by NGS technologies. It is now possible to barcode entire ecosystems in a single run, as this significantly enhances the speed and scope of plant species identification. The cost of DNA sequencing per sample has decreased as a result of NGS, which has made large-scale barcoding initiatives more affordable for research and conservation efforts. Automation in DNA technologies have also enabled high-throughput DNA barcoding, which enables the simultaneous processing of thousands of samples with minimal human intervention.

A new technique of Super-Barcoding offers novel opportunities in the field of molecular plant identification, particularly when single-locus or multi-locus barcoding techniques fail to

adequately differentiate closely related species. The accuracy and scope of species identification have been significantly enhanced by the expansion of exhaustive DNA barcode reference libraries, such as the Barcode of Life Database (BOLD). These libraries now contain millions of DNA sequences for plant species, enabling more dependable comparisons. The efficient management and analysis of large DNA sequence datasets have been facilitated by advancements in bioinformatics. In order to expedite the classification of sequences and their alignment with reference databases, DNA barcoding workflows are incorporating AI and machine learning algorithms[37].

Therefore, there is a need to utilize these techniques for improved efficacy in plant molecular identification and to develop a universal cpDNA barcode which can be applicable to discriminate every plant species thus, ultimately help the law enforcement community to curb various issues related to botanic evidences in crime investigation.

CONCLUSION

Ultimately, although DNA barcoding has achieved notable progress in plant identification, the task of creating a universal DNA barcode for all plant species still presents a problem. Advancements in chloroplast DNA (cpDNA) barcoding, especially using multi-locus methods, have showed potential but remain constrained in their capacity to universally distinguish between plant species. Recent technical breakthroughs, like as Next-Generation Sequencing (NGS) and super-barcoding, provide new opportunities for species identification with greater precision and efficiency. These methodologies have improved the effectiveness of vast-scale plant barcoding and can be used in forensic botany to tackle crucial problems such as connecting suspects to crime scenes, detecting illegal plants, and preserving endangered species. The ongoing incorporation of these sophisticated techniques, together with the expansion of DNA barcode reference libraries like the Barcode of Life Database (BOLD) and the enhancement of bioinformatics tools, will be crucial in attaining the objective of a universal plant DNA barcode and augmenting the significance of forensic botany in criminal investigations.

ACKNOWLEDGMENT

The authors are thankful to Centurion University of Technology and Management, Andhra Pradesh and Odisha, India.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Authors' Contribution

Nisruti Anuja: Writing Manuscript, data collection; Naga Jogayya Kothakota: English Grammer checking, Draft Of Manuscript Preparation, Language reframing; Sheerin Bashar: Writing Manuscript and Reference Collection Compilation Data; Pravallika Vataparti: Helped with manuscript draft and final corrections.

REFERENCES

1. Chloroplast DNA Definition and Examples - Biology Online Dictionary. (2021, July 21). Biology Articles, Tutorials & Dictionary Online. https://www.biologyonline.com/dictionary/chloroplast-dna
2. Ramu, P., Billot, C., Rami, J. F., Senthilvel, S., Upadhyaya, H. D., Ananda Reddy, L., & Hash, C. T. (2013, May 25). Assessment of genetic diversity in the sorghum reference set using EST-SSR markers. Theoretical and Applied Genetics, 126(8), 2051–2064. https://doi.org/10.1007/s00122-013-2117-6
3. Kumar, R., Kumar, C., Paliwal, R., Roy Choudhury, D., Singh, I., Kumar, A., Kumari, A., & Singh, R. (2020, December 9). Development of Novel Genomic Simple Sequence Repeat (g-SSR) Markers and Their Validation for Genetic Diversity Analyses in Kalmegh [Andrographis

paniculata (Burm. F.) Nees]. Plants, 9(12), 1734. https://doi.org/10.3390/plants9121734

4. Tyagi, S., Kumar, A., Gautam, T., Pandey, R., Rustgi, S., & Mir, R. R. (2021, February 4). Development and use of miRNA-derived SSR markers for the study of genetic diversity, population structure, and characterization of genotypes for breeding heat tolerant wheat varieties. PLOS ONE, 16(2), e0231063. https://doi.org/10.1371/journal.pone.0231063
5. Sharma, H., Hyvönen, J., & Poczai, P. (2020, January). Development of chloroplast microsatellite markers for giant ragweed (Ambrosia trifida). Applications in Plant Sciences, 8(1). https://doi.org/10.1002/aps3.11313
6. Wang, S. Q. (2020, November 9). Genetic diversity and population structure of the endangered species Paeonia decomposita endemic to China and implications for its conservation. BMC Plant Biology, 20(1). https://doi.org/10.1186/s12870-020-02682-z
7. *The IUCN Red List of Threatened Species*. (n.d.). IUCN Red List of Threatened Species. https://www.iucnredlist.org/
8. Yang, F., Ding, F., Chen, H., He, M., Zhu, S., Ma, X., Jiang, L., & Li, H. (2018). DNA Barcoding for the Identification and Authentication of Animal Species in Traditional Medicine. *Evidence-based Complementary and Alternative Medicine*, *2018*, 1–18. https://doi.org/10.1155/2018/5160254
9. Saddhe, A. A., & Kumar, K. (2017). DNA barcoding of plants: Selection of core markers for taxonomic groups. *Plant Science Today*, *5*(1), 9–13. https://doi.org/10.14719/pst.2018.5.1.356
10. Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003d). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B Biological Sciences*, *270*(1512), 313–321. https://doi.org/10.1098/rspb.2002.2218
11. Uncu, A. O., & Uncu, A. T. (2020). A barcode-DNA analysis method for the identification of plant oil adulteration in milk and dairy products. *Food Chemistry*, *326*, 126986. https://doi.org/10.1016/j.foodchem.2020.126986
12. Galimberti, A., Casiraghi, M., Bruni, I., Guzzetti, L., Cortis, P., Berterame, N. M., & Labra, M. (2019). From DNA barcoding to personalized nutrition: the evolution of food traceability. *Current Opinion in Food Science*, *28*, 41–48. https://doi.org/10.1016/j.cofs.2019.07.008
13. *Botanical Drug Development Guidance for Industry*. (2016). https://www.fda.gov/files/drugs/published/Botanical-Drug-Development—Guidance-for-Industry.pdf
14. Park, E., Kim, J., & Lee, H. (2017). Plant dna barcoding system for forensic application. *Forensic Science International. Genetics Supplement Series*, *6*, e282–e283. https://doi.org/10.1016/j.fsigss.2017.09.141
15. Agrimonti, C., Vietina, M., Pafundo, S., & Marmiroli, N. (2011, May). The use of food genomics to ensure the traceability of olive oil. Trends in Food Science & Technology, 22(5), 237–244. https://doi.org/10.1016/j.tifs.2011.02.002
16. Ferri, G., Alù, M., Corradini, B., & Beduschi, G. (2009, June 7). Forensic botany: species identification of botanical trace evidence using a multigene barcoding approach. International Journal of Legal Medicine, 123(5), 395–401. https://doi.org/10.1007/s00414-009-0356-5
17. Graham, K., & Houston, R. (2022, July 5). Evaluation of chloroplast DNA barcoding markers to individualize Papaver somniferum for forensic intelligence purposes. International Journal of Legal Medicine, 138(1), 267–275. https://doi.org/10.1007/s00414-022-02862-6
18. Das, S. K., & Joshi, A. (2023, December 27). Development of specific barcodes for identification of Aloe species based on chloroplast DNA barcoding. Asia Pacific Journal of Molecular Biology and Biotechnology, 71–81. https://doi.org/10.35118/apjmbb.2023.031.4.08
19. Srivastava, D., & Manjunath, K. (2020). DNA barcoding of endemic and endangered orchids of India: A molecular method of species identification. Pharmacognosy Magazine, 16(70), 290. https://doi.org/10.4103/pm.pm_574_19
20. Freitas, A., Da Anunciação, R. R., Matielo, C. B. D., & Stefenon, V. M. (2018, March 1). *Chloroplast DNA: A Promising Source of Information for Plant Phylogeny and Traceability*. ResearchGate. https://www.researchgate.net/publication/325271243_Chloroplast_DNA_A_Promising_Source_of_Information_for_Plant_Phylogeny_and_Traceability/citations
21. Opara LU (2003) Traceability in agriculture and food supply chain: a review of basic concepts, technological implications, and future prospects. J Food Agric Environ 1: 101-106.
22. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. (2009, October). Botanical Journal of the Linnean Society, 161(2), 105–121. https://doi.org/10.1111/j.1095-8339.2009.00996.x
23. Doveri, S., & Lee, D. (2007, May 19). Development of Sensitive Crop-Specific Polymerase Chain Reaction Assays Using 5S DNA: Applications in Food Traceability. Journal of Agricultural and Food Chemistry, 55(12), 4640–4644. https://doi.org/10.1021/jf063259v

24. Williams, J., Banta Green, C., & Burgard, D. (2017, November 6). The need for better marijuana sales data. Addiction, 112(12), 2179–2180. https://doi.org/10.1111/add.14037
25. Bever, Ph.D, & Cimino, B.Sc. (n.d.). 18. FORENSIC MOLECULAR BOTANY: IDENTIFICATION OF PLANTS FROM TRACE EVIDENCE . *Promega*.
26. Yoon, C. K. (1993, May 14). Botanical Witness for the Prosecution. Science, 260(5110), 894–895. https://doi.org/10.1126/science.8493521
27. Coyle, H.M., Ladd, C.E., Palmbach, T.M., & Lee, H.C. (2001). The Green Revolution: botanical contributions to forensics and drug enforcement. Croatian medical journal, 42 3, 340-5
28. CBOL Plant Working Group 1, Hollingsworth, P. M., Forrest, L. L., Spouge, J. L., Hajibabaei, M., Ratnasingham, S., ... & Little, D. P. (2009). A DNA barcode for land plants. Proceedings of the National Academy of Sciences, 106(31), 12794-12797
29. Jiao, L., He, T., Dormontt, E. E., Zhang, Y., Lowe, A. J., & Yin, Y. (2018, August 27). Applicability of chloroplast DNA barcodes for wood identification between Santalum album and its adulterants. Holzforschung, 73(2), 209–218. https://doi.org/10.1515/hf-2018-0047
30. Jiao, L., Yin, Y., Cheng, Y., & Jiang, X. (2013, November 8). DNA barcoding for identification of the endangered species Aquilaria sinensis: comparison of data from heated or aged wood samples. Holzforschung, 68(4), 487–494. https://doi.org/10.1515/hf-2013-0129
31. Bolson, M., Smidt, E. D. C., Brotto, M. L., & Silva-Pereira, V. (2015, December 2). ITS and trnH-psbA as Efficient DNA Barcodes to Identify Threatened Commercial Woody Angiosperms from Southern Brazilian Atlantic Rainforests. PLOS ONE, 10(12), e0143049. https://doi.org/10.1371/journal.pone.0143049
32. Yu, M., Jiao, L., Guo, J., Wiedenhoeft, A. C., He, T., Jiang, X., & Yin, Y. (2017, August 19). DNA barcoding of vouchered xylarium wood specimens of nine endangered Dalbergia species. Planta, 246(6), 1165–1176. https://doi.org/10.1007/s00425-017-2758-9
33. Thi Hai, Y. N., Xuan, Q. N., Dinh, T. N., Tien, P. D., & Hoang, M. C. (2023, April 30). Morphology and DNA marker for distinguishing Paphiopedilum hangianum and Paphiopedilum emersonii from Vietnam. Journal of Experimental Biology and Agricultural Sciences, 11(2), 423–435. https://doi.org/10.18006/2023.11(2).423.435
34. Park, E., Kim, J., & Lee, H. (2017, December). Plant dna barcoding system for forensic application. Forensic Science International: Genetics Supplement Series, 6, e282–e283. https://doi.org/10.1016/j.fsigss.2017.09.141
35. Letsiou, S., Madesis, P., Vasdekis, E., Montemurro, C., Grigoriou, M. E., Skavdis, G., Moussis, V., Koutelidakis, A. E., & Tzakos, A. G. (2024). DNA Barcoding as a Plant Identification Method. *Applied Sciences*, *14*(4), 1415. https://doi.org/10.3390/app14041415
36. Park, E., Kim, J., & Lee, H. (2017). Plant dna barcoding system for forensic application. *Forensic Science International. Genetics Supplement Series*, *6*, e282–e283. https://doi.org/10.1016/j.fsigss.2017.09.141
37. Riza, L. S., Zain, M. I., Izzuddin, A., Prasetyo, Y., Hidayat, T., & Samah, K. a. F. A. (2023). Implementation of machine learning in DNA barcoding for determining the plant family taxonomy. *Heliyon*, *9*(10), e20161. https://doi.org/10.1016/j.heliyon.2023.e20161