

DNA BARCODING

Abstract

DNA barcoding is a technique used to identify plant species by using specific sections of DNA. The conventional approach to species identification is gradually diminishing due to constraints such as potential misinterpretation, overlooking morphologically cryptic.

The two key coding regions, *rbcL* and *matK*, serve as primary DNA barcode markers for terrestrial plants. Each plant subjected to DNA barcoding must have a herbarium voucher alongside the *rbcL* and *matK* DNA sequences to create high-quality databases. In plants, both plastid DNA regions (*rbcL*, *matK*, *trnL*, and *trnH-psbA*) and nuclear DNA regions (ITS and ITS2) are commonly used in DNA barcoding. This molecular-based technique helps in exploring, identifying, and documenting plant diversity within biodiversity hotspots. DNA barcoding has become increasingly prevalent in taxonomic research, providing an alternative and practical set of tools for swift and precise identification of various organisms. The Consortium for the Barcode of Life (CBOL) and the multinational Barcode of Life (iBOL) have made commendable efforts in creating DNA barcodes specifically for plants. DNA barcoding has brought a significant revolution in taxonomy by integrating molecularization, computerization, and standardization into various approaches employed. Genetic fingerprinting is a widely accepted technique for ensuring the quality control of multicomponent herbal medicines and their final products. Sequencing enables the identification of genes that share the most recent common ancestor. DNA sequence data can be stored as simple text strings in electronic databases like GenBank and easily

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analyzed using text-based bioinformatics techniques. Next-generation sequencing (NGS) has revolutionized the extraction of sequence data from individual molecules within complex sources, leading to a new level of species resolution known as next-generation sequencing (NGS). DNA microarray has gained widespread popularity as a technique for verifying the authenticity of herbal medicine due to its high sensitivity, accuracy, specificity, and repeatability in transcriptomics applications. Successful DNA barcodes for herbal plant identification include matK, rbcL, trnH-psbA, ITS, trnL-F, 5S-rRNA, and 18S-rRNA. Traditional approaches are being replaced by adulteration detection techniques based on DNA barcoding. Standards for herbal products including DNA barcoding require a robust scientific community. It is necessary for national pharmacopeia authorities and DNA barcoding experts to collaborate. Pharmacovigilance monitoring might be facilitated and herbal business quality could be enhanced by DNA barcoding verification. For cases of poisoning, genus-level identification is appropriate, using ITS2 or trnH-psbA as secondary markers and rbcL as the main marker.

Keywords: DNA, Medicinal Properties, Plants, Barcode.

To identify different species, the technique of DNA barcoding utilizes specific sections of DNA. In order to enhance our comprehension of the world's biodiversity, conserve it, and effectively utilize it, there are global endeavors being undertaken to generate DNA barcodes for all living organisms across various categories, with the objective of making this valuable information accessible to the general public. The two key coding regions, namely *rbcL* and *matK*, serve as the primary DNA barcode markers for terrestrial plants. It is crucial that every plant subjected to DNA barcoding possesses a herbarium voucher alongside the *rbcL* and *matK* DNA sequences, as this ensures the creation of databases of the highest quality. Furthermore, users of such data should have the ability to access additional information pertaining to the quality of the DNA sequences, the primers employed, as well as the trace files. It is imperative that multiple individuals of each species undergo DNA barcoding to ensure utmost accuracy and account for any variations that may exist within a particular species. Regarding plants, both plastid DNA regions (*rbcL*, *matK*, *trnL*, and *trnH-psbA*) as well as nuclear DNA regions (ITS and ITS2) are commonly employed in the process of DNA barcoding.

To identify species, DNA barcoding employs certain DNA sections. To better understand, preserve, and make use of the world's biodiversity, efforts are being made worldwide to create DNA barcodes for all categories of living things and make this information publicly available. The two coding regions that make up the genes *rbcL* and *matK* are the primary DNA barcode markers for terrestrial plants. Each plant that is DNA barcoded must have a herbarium voucher in addition to the *rbcL* and *matK* DNA sequences in order to produce high-quality databases. Users of the data should also have access to information on the DNA sequence quality, the utilized primers, and trace files. For each species, DNA barcoding should be performed on many individuals to ensure accuracy and account for intraspecific variation. In plants, plastid DNA (*rbcL*, *matK*, *trnL*, and *trnH-psbA* regions) and nuclear DNA (ITS and ITS2 regions) are often used in DNA barcoding[^]

The application of DNA barcoding to plants has become increasingly significant in contemporary scientific advancements, representing a recent development in the field. This technique is frequently utilized as a means of taxonomical classification or species identification. In the context of medicinal plants, the implementation of DNA barcoding holds crucial importance, serving the dual purpose of product verification and identification. It is a molecular technique aimed at species identification through standard DNA sequencing, has been a recurrent concern in the scientific community. In the realm of land plants, a consensus has been notably absent with regards to the region(s) that should be employed for barcoding purposes. In order to address this issue, a detailed and comprehensive evaluation of seven prominent candidates derived from the plastid DNA has been undertaken. The purpose of this assessment is to offer the scientific community with a recommendation for a standardized barcode that can be universally implemented. In accordance to achieve this particular objective, an assessment was carried out to measure the performance of these candidates in terms of recoverability, sequence quality, and species discrimination. The results of our investigation demonstrate that the combination of the *rbcL* and *matK* loci is particularly exceptional and stands out as the optimal plant barcode. Therefore, we propose that this core 2-locus barcode be adopted as a universal framework for the routine utilization of DNA sequence data in the identification of specimens and the facilitation of the discovery of previously overlooked land plant species.

I. SIGNIFICANCE AND IDENTIFICATION OF MEDICINAL PLANT

The need for environmentally friendly solutions has recently increased across all trade sectors. As the worldwide society shifted increasingly toward complementary and alternative medicine, it also had an influence on the medical and healthcare sectors. Traditionally, plant identification has been based on clearly observable physical traits (Miller et al., 2016). In the realm of land plants, a consensus has been notably absent with regards to the region(s) that should be employed for barcoding purposes. In order to address this issue, a detailed and comprehensive evaluation of seven prominent candidates derived from the plastid DNA has been undertaken. The purpose of this assessment is to offer the scientific community with a recommendation for a standardized barcode that can be universally implemented. In accordance to achieve this particular objective, an assessment was carried out to measure the performance of these candidates in terms of recoverability, sequence quality, and species discrimination. The results of our investigation demonstrate that the combination of the *rbcL* and *matK* loci is particularly exceptional and stands out as the optimal plant barcode. Therefore, we propose that this core 2-locus barcode be adopted as a universal framework for the routine utilization of DNA sequence data in the identification of specimens and the facilitation of the discovery of previously overlooked land plant species.

The demand for environmentally friendly solutions in various sectors of trade has recently witnessed a significant increase. This increase can be attributed to the global trend towards complementary and alternative medicine, which has had a significant impact on the medical and healthcare sectors as well. The environment plays a crucial role in shaping a plant's metabolism, particularly its secondary metabolism, which is responsible for the majority of its therapeutic properties, as highlighted by Briskin (2000). In earlier times, the primary methods of plant identification involved detailed descriptions of morphological and anatomical features. This task was typically carried out by expert taxonomists and trained technicians who relied on their experience to describe variations in morphological attributes. However, this conventional approach to species identification is gradually diminishing due to a number of constraints. These constraints include the potential for misinterpretation resulting from a lack of advanced knowledge, the overlooking of morphologically cryptic taxa, and the inefficiency caused by a shortage of morphological keys for certain stages of a plant's life cycle, as emphasized by Vohra and Khera (2013).

II. SIGNIFICANCE OF PLANTS WITH MEDICINAL PROPERTIES

The utilization of herbal medicine in the field of healthcare has experienced a significant surge in growth during the previous few decades. Because of this expansion, there has been a remarkable surge in the global trade of unprocessed medications. The market for herbal commodities is currently experiencing a period of expansion due to the worldwide resurgence of traditional healthcare systems and the active promotion of a healthy lifestyle. According to a report from the World Health Organization (WHO), around 80% of the global population relies on plant-based or herbal medical systems to fulfill their fundamental healthcare requirements. Furthermore, it is worth highlighting that a staggering 80% of the fundamental components required for the production of traditional medicines are derived from medicinal plants. Mentioned their numerous health benefits and practical experience, herbal medicines have become a topic of increased recognition on a global scale. India, with its vast array of medicinal plant species (approximately 45,500), accounts for roughly 7%–

8% of the world's biodiversity. Out of this impressive figure, it is believed that over 8,000 species from both upper and lower plant groups possess medicinal value. Additionally, there are 960 species of medicinal plants that are considered to be traded, with 178 species having annual consumption levels exceeding 100. These figures have been documented in various sources such as Aneesh et al. (2009), Efferth and Greten (2012), and the website www.cbd.int. The successful utilization of these medications is heavily reliant on the correct utilization of authentic raw ingredients and their ongoing availability. However, it is crucial to acknowledge that the chemical compounds found in plants may vary even within the same species due to external factors such as humidity, light, soil composition, temperature, pH, and storage conditions, which are often beyond human control.

Challenges: DNA barcoding can be employed as a molecular technique for the identification of different species due to the fact that when plants are consumed, they undergo significant degradation, making visual inspection impractical. To conduct the molecular identification, the selection of *rbcL* DNA marker was based on several factors which includes the availability of universal primers, the success rate of PCR and sequencing, and the wider range of hazardous plants. Consequently, a DNA barcode library consisting of *rbcL* DNA barcodes was developed, specifically focusing on 100 hazardous plant species. The evaluation of the library revealed that a complete differentiation of species was achieved at the genus level, while a differentiation rate of 89% was observed at the species level for the dangerous plants incorporated in the library. It is worth noting that all undifferentiated species belonged to the same genus.

III. DNA BARCODING

DNA barcoding, which utilizes a limited and standardized segment of the genome, is a scientific approach employed for the purpose of species identification. This method is known as DNA barcoding and it holds a significant potential and serves as a dependable technique for expeditiously identifying various species. In the sphere of scientific exploration, DNA barcoding is proved as an valuable asset as it enables researchers to rapidly identify plant species, including those that possess characteristics that have not yet been documented in the realm of scientific knowledge. Attaining this rapid identification is made possible by refining in on precise DNA sequences derived from a clearly defined segment of the genome. The appropriate use of DNA barcoding facilitates the exploration, identification and documentation of plant diversity within biodiversity hotspots. This molecular-based technique allows for a comprehensive understanding of the distribution patterns of plant life in particular ecologically important areas. It strengthens our capacity to conserve and defend these priceless natural resources and adds to the corpus of knowledge on biodiversity hotspots (Lahaye et al. 2008). Standardization, simplicity, and scalability are the three main tenets of DNA barcoding. Rapid, time-saving, and automated species identification from a variety of herbal items is possible with DNA barcoding. When using this technique, the target gene's DNA sequence analysis is followed by the extraction of the sample's DNA, which contains high rates of nuclear substitutions that can differentiate between species which are closely related while remaining more or less the same for all individuals of the same species. The initial proposal and subsequent widespread acceptance of DNA barcoding occurred within the context of animal systems. DNA barcoding is an approach which involves the sequencing of a particular segment of the mitochondrial cytochrome c oxidase subunit I (COI) gene, which is known as "DNA barcodes," from those specimens whose taxonomic

classification remains unknown. The obtained DNA barcodes are then compared with a comprehensive library of DNA barcodes of those organisms with well-established taxonomies. This comparative analysis serves as a means to obtain taxonomic information regarding the unidentified organisms in question. As a result of its potential, DNA barcoding has become increasingly prevalent in the domain of taxonomic research, simultaneously providing an alternative and practical set of tools for the swift and precise identification of various organisms. Now DNA is also utilized to create bioinventories and contribute in species identification. [1] The use of herbal medicine in healthcare has grown significantly during the last few decades. The global traffic in unprocessed medicines has increased as a result. The success of the herbal medication industry depends on quick and accurate scientific identification of the plant or plants. At the industrial scale, the process of the identification of bulk samples is a task which requires a enormous amount of time and effort. However, there is an alternative method known as DNA barcoding, which offers a feasible and effective taxonomic toolkit for obtaining quick and accurate results. These two multinational ventures, named as Consortium for the Barcode of Life (CBOL) and the multinational Barcode of Life (iBOL), have made commendable efforts in the creation of DNA barcodes specifically for plants. CBOL is a group of scientists with a great expertise and they have been actively engaged in the exploration of flora and fauna across the globe in the search of DNA barcodes. Furthermore the iBOL stands as the largest organization in the field of biodiversity genomics, with their primary objective being the establishment of DNA barcoding as a recognized discipline within the international scientific community. Kress and Erickson (2007) presented that the most ideal region for DNA barcoding should reflect a significant level of divergence among species rather than within species. Now a days DNA barcoding is currently being recognized as an highly effective and extensively utilized tool that greatly facilitates the rapid and precise identification of various plant species. The second largest family of flowering plants is Orchidaceae, which shows a remarkable and astonishing diversity of more than 700 genera and almost 20,000 species, all of which are spread across the earth's surface.

DNA barcoding of four chloroplast genes specifically, *matK*, *rbcL*, *ndhF*, and *ycf1* was considered under a study conducted with the primary goal of providing a comprehensive and well-organized theoretical framework for the identification of orchid species, germplasm conservation, as well as innovative orchid utilization. With careful examination, the nucleotide replacement saturation of either single or combined sequences among the four genes, this study was able to determine that these sequences had reached a state of saturation and were therefore suitable for phylogenetic relationship analysis. As a result, this study represents an invaluable and highly significant resource for researchers as it presents the fundamental groundwork for the conservation, evaluation, innovative utilization, and protection of Orchidaceae germplasm resources.

The botanical and descriptive aspects of pharmacognosy were enhanced by the incorporation of medicinal and pharmaceutical chemistry in order to ensure the quality of drugs through extensive testing. Different phytochemical analysis techniques, such as Fourier transform infrared spectroscopy, high-performance liquid chromatography, mass spectrometry, nuclear magnetic resonance spectroscopy, and thin-layer chromatography, are frequently used to verify the authenticity of plant materials.

These techniques have been studied and are widely recognized in the field (Harborne, 1998; Meena Devi et al., 2010). Since last few years, the Consortium for the Barcode of Life

(CBOL) has devoted its efforts in identifying a plant barcode that can be universally informative. The introduction of DNA barcoding has brought a significant revolution in taxonomy by integrating molecularization, computerization, and standardization into the various approaches employed (Casiraghi et al., 2010).

The quality control of a product has always been a major concern in the creation and verification of herbal medicines. In order to address and overcome the traditional problems associated with the identification of these products, various molecular techniques have been developed. These techniques have emerged as a solution due to the close relationship between taxonomy and quality control of herbal medicines. Consequently, several molecular approaches have been developed, improved, and are currently dominating the scientific community for the taxonomic identification of plants. Furthermore, in the pharmaceutical sector, chemotaxonomy is frequently employed in addition to taximetrics. The limitations inherent in the information provided by morphological features led to the creation of biochemical and molecular approaches for plant identification. There is a growing interest in the use of molecular markers which are DNA- and protein-based in order to achieve more precise identification of plants, attracting global attention (Buriani et al., 2012).

IV. TECHNIQUES USED

The highly effective technique of genetic fingerprinting has now gained widespread acceptance in many developed countries as a means of ensuring the quality control of multicomponent herbal medicines and their final products. Numerous approaches have been employed to authenticate medicinal plants based on their genome, presenting a range of potential applications. Most of these approaches rely on methods that can identify unique nucleotide sequences specific to a particular species, allowing for the determination of the nucleotide sequence of one or more genetic loci in the plants of interest. By comparing the relatedness of gene sequences in the samples, sequencing enables the identification of genes that share the most recent common ancestor. Unlike gel-based fingerprints, which require sophisticated image analysis software, DNA sequence data can be stored as simple text strings in electronic databases like GenBank and easily analyzed using text-based bioinformatics techniques. The remarkable progress made in massively parallel and clonal sequencing systems has revolutionized the extraction of sequence data from individual molecules within complex sources, leading to a new level of species resolution commonly referred to as next-generation sequencing (NGS) (Mardis, 2013; Metzker, 2010). The introduction of sequencing-based technology and automated DNA sequencers has significantly reduced the cost of gel-based fingerprints. Sequences provide the only suitable approach for taxonomic investigations as they offer a comprehensive record of their own history, although molecular data faces similar challenges of homology as morphological data (Karp et al., 1997). DNA microarray has gained widespread popularity as a technique for verifying the authenticity of herbal medicine due to its ability to analyze a large number of samples simultaneously, its high sensitivity, accuracy, specificity, and repeatability in transcriptomics applications (Chavan et al., 2006; Lo et al., 2012). Professionals in this field now have the opportunity to utilize a wide range of innovative approaches that have emerged as a result of recent developments in molecular genetics. These approaches are designed in such a way that they facilitate the quick and accurate identification of plant species. Additionally, a reliable method has been developed, largely driven by the expanding advancements in taxonomy and biotechnology. This method is known as DNA barcoding and

its been suggested as a valuable addition to the taxonomic toolkit by Hebert et al. (2003a). The concept of DNA barcoding involves the utilization of short DNA sequences, specifically derived from a particular region of the genome, for the purpose of biological identification. It proposes the use of standard DNA locus sequencing as a means of identifying species. To be considered a standard DNA barcode, it is essential for a sequence to possess the capability of effectively discriminating across various species, be easily retrievable using a single primer pair, and be amenable to bidirectional sequencing. Many authors have put forth the idea of DNA barcoding as an integrated approach alongside classical taxonomy, particularly for species identification and authentication in the post genomics era. However, the introduction of this invention has given rise to numerous contentious issues pertaining to the nature and objectives of systematics and its numerous subdisciplines (DeSalle, 2006; Lipscomb et al., 2003; Rubinoff et al., 2006). The universality and high resolution of DNA barcoding can be attributed to its utilization of different DNA sections as markers. For a marker to be effective, it must exhibit both a high level of inter-specific variability and a low level of intra-specific variability. This characteristic is crucial in order to possess the necessary discriminating power. The concept of the 'DNA barcoding gap' refers specifically to the variation in distance that exists between intraspecific and interspecific groups. The task of defining DNA barcodes in the realm of plant taxonomy has proven to be significantly more difficult when compared to the realm of mammalian taxonomy. In 2009, the Consortium for the Barcoding of Life (CBOL) that operates on a global scale proposed a pair of chloroplast genes, namely *rbcL* and *matK*, as the recommended official barcode for plants. However, this particular method does come with noteworthy limitations. Primarily, in order for any barcode system to be effective, it is imperative to have a discernible barcode gap and a monophyletic species. In the animal kingdom, the COI gene is utilized in the mitochondria and chloroplasts, respectively. Additionally, it has also been demonstrated that the utilization of chloroplast regions yields a maximum species discrimination rate of approximately 70%, which exhibits significant variation across different plant groups. Due to the fact that the nuclear ITS region showcases a greater number of variants and enables the resolution of hybrid or closely related species, a multitude of scientists have recently presented arguments in favour of incorporating the nuclear ITS region into the barcode system. In order to validate the efficiency of various barcode schemes, examination was conducted of the nuclear ITS region and various chloroplast genes (*rbcL*, *matK*, *psaB*, and *psbC*) within the genus *Vanilla*, which is a taxonomically complex group. It was determined that the official CBOL barcode system only functioned with an efficacy rate of 76% in *Vanilla*. Further study showed that the inclusion of the ITS region in this barcode system not only enhances the resolution capabilities, particularly for closely related species and even down to the subspecies level, but also facilitates the identification of hybrid species.

The barcode locus must include enough data to enable clear differentiation between closely linked plant species and the discovery of new cryptic species for DNA barcoding to be successful. Successful DNA barcodes for herbal plant identification include *matK*, *rbcL*, *trnH-psbA*, ITS, *trnL-F*, 5S-rRNA, and 18S-rRNA. New developments in DNA barcoding, together with next-generation sequencing, high-resolution melting curve analysis, and other techniques, have made it possible to successfully recover species-level resolution from finished herbal products (2017) Mishra et al.

Some Molecular Techniques and its Potential Function

1. DNA sequencing: It uses dideoxynucleotide triphosphates and thermostable DNA polymerase to create chain-termination sequences in a temperature-cycling configuration. (Kretz et al. 1994)
2. Arbitrarily primed (AP)-PCR and the direct amplification of length polymorphism (DALP): The technique of Arbitrarily Primed Polymerase Chain Reaction (AP-PCR) and the direct amplification of length polymorphism (DALP) involves the identification and sequencing of the nucleotide sequences of one or more genetic loci. These sequences are then analyzed to identify those that are specific to a particular species. This methodology has been extensively employed by researchers such as Cao et al. (1996), Desmarais et al. (1998), and Ha et al. (2001) to elucidate the genetic makeup of various species.
3. DNA microarray. In this approach, Species-specific oligonucleotide probes are designed based on the sequence of the 5S ribosomal RNA gene. These probes are then immobilized onto a silicon chip. The target sequences are subsequently amplified and fluorescently labeled through the application of asymmetric PCR. This methodology has been employed by researchers such as Carles et al. (2005) and Schena et al. (1998) to investigate the genetic composition of different species.
4. The polymerase chain reaction-short tandem repeats (PCR-STR) technique, a powerful tool in forensic genetics, is a method that utilizes the amplification of short tandem repeat markers to analyze DNA samples. This approach, which involves the amplification of specific STR loci using PCR, has proven to be highly effective in identifying individuals in criminal investigations and paternity testing. In the study conducted by Qin et al. (2005), the PCR-STR method was further enhanced by combining it with the microchip electrophoresis method. This innovative integration allowed for the simultaneous amplification and separation of STR markers on a microchip platform, streamlining the overall process and increasing the efficiency and accuracy of DNA analysis. The results obtained from this study demonstrated the potential of this advanced technique to revolutionize the field of forensic genetics, providing a more rapid and reliable means of DNA profiling. Therefore, the combination of PCR-STR with microchip electrophoresis represents a significant advancement in forensic genetics and holds great promise for future applications in criminal investigations and paternity testing.
5. Loop-mediated isothermal amplification (LAMP): Amplification is the process of increasing the amount of a specific DNA sequence and is typically conducted under an isothermal reaction condition, which refers to a constant temperature maintained throughout the reaction. This approach ensures that the reaction occurs at a single, stable temperature, which is advantageous for the amplification process. The design of allele-specific primers, short DNA sequences that specifically bind to a target DNA region, plays a crucial role in the success of the amplification. In this particular study, the researchers employed allele-specific primers that were specifically designed based on the sequence of the 18S ribosomal RNA gene, which is a highly conserved gene found in all eukaryotic organisms. The choice of this gene as the target for amplification is significant as it allows for the detection and analysis of a wide range of organisms, in turn making it a versatile tool in various fields such as ecology, microbiology, and evolutionary biology.

The use of the 18S ribosomal RNA gene sequence as a basis for primer design ensures a high level of specificity, enabling the amplification of only the desired target sequence. This approach minimizes the risk of amplifying non-target sequences, which can lead to erroneous results and misinterpretation of data. The study conducted by Sasaki et al. in 2008 utilized this method, highlighting the importance of careful primer design in achieving accurate and reliable amplification results.

6. Intersimple sequence repeat (ISSR) and simple sequence repeats (SSR): Utilizing PCR primers that are designed to be complementary to two microsatellites that are located in close proximity to each other, the process of ISSR amplification is carried out. This amplification process involves the creation of SSR, which are short DNA sequences that contain repetitive motifs, using a genetic library that has been specifically enhanced with these motifs. Subsequently, after the isolation and sequencing of clones that contain the microsatellites of interest, the design of primers that will be used in PCR amplification is conducted. This amplification is performed by employing the chosen primer pair, which has been carefully selected based on its compatibility with the microsatellite sequences. It is worth noting that this methodology has been extensively documented in studies conducted by Sharma et al. in 2008, Su et al. in 2008, and Tamhankar et al. in 2009.
7. Quantitative real-time PCR (Q-PCR/qPCR) : Real-time PCR, a commonly employed molecular biology technique, is utilized in conjunction with a diverse array of sophisticated analytic software applications, with the primary objective of accurately and precisely identifying the presence of DNA molecules within a given biological sample. This innovative methodology operates by specifically targeting and interrogating a selected genomic location, thereby enabling researchers to ascertain the presence or absence of specific DNA sequences of interest. The exploration of this powerful technique has been the focus of numerous studies, with notable contributions made by Xue et al. in 2009, as well as Xue and Xue in 2008. Through their significant contributions, these researchers have enhanced our understanding of the capabilities and limitations of real-time PCR in the context of DNA identification, further advancing the field of molecular biology.
8. Random amplified polymorphic DNA (RAPD) and sequenced characterized amplified region marker (SCAR): Specific marker-defined polymorphic termini undergo sequencing in order to obtain their genetic information, which enables the identification of specific genetic markers. These genetic markers are then utilized to design primers that are specifically engineered for the amplification of a particular locus within targeted species. These investigations have provided valuable insights into the design and application of primers for targeted amplification, thereby enhancing our capability to study and analyze genetic diversity in various species.
9. Restriction fragment length polymorphism (RFLP): A particular gene of interest, which has been amplified using a polymerase chain reaction (PCR), is subjected to a process known as restriction digestion, wherein a variety of restriction enzymes are employed to cleave the DNA at specific recognition sites. The resulting fragments of DNA are then separated from one another by means of agarose gel electrophoresis, a technique that takes advantage of the differential migration of DNA molecules through an electric field. Next, these fragments are transferred onto a membrane using a blotting method, a

procedure that involves the capillary action of a buffer solution. Finally, in order to visualize the presence of the gene of interest, labelled probes are utilized, which specifically binds to the target DNA fragments, and the resulting signal is captured on X-ray film. These experimental steps were conducted by Biswas et al. in the year 2013, as well as by Lin et al. in 2012.

10. **Amplification refractory mutation system (ARMS) and multiplex ARMS (MARMS):** On the foundation of the sequencing data pertaining to the specific species of interest, the mutation site was identified. Subsequently, a set of primer pairs, which are specific to the different alleles, were generated. The purpose of these primer pairs is to bind to the target DNA sequences and facilitate the identification of the mutations. In order to achieve this, a technique known as Multiplex Amplification Refractory Mutation System (MARMS) was employed. This technique is widely used in genetic research to detect specific mutations in DNA samples. Several studies including those conducted by Chiang et al. (2012), Diao et al. (2009), and Wang et al. (2011) have successfully implemented this approach to identify and characterize mutations in various species. The utilization of MARMS in conjunction with allele-specific primer pairs has proven to be an effective strategy for identifying and analyzing mutations in genetic sequences.
11. **Specific expression subset analysis (SESA):** The process of Suppression Subtractive Hybridization (SSH) Analysis, hereinafter referred to as SESA, was undertaken in this study. This scientific technique involved the utilization of cDNA populations acquired from the active component of the target plant species, which is known for its therapeutic value. Moreover, other significant tissues were also employed as the tester and driver in this experimental setup. The purpose of using these tissues was to differentiate and identify the specific genes and molecules that were active in the target plant species' active component, as compared to other important tissues. To achieve this, the collected Expressed Sequence Tags (ESTs) were subjected to a comprehensive computer analysis, as described in the research conducted by Shukla et al. in the year 2013. This analysis aimed to unravel the intricate details of the genetic composition and expression patterns within the target plant species' active component, ultimately leading to a deeper understanding of its therapeutic potential and biological significance.

V. THERE ARE SOME STRATEGIES USED TO OVERCOME THE DIFFICULTIES OF DNA BARCODING IN PLANTS HAVE BEEN REVIEWED BELOW TO FAMILIARIZE RESEARCHERS WITH THE CURRENT DEVELOPMENTS

1. **Single-Locus Strategy:** The goal of (Wicke and Quandt's 2009) work was to create universal primers that would effectively amplify the trnK/matK region in terrestrial plants. DNA barcoding, which identifies plant species, depends on this area of the genome. For the primers to have a wide range of applications, the researchers created and tested them on a variety of plant species. The study is a useful resource for taxonomy and plant identification and matK distinguishes out among all the plastid areas employed in plant systematics because of its faster pace of change. Another study by Barthelet and Hilu (2007) focused to work on the expression of the matK gene and its function. The essential gene for the proper functioning of the trnK/matK region in land plants is matK gene, which is widely used in DNA barcoding to identify plant species. The researchers

investigated the expression patterns of the *matK* gene in many different plant species and tried to identify its role in different plant tissues. They also examined the evolutionary changes and adaptations that might have influenced the *matK* gene over time. The work offers important new insights into the molecular mechanisms underlying plant genetics and evolution by analyzing the functional and evolutionary properties of the *matK* gene. These discoveries help us understand plant biology more broadly and might have applications in the future for DNA barcoding and species identification. (Barthet and Hilu, 2007; Hilu et al., 2003; Wicke and Quandt, 2009). The investigation performed by Lahaye et al. (2008) scrutinized a total of 1084 plant species, which accounts for nearly 96% of the entire orchid species. This meticulous study has successfully demonstrated that a particular segment of the plastid *matK* gene carries the potential to serve as a universally applicable DNA barcode for flowering plants. According to the research conducted by Johnson and Soltis (1994) and Olmstead and Palmer (1994), it has been postulated that the substitution rates among the three codon positions are almost evenly distributed in *matK*, in contrast to most protein-coding genes where the rates tend to be skewed towards the third codon position. As a result, the rate of substitution in *matK* at the nucleotide level is three times higher, and at the amino acid level, it is six times higher compared to that of *rbcL*. Despite numerous efforts, some researchers have encountered challenges in developing universal primer sets that can be applied to all taxa, and these challenges have further complicated the PCR process. Consequently, it has led to the hypothesis that *matK* may not be functional in certain taxa, particularly nonangiosperms. The lack of universal primer sets specifically for nonangiosperms has resulted in a low rate of success in PCR amplification as well as an accelerated rate of substitution (CBOL, 2009; Hidalgo et al., 2004; Kugita et al., 2003).

- 2. Multi-Locus Barcode:** Because *in silico* and laboratory-based assessments yielded unsatisfactory results for individual loci, the most recent successful approach involved the utilization of a combination of barcodes. The Plant Working Group of the Consortium for the Barcode of Life (CBOL) meticulously evaluated numerous potential markers to determine their suitability, ultimately recommending the two-locus combination of *matK* and *rbcL* as the primary plant barcode. This recommendation was supported by the research conducted by CBOL (2009), Fazekas et al. (2008), and Newmaster et al. (2008). Another combination, specifically the pairing of *rbcL* and *trnH-psbA*, which represents the internal transcribed spacers of nuclear ribosomal DNA (*nrITS/nrITS2*), has also been subjected to evaluation to determine its potential, as documented by Ferri et al. (2008). Upon examining the case of two loci, it was discovered that the conserved coding locus aligns aptly with the taxa of a community sample, facilitating the creation of deep phylogenetic branches. On the other hand, the hypervariable region of the DNA barcode readily aligns within the subclades of closely related species. In several instances, the combination of three loci did not yield improved discrimination compared to the best two-loci barcodes. Furthermore, to economize and avoid the associated expenditures of combining three loci for large datasets, it was decided that the two-loci barcode would be established as the standard barcode for terrestrial plants. DNA barcode technology is employed in the forests across tropical and temperate zones for the purpose of swift and precise identification of various species. To carry out one particular study, a total of 183 plant species present in the National Nature Reserve of South China were selected and subjected to sampling and sequencing. In this process, the *matK*, *rbcL*, and *psbA-trnH* genes were utilized to generate multi-locus barcodes. It was observed that the *psbA-trnH*

gene exhibited the highest level of success, which was determined by the combination of sequencing recovery and accurate identification of the species, with a rate of 75%. This was followed by the matK gene with a success rate of 70%, and the rbcL gene with a success rate of 56%. Moreover, it was found that a combination of three-locus barcode (matK, rbcL, and psbA-trnH) had the ability to identify over 87% of the total species. This was then followed by a two-locus barcode, which was able to identify 85% of the species when matK and psbA-trnH were combined, 83% when rbcL and psbA-trnH were combined, and 81% when matK and rbcL were combined. In order to evaluate the universality and accuracy of species identification of the proposed DNA barcodes, a comparison was made with the previously published results from three forest plots: one subtropical forest plot in Puerto Rico (LFDP) with 143 species, and two tropical forest plots in Panama (BCI) with 296 species, and French Guiana (NRS) with 254 species. The results showed that the success rates of sequencing for rbcL, psbA-trnH, and matK were 93% and 95.1%, 91.5% and 94.6%, and 68.5% and 79.7%, respectively, for the plots in tropics and subtropics. Furthermore, it was observed that the combination of matK and rbcL demonstrated a high capacity for identification in geographically restricted regions within taxonomic groups. On the other hand, the three-locus barcode exhibited a high rate of accurate species identification in both tropical regions (84%) and subtropical regions (90%) (Pei.N.C.2012).

- 3. Next-Generation Biodiversity Assessment using DNA Metabarcoding:** Species identification, which is the process of determining the specific type of a species, is an essential requirement for nearly all empirical ecological investigations when gathering data. In cases where numerous species are identified automatically from a singular bulk sample of complete organisms or from a singular environmental sample, such as soil, water, or feces, that includes degraded DNA, this technique is known as DNA metabarcoding. Moreover, DNA metabarcoding can be utilized with environmental samples obtained from both the present and the past. The progress and promotion of DNA metabarcoding has been greatly aided by the accessibility and availability of next-generation sequencing technologies, as well as the increasing demand from ecologists for a high-throughput method of taxon identification. However, it is important to acknowledge that the current utilization of DNA metabarcoding is limited by its dependence on PCR (polymerase chain reaction) and the substantial expenditure required to establish comprehensive taxonomic reference libraries. Nevertheless, the reliance on the DNA amplification step will eventually become obsolete, as comprehensive taxonomic reference libraries can be constructed using carefully curated DNA extract collections that are maintained by standardized barcoding initiatives. These libraries will consist of repetitive ribosomal nuclear DNA and entire organellar genomes.
- 4. Microfluidic Enrichment Barcoding (ME Barcoding):** As we are already familiar, there are numerous applications for DNA barcoding in various fields such as classical taxonomy, ecology, forensics, food analysis, and environmental research, all of which contribute to the identification of different species. This particular technique, Microfluidic Enrichment Barcoding, has become widely adopted due to its effectiveness in generating a large number of DNA barcodes in plants, and it has also been recognized for its cost-efficiency and high efficiency. The method itself incorporates cutting-edge technologies such as the Fluidigm Access Array and Illumina MiSeq, which enable the simultaneous amplification of DNA from multiple samples, thereby facilitating the creation of

extensive plant DNA barcodes and comprehensive databases. By employing this methodology, we are able to present a powerful alternative to traditional PCR and Sanger sequencing, as it allows for the generation of barcode libraries across a wide range of plant lineages. Furthermore, this approach also involves the utilization of a microfluidic enrichment device that utilizes electroosmosis-induced pressure flow in order to enhance the efficiency of enrichment, ultimately enabling the detection of trace substances. This innovative approach also enables the co-localization of DNA or RNA particles with known barcode oligonucleotides, thus providing the opportunity to correlate a cell's transcriptome or DNA with its phenotype or the effects of drugs. Overall, Microfluidic Enrichment Barcoding is a cutting-edge technique that has revolutionized the field of high-throughput DNA barcoding in plants and shows great potential in various biological applications. Within a single thermal cycling cycle, MEBarcoding employs the Fluidigm Access Array, a state-of-the-art technology, to simultaneously amplify specific regions for 48 DNA samples and hundreds of PCR primer pairs, resulting in the generation of up to 23,040 PCR products. To validate the feasibility of this approach, we designed and executed a microfluidic PCR process utilizing the Fluidigm Access Array in combination with the Illumina MiSeq platform. In order to assess the efficacy of this method, we analyzed a total of 96 samples for each of the four major DNA barcode loci in plants, namely *rbcL*, *matK*, *trnH-psbA*, and ITS. By employing this approach, we successfully constructed a reference library consisting of 78 families and 96 genera, spanning across all major plant lineages. It is worth noting that many of these taxa are currently absent from public databases. The results obtained from our study clearly demonstrate that this method serves as a highly efficient alternative to conventional PCR and Sanger sequencing, enabling the generation of an extensive collection of plant DNA barcodes and the construction of more comprehensive barcode databases.

Some Recommended DNA Barcode Loci for Families of Medicinal Plants

- *rbcLa* + *matK* + *trnH-psbA* : Combretaceae (Gere et al. (2013))
- ITS/ITS2 + *psbA-trnH*, ITS and ITS2: Apiaceae (Liu et al. (2014))
- *atpF-atp* + *psbK-psbI* + *trnH-psbA*: Orchidaceae (Kim et al. (2014))
- ITS2: Araliaceae (Liu et al. (2012b))
- ITS2; *matK*; *rbcL*: Zingiberaceae (Shi et al. (2011), Vinita et al. (2014))
- ITS2: Rutaceae (Luo et al. (2010))
- *trnH-psbA*: Polygonaceae (Song et al. (2009))
- ITS2: Rosaceae (Pang et al. (2011))
- *trnH-psbA*: Polygonaceae (Song et al. (2009))
- ITS: Nyssaceae (Wang et al. (2012))
- *atpF-atpH*: Lemnaceae (Wang et al. (2010))
- *trnL* and ITS2: Leguminaceae (Madesis et al. (2012))
- *psbA-trnH*: Lauraceae (Liu et al. (2012c))
- *matK* and *trnH-psbA*: Lamiaceae (Theodoridis et al. (2012))
- ITS2: Fabaceae (Gao et al. (2010a))
- *matK*: Juglandaceae (Xiang et al. (2011))
- ITS2: Asteraceae (Gao et al. (2010b))
- *matK* + *rbcL* + *trnH-psbA*: Arecaceae (Yang et al. (2012))
- *rbcL* + *matK*: Angiosperms (CBOL (2009))

VI. CONCLUSION

To replace the traditional identifying methods, DNA barcoding-based adulteration detection is still being developed. In contrast to DNA marker-based identification, which is better for validating the original species, chemical studies are used to find foreign substances and to check the quality of herbal medicines. To fully comprehend adulteration in herbal medications, DNA barcoding-based authentication must be combined with metabolomics, transcriptomics, and proteomics technologies. In order to include the DNA barcoding process in the standards for certifying herbal goods, this sector needs a strong scientific community. To test DNA barcoding-based herbal pharmacovigilance, close cooperation between national pharmacopeia authorities and university or commercial institutes specialists in DNA barcoding should be promoted. Along with chemical analytical techniques, regular DNA barcoding authentication might improve the quality and authenticity of the herbal business and make pharmacovigilance monitoring and signal detection easier. All herbal businesses will eventually adopt DNA barcoding-based authentication, with biomonitoring employing readily accessible barcodes to identify adulterants. As biological data is developing quickly, many DNA barcoding difficulties will also be overcome.

In the practical application of DNA barcoding in poisoning cases, it may be deemed appropriate to utilize genus-level identification. Through our rigorous analysis, we have derived that *rbcL* can serve as a primary marker in such cases. Furthermore, should the need arise, *ITS2* or *trnH-psbA* may be employed as a secondary marker to facilitate identification of the poisonous plants. This comprehensive investigation serves as a fundamental basis for the development of a dependable molecular approach that can be utilized to accurately discern the poisonous species from the vomit samples of poisoning cases.

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