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DNA barcoding: An effective molecular tool for species identification, molecular authentication and phylogeny studies in plant science research

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Abstract

DNA barcoding is a technique for identifying specimens using brief, standardised DNA segments. In a variety of fields, including phylogeny, ecology, population genetics and biodiversity, DNA barcoding has become a successful method for precisely distinguishing species. The method is straightforward, efficient in both time and money and accurate. The key to successful DNA barcoding is choosing the right DNA marker. Since the idea of a quick approach for species identification was first put up in 2003, the scientific community has been keen to realise the potential of DNA barcodes. Cytochrome c oxidase, I (COI) region of the mitochondrial genome is mostly recognised as a standard barcoding region in animals. Later, rbcL + matK pairing, with a 70 % discriminating efficiency, was suggested by the Plant Working Group (PWG) of the Consortium for the Barcoding of Life (CBOL) as the standard barcode in plants. Three conditions must be met for a gene region to be an efficient DNA barcode: it must have sufficient species-level genetic divergence and variability, it must have conserved flanking regions for the widest taxonomic use and for generating universal PCR primers and it should be long enough to facilitate current capability for sequencing and extracting DNA. Different combinations of plastid coding, non-coding and nuclear markers are utilised as supplemental markers to boost the degree of plant species differentiation. The reliability of different barcodes in distinguishing species varies among different groups of plants. As DNA barcoding approaches its twentieth anniversary, technologies are still being developed that make use of this resource, which is constantly expanding in a variety of biological disciplines. Plant DNA barcoding, which became a scientific advance during the last ten years, is frequently employed as a taxonomical aid in identifying species. It is a way of choosing genetic loci that identifies and distinguishes an organism's membership from specific species, variations or even intervarieties. It varies from molecular phylogeny, which identifies an unknown sample from an existing classification rather than identifying patterns of association.

Keywords

CBOL; DNA barcoding; DNA marker; rbcL; matK; ITS; trnH-psbA

Introduction

As a distinctive identifying marker to detect and classify species, DNA barcodes are gene sequences of between 400 and 800 base pairs (bp) that are

taken from a specified region of the genome (1-3). Using these brief DNA segments and techniques that have been authorised by international agencies, DNA barcoding is a tool for species identification that builds a worldwide record of living things (4-6). It is reported that the first accurate (100 %) identification of 200 closely related Lepidopteran species using the mitochondrial cytochrome c oxidase, I (COI) region (4, 5). The basic objective of DNA barcoding is to provide comprehensive online datasets of all known species. DNA barcoding fulfills the need for precise identification of species for the preservation and utilisation of plants. This method can help to mitigate some of the inherent problems with traditional taxonomic identification (7).

DNA barcoding has broader applications as it enables the researcher to identify species from any part of the plant, like spores, ovules, roots, or samples collected from the air, water, land or even processed products. The use of roots to reveal previously undiscovered information on the diversity of underground plants and the use of soil remnants to recreate previous vegetation and climates are some other aspects of DNA barcoding. Using genetic sequences provided by DNA barcoding, phylogenetic trees have been constructed for use in phylogenetic community ecology. The two main issues causing inaccuracy in plant identification by barcodes are pseudogenes and hybridization. Some biological phenomena that occur at different degrees, like heteroplasmy, paternal leakage, introgression, polyploidization, recent speciation, incomplete lineage sorting, polymorphism, error in specimen identification and incorrect taxonomy, also interfere with barcoding (8). Studies on DNA barcoding have been progressing day by day since its introduction (Fig. 1). Advanced techniques like Affordable high-throughput DNA barcoding technique using Microfluidic Enrichment Barcoding (ME Barcoding are proven to be efficient alternatives to traditional PCR and Sangers sequencing to create a vast number of plant DNA barcodes and expand barcode databases. Plant genome research has advanced more quickly owing to the development of high-throughput sequencing technology, especially in the area of chloroplast genomics. Primarily utilised in phylogenetics, breeding, domestication and conservation research, these plastome data have also been suggested as the “super-barcode” for plants. It has also been demonstrated that complete chloroplast genomes can effectively distinguish between closely related species. However, there are still certain issues to take into account when using “super-barcodes” for large-scale biodiversity research, such as data management and bioinformatic difficulties. Furthermore, because of variations in gene structure, length and organisation, whole chloroplast alignments between genetically distant populations can be challenging. Hence, researchers began to rely on Sanger’s sequencing-based barcoding for species discrimination, authentication or phylogeny studies of plants.

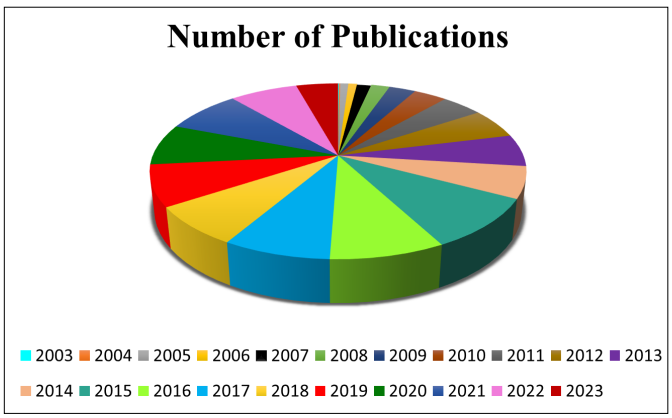

Fig. 1. Estimate on yearly increase in the published literature on DNA barcoding for last two decades. The pie diagram is based on the detailed literature searched in popular sources of scientific information viz. Pubmed, google scholar, and research gate. In August 2023, data spanning from 2013 to 2023 was gathered in preparation for the drafting of this article.

This review outlines the characteristics, benefits and drawbacks of the markers used to identify plant species as well as the suggested standard DNA barcodes for plant species. Also, the paper focuses on the operational procedures, process and data analysis followed in the DNA barcoding studies, in addition to its multiple positive impacts on taxonomy and biodiversity research or barcoding operational procedures and processes.

Software tools used for post-sequencing data analysis in DNA barcode studies

The laboratory method of DNA barcoding includes sample selection, DNA extraction and amplification using PCR and the success of PCR can be tested on Gel electrophoresis followed by sequencing of DNA (Fig. 2). Aligning sequences and assigning barcodes to sequences for enhanced identification are both included in data management. By amplifying a highly variable section of the nuclear, chloroplast, and mitochondrial genomes in plants, polymerase chain reaction (PCR) aids in finding a species' identity (9). Software tools like BLAST are used for sequence alignment. Numerous software programs, such as ABGD (10), Taxon DNA (11) and MEGA 7.0 (12), are used in the analytical examinations of sequenced DNA.

A widespread DNA barcode should be legitimate and its reference database should contain high-quality sequences. But in some cases, incorrectly labeled ones are also present in GenBank due to several possibilities, such as contamination; e.g., ITS primers are universal for plants and fungi, and sometimes they may be altered by endogenous fungal contamination. Extracted DNA gets confounded identification or by the low quality of the sample, DNA is misled. The actual aim of barcodes is to minimise this type of problem, promote clear and credible data for comparison, and make it easy to remove the labels from barcodes of legitimate data records. The most widely used methods for barcoding queries can be roughly divided into two categories: In contrast to clustering approaches like parsimony and neighbour joining, which are currently significantly slower and presumably are not practicable for a worldwide plant query system, similarity methods include BLAST, mega BLAST and FASTA, which all produce answers rather quickly. When managing some of the situations that emerge in barcoding, these strategies are particularly prone to errors and inconsistencies (13).

The frequency with which nearest neighbour distances exceed intraspecific divergences, suggesting the

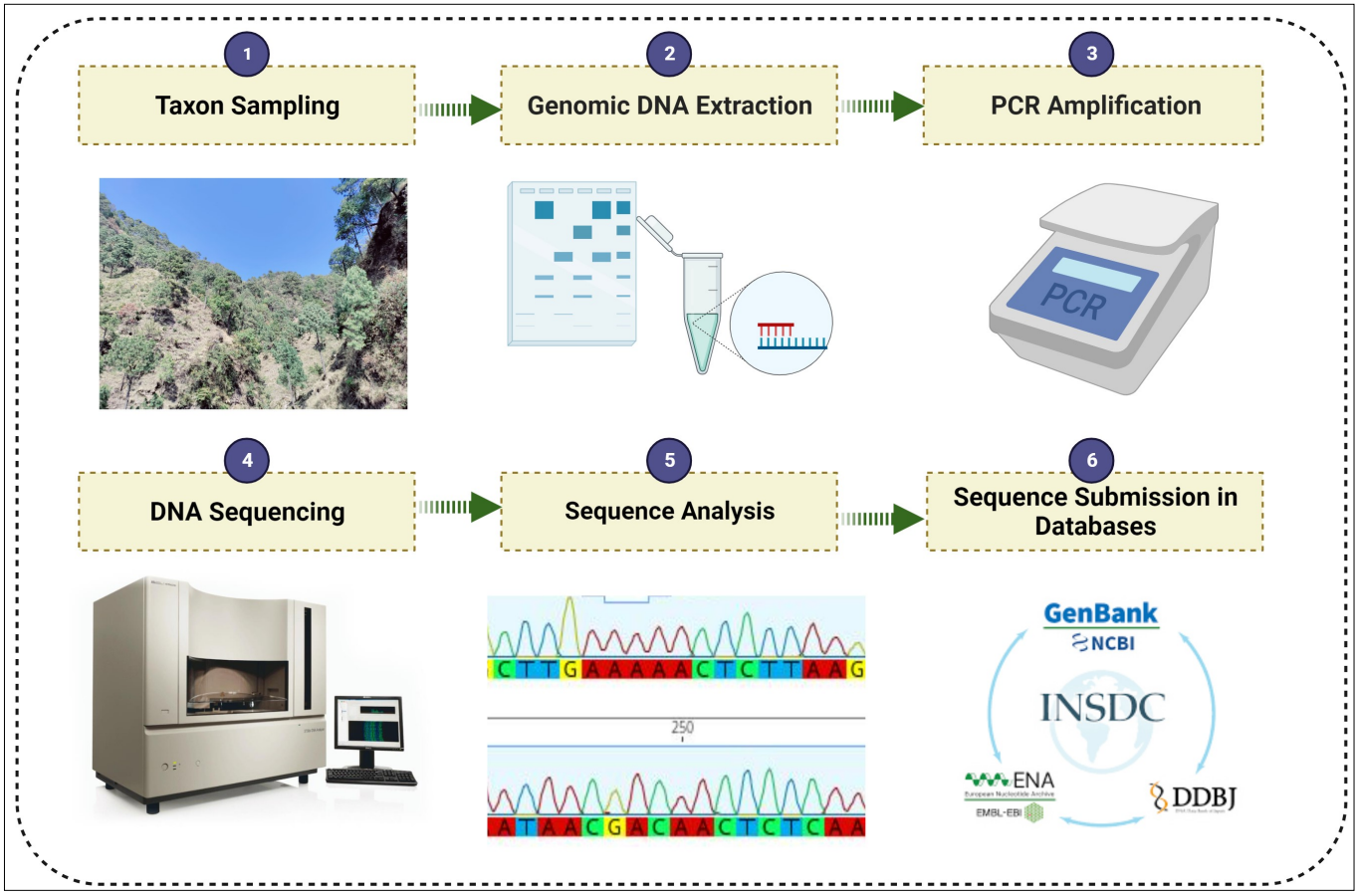

Fig. 2. Pictorial representation of various steps of DNA barcoding procedures for plant species.

occurrence of a barcode gap, is being determined by plotting maximum intraspecific divergence versus nearest neighbour distance. Additionally, variation in GC content among species is investigated using the 'Sequence Composition' tool on BOLD. In order to find out whether the number of samples taken from a genus or species is affecting the mean distances between nearest neighbours, linear regressions are carried out in Revolution R using the Picante and VEGAN packages (14). The mean closest neighbour distance and mean GC content are compared using the boot and Hmisc packages in Revolution (15). By using the Kimura-2-Parameter (K2P) distance measure, genetic distances can be estimated using the BOLD Management and Analysis System (16). An inadequate reference dataset may decrease the likelihood of a match but may potentially misidentify an unknown species. If a query's intraspecies variability exceeds or equals its interspecies variability or if it more closely resembles the barcodes of another species than the one to which it actually belongs, the query may be incorrect (17).

Common loci used for plant genome studies

The cytochrome c oxidase I (COI) region of mitochondria, widely known as a characteristic barcoding region, can be used to identify animal species (18). There are not enough variations in the COI among various plant groups to be used for plant identification (19). There are numerous variations in the entire plastid genome as there are in the COI location in animals and it is widely used in plant DNA barcoding (20). The chloroplast barcode regions can distinguish between closely related plants with greater diversity since it is substantially longer (between 110 and 160 kb) than other DNA barcodes (21). The large copy number, conserved structure and variety of substitution rates among genes, introns and intergenic spacers make the chloroplast genome ideal (22). The utilisation of 2 or more barcoding loci is necessary to achieve the level of species discrimination and universality for plants because a single locus combining both features is still not known (23). The "Consortium for the Barcode of Life (CBOL) Plant Working Group" suggested two coding areas from the chloroplast genome, rbcL and matK, as a "core barcode" for plants in 2009 (24). This "core barcode" was to be extended with additional parts. There are reports for the assessment of 7 markers—trnH-psbA, matK, rbcL, chloroplast RNA polymerase subunit (rpoC1), ycf5, ITS2 and ITS—from different species of medicinal plants. In addition to DNA sequences, each plant that is DNA barcoded must also have a herbarium record in order to build superior databases. Users of the data should also have access to information on the DNA sequences' quality, the primers used and the trace elements. For each species, many individuals should have their DNA barcoded in order to check for flaws and account for intraspecies variation (25). It is becoming clearer from several studies that DNA barcoding has improved other conventional scientists' access to and comfort with the Linnaean taxonomy system. The core process will be better understood with the support of current DNA barcoding research, which will also show applicability for this strategy in several fields. First and foremost, these methods give conventional taxonomists a wonderful chance to increase the precise inventory of the variety of ecosystems and plant life on Earth (26).

The ideal DNA marker should be appropriate for a variety of taxa, have significant interspecific diversity and be highly preserved within the species. It should be easily amplified by PCR using just one primer pair and should also be able to be sequenced in both directions with the least amount of human alteration (27). Common loci used in plant DNA barcoding studies are as follows:

The rbcL region

The plastid genome's ribulose 1, 5-bisphosphate carboxylase/oxygenase large subunit coding region (rbcL) is 600–750 bp long. It is the most abundant protein on the earth, with 476 amino acid residues (28). The gene for ribulose 1, 5, bisphosphate carboxylase, or rbcL, can identify genera-level evolutionary links. The rbcl region has the potential for plant barcoding (29). The functional locations and conserved sections of this protein have been identified by structural and mutational research. The chloroplast gene, rbcL, has been utilised to build the phylogeny of plants. This gene segment is readily amplifiable and the phylogeny of angiosperm and other photosynthetic plants at family and subclass levels was effectively constructed using it (30). Therefore, it is not particularly useful for making species-level distinctions. Nevertheless, rbcL is still widely employed for plant barcoding despite its drawbacks because of the abundance of readily available data and the simplicity with which the whole gene sequence can be recovered. rbcL alone does not have the necessary characteristics of a barcoding locus (31). rbcL is not a good marker for distinguishing between organisms at the species level because there is variation above the level of the species and little variation at the level of the species; however, it can be used in conjunction with other plastid or nuclear loci to provide accurate identifications (32). Despite lacking a number of essential characteristics, rbcL enables for accurate identification when combined with another plastid or nuclear marker (33). rbcLaF and rbcLr590 were used for the initial PCR in a variety of species and were successful (25).

The matK region

With applications in plant molecular systematics and evolution, the matK gene, previously referred to as orfK, is a 1500 bp long region found within the intron of the chloroplast gene trnK. Its 5' region is highly variable compared to the conserved 3' end (34). The chloroplast lysine tRNA (trnK) gene is located next to the matK core region. This gene encodes the maturase K protein, which facilitates RNA editing. The CBOL Plant Working Group (2009) claims that the matK barcoding area was chosen due to its extensive diversity and ability to distinguish across species (24). Researchers have reported its high rate of substitution and the existence of conserved areas (34). The maturase protein, or matK, is a degenerated version of the reverse transcriptase enzyme. One of the plastome's rapidly developing protein-coding sites is this marker (35). Despite the fact that it commonly displayed discrepancies between forward and backward readings compared to other coding areas (36). The pace and kinds of nucleotide changes in the gene, as well as the utilisation of sequence variation in building phylogenies from the level of the tribe to that of the division, are the features of this region that are being examined (37). Primers developed from matK loci have proven to be powerful markers in ferns (38). Researchers assert that matK has a low transition/transversion rate, an appropriate length, clear interspecific divergence and a strong evolutionary pace (29). Unfortunately, using the primer sets that are currently available, matK is difficult to uniformly amplify. In later studies, it is reported that the matK gene was successfully amplified in all 1667 samples of angiosperm plants (39). However, another study is reporting that the discriminating rates of various taxonomic groups present an additional difficulty. Only 56 % of the 92 species from 32 genera that sought to classify were successfully identified using the matK barcode (32). These studies demonstrate that the matK barcode by itself is frequently insufficient as a universal barcode.

The trnH-psbA region

Most plants include a non-coding spacer called trnH-psbA, which exhibits several characteristics that would make a good barcode between tRNA-His and photosystem II protein D1. In the intron of the chloroplast DNA, the trnH-psbA has amplicons that are 318 to 820 bp in size with a high degree of insertions and deletions as well as sequence divergence. Its length variance is mostly caused by minor, dispersed insertions and deletions that do not appear to have a taxonomic pattern (40). According to the existing reports, almost all angiosperms can likely be multiplied by a single trnH-psbA primer pair (41). All species in plant group members like *Dendrobium*, Pteridophytes and *Hydrocotyle* may be recognised by the trnH-psbA region (19). Since some plant ancestry has multiple inversions, the trnH-psbA barcode has the disadvantage of overestimating genetic variation and leading to incorrect phylogenetic classification (42). These mononucleotide repeats have the additional drawback of prematurely terminating sequencing reads, which makes it difficult to recover longer parts without internal sequencing primers. Its use as a core barcode is further prohibited by the widespread incidence of insertions within species, large poly structures that make sequencing challenging and relatively short lengths (2). The trnH-psbA can be coupled with other markers, such as rbcL and matK as a multi-locus system in order to obtain adequate resolution (41).

The ITS region

The nuclear ribosomal DNA's internal transcribed spacer (ITS) located between 16S and 23S rRNA genes and its specific ITS regions may serve as possible barcodes (43). It is solely suggested by CBOL as an additional locus. As a potential universal DNA barcode in eukaryotes, it appears to be one of the strongest contenders. It is not permissible for an ITS area to serve as a core barcode marker for the following reasons: 1) variation in amplification and sequencing success, 2) its concerted evolution is insufficient and 3) as the ITS region is found in both fungi and plants, the fungal ITS sequence also gets amplified (44). It is advised that a core plant DNA barcode be created using the ITS of nr DNA, one of the DNA markers utilised most frequently in

plant phylogenetic and DNA barcoding research, which is suggested as a fundamental plant DNA barcode (45). It has been suggested that the ITS2 sequence is suited for DNA barcoding applications in plants due to its shorter length (300 bp) (46, 47). Due to ITS2's excellent discrimination strength in both post-DNA barcoding and High-resolution melting analyses (HRM), it was shown to be a more credible DNA barcode than rbcL. Along with separating counterfeit goods from genuine ones, the DNA barcode and HRM showed how sensitive these approaches are in detecting them (48). Due to its conserved sequence, ITS 2 can help alleviate issues with ITS area amplification and sequencing. Later, ITS 2 was acknowledged as a cutting-edge universal barcode for a variety of plant species. The ITS region is divided into 3 partitions: the ITS1 partition, the 5.8S partition and the ITS2 partition. The 5.8S region is the least frequently employed for phylogenetic analyses and DNA barcoding because it is too well preserved to include a significant number of relevant sites (49). According to the China Plant BOL Group (2011), the core barcode for seed plants should include the ITS/ITS2 regions (50). It is trustworthy, exhibits considerable intraspecific variation and is more discriminatory at lower taxonomic levels. There is a report regarding studies demonstrating that there is nrITS discrimination between plant species that share plastid haplotypes (51). However, CBOL has designated ITS as a supplemental locus because of constraints such as improperly regulated evolution, fungal attack, amplification, and sequencing concerns (19, 24). The study of the discriminatory power of ITS2 has shown a 90 % potential at the species level while evaluating about 4800 species from 800 genera (46). Several reports are there regarding successful species discrimination and adulteration studies using ITS (51, 52).

Multi locus barcodes

matK, rbcL and ITS are single locus DNA barcodes. As single locus modifications are insufficient for significant species discrimination, many researchers have proposed a multi-locus approach (24, 40). rbcL + trnH-psbA, rpocL + matK + trnH - psbA, or rpocL + rpoB + trnH-psbA are just a few examples of the many combinations of plastid loci that have been discovered (40). Researchers revealed that a 2-locus combination has been suggested as a high resolution marker (53). According to different researchers, different groups of plants demand different markers. In the evaluation of several genera of land plants, it was observed that different multi-locus combinations comprising 4-7 markers showed only a slight increase in success rates (32). The TrnH-psbA+ rbcL, a 2-locus combination, acts as a universal barcode for land plants with sufficient species discrimination (40).

DNA that has been transferred from ancestor plastids into mitochondrial genomes is known as mitochondrial plastid DNA (MTPT). Research on MTPTs has showed that DNA markers like rbcL, atpB, rpl2, rpl23, psaA, psaB, psbC, psbD, rpoB, rps7, rps12 and ycf2 were all found in the mitochondrial genomes of more than 20 plant species and can cause misidentification of species during DNA barcoding. Therefore, the use of multi-marker combinations was suggested to avoid such barcoding contradictions (54).

Data analysis of DNA barcoding

The use of DNA barcoding as an identifying method requires the development of top-notch reference databases of sequences (19). The procedure entails classifying the DNA of recognised species in a barcoding library and comparing the DNA of unidentified species to the genetic information stored in the library (40). For species identification and taxonomic clarification, the database can be accessed online (55), namely through the NCBI GenBank and the Barcode of Life Data (BOLD). Each DNA sequence must be linked to the plant specimen from which it originated as well as the time, location and person(s) responsible for collecting and identifying it. Creating a herbarium voucher for each DNA sample is also important, but in some circumstances, especially for rare and endangered species, a photograph can be adequate (56). A project management tool called BOLD enables the storage of DNA sequences with trace files, herbarium specimen images and pictures to submit information to the Barcode of Life Data system in addition to GenBank, which serves as a repository for DNA sequences and makes all data accessible to the public (57). It's crucial to record the lab procedures used to generate a sample so that the data's ultimate users can access the details of primers, trace files and quality parameters for its DNA sequence (56). The ITS2, matK and rbcL barcode markers underwent bidirectional sequencing. The acquired sequences can be put together and aligned in MEGA X and Geneious Prime 2021 (accessed on 27 December 2021) using the Muscle Algorithm (12). With the help of the online sequence submission tool "BankIt", the sequences can later uploaded to NCBI GenBank and access numbers for all the analysed barcode markers can be acquired (58). Utilizing the NCBI Gen-BLASTn Bank's programme, the sequences can put through a taxonomic examination to find homologies between the fragments. Additionally, unsupervised OTU selecting techniques can be used. MEGA can use for the phylogenetic analysis, while ABGD and ASAP can use for the OTU evaluation (59). Supervised Machine Learning (SML) techniques can use in conjunction with the unsupervised OTU selecting techniques to identify divergent taxa. With the help of the FASTA to WEKA converter, the aligned datasets can be converted to the WEKA's necessary file format (60). The sites with gaps and missing values can be taken out of the data set for the pairwise distances analysis (complete deletion option). The neighbour-joining (NJ) approach is used to create phylogenetic trees in accordance with the Kimura 2-Parameter (K2P) model, which is being evaluated using MEGA 7.09 (61). In these trees produced using the NJ techniques, the clade dependability can be investigated using bootstrapping, which determines the support values of the clade nodes through 1000 repeated sample tests. Librado and Rozas, performed polymorphic sites, genetic diversity indices and neutrality tests (62). Researchers have also successfully created a few DNA barcode reference libraries for natural medications. For example, the first Medical Materials DNA Barcode Database (MMDBD), which has 62011

sequences from 2111 species (http://www.cuhk.edu.hk/icm/mmdbd.htm) was established in 2010 (63). Several of the sampled species were not in the DNA BOLD or PubMed databases. This suggests that the organism is not currently included in any database since the alignment of these samples was less than 90 %. The identification of these organisms at the species level is not possible, yet they may be able to be placed in a certain genus. An official identification for these materials will require collaboration with a taxonomist. It would also be important to create a voucher that could be added to the DNA BOLD database (64). The main databases providing reference barcodes are GenBank, the DNA Databank of Japan (DDBJ) and the European Molecular Biology Laboratory (EMBL) (65).

Future perspectives of DNA barcoding

Living things have frequently been barcoded using DNA. The BOLD system has produced more than one million barcodes thus far (DNA barcode data depository). IBOL speeds up the barcoding process even further by building massive libraries that make it easier to correctly identify living things. Barcoding plays an important role in taxonomic research, but it also facilitates significant cooperation and communication across many scientific groups, particularly taxonomists, population geneticists, phylogeneticists and applied biologists (26). The critical stage in constructing a high-quality reference library of DNA sequences of all known plant species on Earth will be the collection and compilation of well-identified sample sets that are acceptable for DNA sequence analysis. Taking into account technological developments, these sample sets will be reasonably simple to resequence for new loci once they have been assembled (19). Plant barcoding necessitates additional markers like trnH-psbA and ITS in addition to the essential DNA barcodes rbcL and matK. Furthermore, DNA barcoding is sometimes imprecise and necessitates the use of extra group-specific markers in species that are closely related and cryptic. However, molecular phylogeny, population genetics, evolution and the field of ecology, biological safety and food-related product regulation are all significantly impacted by DNA barcoding. The resolution of cryptic taxa may be done quickly, accurately and affordably using recently developed technologies like metabarcoding in combination with high throughput sequencing (HTS). All gene variants can be sequenced using next-generation sequencing (NGS), even in the presence of microsatellites, homopolymeric areas, insertions/deletions (indels) and single nucleotide polymorphisms (SNPs). NGS is a priceless instrument with a wide range of applications in the DNA barcoding field because it can efficiently sequence hyper variable markers. It also sheds light on the shortcomings of earlier research and methods. Unlike Sanger sequencing, NGS technology can sequence single DNA molecules in enormous parallel, producing high-throughput data. All areas and variants of the gene are read by parallel sequencing, which eliminates the difficulty of working with multiple templates and enables the discovery of contaminants, pseudogenes and allelic variation within organisms. While one of the first applications of NGS technology was whole-genome sequencing, several techniques have since been developed to produce multilocus sequence data for a range of purposes, including phylogenetics and genotyping. Direct Sanger sequencing of DNA barcode amplicons, which is the method used in the majority of DNA barcoding procedures, is hindered by the requirement for a relatively high yield of target amplicons, nuclear mitochondrial pseudogene coamplification, sequence confusion with intracellular endosymbiotic bacteria (like Wolbachia), and intraindividual variability (heteroplasmy). Failures in Sanger sequencing attempts or unclear DNA barcodes produced can result from any of these circumstances. Because of its multiplexing ability, NGS is an affordable DNA barcoding technique.

A further effective method for defining biological groups from samples of terrestrial and aquatic habitats is environmental DNA (eDNA) metabarcoding, which combines universal DNA barcodes with HTS (66). According to the China Plant BOL Group (2011), the advantages of employing nrDNA ITS for species resolution are expected to exceed the increase in our capacity to identify between different plant species (50). In order for experts to make taxonomic determinations and incorporate the specimen information into floras or species descriptions, the herbarium provides an organisational framework for sharing specimens with other institutions. Curators and systematists who work in herbariums can identify uncommon or unusual species and mark them for field collections or observations (67). One of the most difficult tasks for the upcoming ten years is to fill the world's plant DNA barcode collection. As they frequently contain well-verified identifications, vouchered collections and individually tagged specimens, these ecological monitoring plots offer an invaluable resource for creating the plant DNA barcode library (68). Metabarcoding, also referred to as environmental DNA, is an innovative variation of DNA barcoding that employs genetic markers to identify living entities in environmental samples (69). Due to technological advances in the recovery, amplification and sequencing of minuscule DNA fragments and even damaged ones, the field of metabarcoding is expanding quickly. Furthermore, creating new bioinformatics tools that can translate a list of DNA sequences discovered in a sample into a list of species that can be identified is difficult, but this issue could ultimately be resolved (68).

Challenges of DNA barcoding

Taxonomic congruence

For the DNA barcoding proposal to be successful, there must be a strong correlation between the barcodes and the group's species classification. Although it may be more important, there should not be any overlap in the absolute barcode sequences of distinct species. The ideal condition would be for interspecific variation to dominate intraspecific variation. These prerequisites have so far been accomplished in fewer studies on plants than on animals. This may be brought about by reasons such as hybridization and polyploidy, inadequate sorting of ancestral polymorphisms, faulty taxonomies and poorly described species, in addition to the putative plant DNA barcode areas'

lower variability and the less well-defined borders between plant genomes (32).

Implications of using plastid regions

Given that the plant DNA barcode is made up of 2 plastid regions, which are key factors in species identification, hybridization and polyploidy may have a greater impact on species identification in groups with high levels of apomixis.

Hybridization

In the majority of angiosperms, back cross after hybridization can cause introgression of plastids from one maternal parent into another. But in conifers, it is from the paternal parent (70). The subsequent generation may contain the plastid genome of the second species but a more or less "pure" nuclear genome from the first species following multiple generations of backcrossing. The "erroneous" response will occur if the common plant barcoding loci (or any other plastid loci) are used. However, certain populations that were previously believed to be pure examples of one species include the plastid genome of a distinct species, according to morphological and nuclear DNA investigations.

Polyploidy

More than 70 % of angiosperm species may have experienced one or more polyploid occurrences in the course of their evolutionary history, making polyploidy a widespread phenomenon. This can make plastid genome-based barcoding problematic (71). The plastid genome of recently generated auto polyploids will be identical to that of the parental diploid. Barcoding won't be able to detect the difference between the diploid and the polyploid lineages that have been created due to reproductive isolation. Also, barcoding alone will not be able to distinguish between the plastid donor parent and the polyploid since newly created allopolyploids will only contain the plastid genome of one of the progenitors. When sufficiently intact specimens are available for examination, morphological study combined with barcoding may be able to solve the allopolyploid problem, but auto polyploids and parental diploids are frequently difficult to distinguish on the basis of gross morphology and have been treated as cytotypes of the same species (72). The usage of "traffic light" systems, such as those found at https://www. isisintegration.com, might be made possible (43). In such a system, a green light indicates that the DNA barcode completely matched another species' barcode that was known to be unique. A red signal would indicate that this particular taxon does not work with barcoding, while an amber light would say, "Continue with care." There may not be a match in the current database, the supplied sequence is of low quality or there are many matches, among other possibilities, for this. The standard and completeness of the barcode database, as well as knowledge of genetic trends, will surely affect how accurate such a traffic light system is (1) Key challenges such as collection of specimens for rare or ephemeral species (2) limited availability of taxonomic knowledge required for accurate identification of reference specimens and (3) issues with amplifying and matching barcode data at the molecular level. Low populations of uncommon species can also be troublesome because it is impractical to collect them when sampling could have a detrimental impact on population survival; as a result, workers must spend valuable time hunting for alternative sources of plant material (73). With the help of well-built barcode libraries, end users may classify plant material according to species or genus utilising tissue pieces (such as a single leaf, root or stem) that are hard to identify using conventional taxonomic keys. However, because barcode matches are based only on sequence data and not morphology, end users are unable to identify flaws in the initial species identification. Nevertheless, digital photos and herbarium specimens can be checked again in the future to confirm identifications. Therefore, it is crucial that reference specimens are identified correctly (74). It is possible to construct phylogenetic hypotheses for taxa that have some barcode data utilising incomplete or skewed molecular matrices; nevertheless, a lack of sequence data may increase phylogenetic uncertainty and result in unresolved nodes (i.e., polytomies). This lack of phylogenetic precision will have various implications depending on the problems to which the phylogenetic theories are applied (75).

Applications of DNA barcoding

The advances in sequencing techniques have enhanced the applicability of DNA barcoding for various biomonitoring applications (76). Species identification has been the main application of DNA barcodes. By extending the diagnostic range to include all stages of an organism's life history, unisexual species, damaged specimens, stomach contents and fecal samples, barcoding is a research technique that helps taxonomists identify different species. DNA markers have quickly risen to the top as the most popular tools for genetic evaluations of crops and cultivars, in addition to tracking and verifying the unprocessed ingredients used in food (77).

Identification and discrimination of species

The DNA barcoding method is used to determine the identity of biological items, their purity and the identification of controlled species, including invasive and endangered species (78). Especially for undescribed and cryptic species, barcoding serves as a biodiversity discovery method that can help to identify species that may be novel to science. DNA barcodes are currently being used to address significant ecological and evolutionary problems, such as the species composition of plant communities and the degree of specialisation in tropical versus temperate zone herbivores (40). Compared to traditional morphological identification, DNA barcoding can distinguish between species and guarantee that a product is legitimate and not a duplicate; it is helpful for identifying medicinal plant (MP) species for conservation and use (59). Based on phylogenetic analysis and genetic distance, a 2-locus combination of matK + ycf1 and ndhF + ycf1 or ndhF and ycf1 alone has proved to be markers for the identification, conservation and utilisation of members of Orchidaceae (79). For *Clerodendrum* species, a 2-locus combination of ITS2 + matK has been proposed as the core barcode (80). Simi-

larly, identification at the level of family, species as well as subspecies level has been done using DNA barcoding (81) (Supplementary Table 1).

Traditional biologists have the ability to recognise the species of vectors harming humans, animals and crop plants most severely and to comprehend the mechanisms of management (26). DNA barcoding is a method for monitoring and regulating the illegal trade of natural resources. For the purpose of barcoding hardwood trees, scientists have created a database that serves as a reference barcode library. This will help to further improve natural resource management and conservation techniques (82). Controlling the spread of water-borne illnesses requires careful examination of any possible microflora in drinking water. DNA barcoding has made it possible to identify microbes quickly while less expensive and in minimal time by avoiding the need for elaborate methods. Environmental authorities utilise DNA barcoding to assess the water's quality and guarantee that the water being delivered is fit for human use (26). The identification of many illnesses and disorders has been aided by DNA barcoding. By recognising foreign species, DNA barcoding has aided in medicine authentication and improved biosecurity. Species barcoding is thought to be a reliable and trusted identification strategy for determining the status and conservation of biodiversity at the level of species and populations (43). The use of DNA barcodes as a tool has significantly increased cooperation between ecologists and systematists, who study patterns of association and species interactions as well as the identification of species and their evolutionary links (83). Ecologists made great strides with the development of Phylomatic (84), a tool for calculating phylogenetic trees for plant communities. Genetic markers known as "DNA barcode forensics" are being used to safeguard endangered species from being trafficked illegally, to ensure the identification and purity of commercial products and to track how locals use forest plants. One of the motivating factors behind the current uses of DNA barcode technologies in different locations of the world is the need for an efficient, trustworthy and affordable instrument for the detection of illicit wood products (68). The use of these identifiers are in the identification of wild-collected goods, including animal items, that are sold in marketplaces throughout the world (85). Today, it is common practice to utilise DNA barcodes to identify the species that cause bird attacks on commercial aeroplanes (86). DNA barcoding, a tool primarily for species identification, can be applied to biodiversity conservation in 2 ways: 1) to increase the accuracy and speed of biodiversity monitoring both before and after conservation actions and 2) by providing information to help determine estimates of phylogenetic diversity for allocating conservation priorities (87).

Identification of diseases and pests

By accurately identifying pests, DNA barcoding assists in pest control and helps to reduce costs caused by agricultural pest infestation. DNA barcoding may be used to quickly create containment and suppression tactics for disease incursions before their numbers get out of control (88). Traditional biologists have the ability to recognise the species of vectors harming humans, animals and crop plants most severely and to comprehend the mechanisms of management (26). DNA barcoding is a method for monitoring and regulating the illegal trade of natural resources. For the purpose of barcoding hardwood trees, scientists have created a database that serves as a reference barcode library. This will help to further improve natural resource management and conservation techniques (82). Controlling the spread of water-borne illnesses requires careful examination of any possible microflora in drinking water. DNA barcoding has made it possible to identify microbes quickly while less expensive and in minimal time by avoiding the need for elaborate methods. Environmental authorities utilise DNA barcoding to assess the water's quality and guarantee that the water being delivered is fit for human use (26). The identification of many illnesses and disorders has been aided by DNA barcoding. Numerous fungi infections have dramatically grown, including aspergillosis, candidiasis and cryptococcosis. Fungal DNA barcoding, which traditionally depended on a single barcoding area, is frequently used to identify these infections. However, only a few of the fungi were successfully identified. The translational elongation factor 1 (TEF1), a supplemental barcoding area that ensures efficient and accurate detection of invasive fungal infections, has most recently been introduced to fill this gap (89). By recognising foreign species, DNA barcoding has aided in medicine authentication and improved biosecurity.

Molecular traceability of agricultural products

Agricultural products go through extensive processing and manufacture before they are distributed to the market. These activities modify plant structure, making it difficult to identify most agricultural products using visual characteristics. To circumvent this limitation, the study of proteins and/or DNA is increasingly utilized as the primary tool for plant tracking. DNA barcoding is a genuine functional tool for agricultural products' molecular traceability because the majority of minor crops have not yet been identified with specific markers like SSR or SNP that would enable a precise DNA fingerprinting system. Reports are there regarding the application of DNA barcoding using nuclear and plastid regions for authentication of minor crops like spices, aromatics plants, legumes, herbal infusions, fruits and also for major crops (90).

Identification of plants causing intoxication

DNA barcoding is a useful technique for identifying the plant species that are responsible for consumer poisoning or intoxication (91). ITS1 and ITS2 primers were successfully designed for the identification of toxic substitutes of the Apiaceae family. Such DNA barcode regions have a size of 140 and 80 bp respectively, termed as mini barcodes (92). A reference DNA barcode library for 100 poisonous plant species has recently been created employing rbcL DNA barcodes (93).

Detection of adulterants in food commodities and herbal medicines

The ability to identify contaminated food products preserved once was made possible by DNA barcoding (http://www.dnabarcoding101.org). The detection of adulterants in adulterated spices is a very difficult process; however in

the literature, it has been discovered that DNA barcoding is helpful in locating unethical product substitution. This method has become a reliable tool for identifying tainted processed seafood and meat products (94). Species from the same genus, less expensive imitations with a similar colour or appearance or crop-based goods like rice or wheat flour were frequently used to adulterate spices (95) (Supplementary Table 2). Detection of spices as well as their adulterants has been done by many researchers (52). Similarly, the authentication of medicinal plants, including laxative-yielding plants like Cassia and *Senna,* has been done (96). With the aim of authenticating herbal items, a standard Biological Reference Materials (BRM) herbal DNA library has been created, including 180 species of plants (97). A standardized method for the application of DNA barcoding in the detection of adulterants has been given by Howard *et al.,* using *Hypericum perforatum* L. According to researchers, the ITS2 intergenic spacer is useful for detecting medicinal components in processed medicinal plants from different families (98).

Phylogenetic diversity analysis and plant biodiversity conservation

Prioritising and managing the protected area successfully requires an assessment of species variety and richness. Phylogenetic diversity is used to evaluate taxonomic divergence in various species (99). The improvement and acceleration of phylogenetic diversity analyses are made possible by DNA barcoding. Comparing DNA barcode-based data on phylogeny with a larger evolutionary repository is required in this (68). Plant DNA barcodes have proven to be essential for assessing species richness in previously unexplored regions, such as the tropical forests of Australia (100). Reports are there for authentication and development of conservation strategies for threatened species of the genus *Decalepis* of the Apocynaceae using DNA barcode studies (44). With the help of this innovative technique, it is possible to simultaneously identify the majority of species from a specific biotope and assess the biodiversity of ecosystems with limited accessibility. Molecular methods and DNA barcoding may allow scientists to precisely recreate the past habitats of flora and fauna (101).

Specific barcoding is thought to be a reliable and trusted identification strategy for determining the status and conservation of biodiversity at the level of species and populations (43). The use of DNA barcodes as a tool has significantly increased cooperation between ecologists and systematists, who study patterns of association and species interactions as well as the identification of species and their evolutionary links (83). Ecologists made great strides with the development of Phylomatic (85), a tool for calculating phylogenetic trees for plant communities. Genetic markers known as "DNA barcode forensics" are being used to safeguard endangered species from being trafficked illegally, to ensure the identification and purity of commercial products and to track how locals use forest plants. One of the motivating factors behind the current uses of DNA barcode technologies in numerous different locations of the world is the need for an efficient, trustworthy, and affordable instrument for the detection of illicit wood products (68). The use of these identifiers in the identification of wild-collected goods, including animal items, that are sold in marketplaces throughout the world (85). Today, it is standard procedure to employ DNA barcodes to pinpoint the species responsible for bird attacks on commercial aircraft (86). DNA barcoding, a technique primarily used for species identification, can be used to support biodiversity conservation in two ways: 1) by improving the precision and efficiency of biodiversity monitoring and 2) by providing data to support estimates of phylogenetic diversity for determining conservation priorities (87).

Conclusion

DNA barcoding is a rapid succession procedure that enhances species identification by comparing the barcodes from the DNA barcode library. DNA barcoding makes the analysis of samples easy and helps with classification. The main aim of DNA barcoding is to prepare a barcode library for reference and taxonomical identification. The barcode library paves the way for the reference of samples and is open to other researchers. It also gives clarity on the species' discovery and it aids in certain other impacts in the biological field. Phylogenetic analysis, ecological forensics, medicinal plant authentication, endangered species conservation, helping in the discovery of new species in particular plant groupings and performing identifications when taxonomic knowledge is lacking are just a few of the many applications that find great utility in DNA barcoding. Physical procedures for the detection of adulterants are easy and inexpensive, but the main disadvantages are the inaccuracy and repeatability of results. This emphasises the need for precise molecular authentication techniques. Numerous taxonomists might be replaced by a DNA barcoding technician for basic identification, freeing up taxonomists to focus on locating reference specimens to build trustworthy databases. DNA barcoding is now a widespread practice across the entire tree of life and is a recognised and integrated tool in the study of biodiversity. Even though the discriminating efficiency of single locus markers in plants is less than that of animals' multiple locus combinations, it gives an enhanced resolution. Different combinations of markers show different degrees of identification efficacy in various groups of plants. In biodiversity research, the potential for accurate identification or library preparation could be considerably increased by using the next-generation sequencing technique for DNA barcoding.

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Authors' contributions

DN conceived, designed and edited the manuscript, critically reviewed and approved the final content of the manuscript. AP supported in the formatting of the manuscript. KC contributed for the Fig. 2 of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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Supplementary data

Table 1. Reports on successful identification of plant species using DNA barcoding techniques and the primers used.

Table 2. Application of DNA barcodes and DNA sequence-based markers in the authentication of herbal medicinal ingredients.

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