

REVIEW ARTICLE

PLANT DNA BARCODES: APPLICATION IN HERBAVIGILANCE

Yogesh Murti^{a,b}, Sonia Singh^{a,b}, Jyoti^c and Krishn K. Agrawal^{d*}

(Received 31 December 2020) (Accepted 09 May 2022)

ABSTRACT

Globally, 80% of the world population uses the herbal medicines. Wellbeing is a key guideline in arranging natural prescriptions and herbal products for medical care, and a basic segment of value control. Among consumers, there is a widespread misconception that “natural” always mean “safe.” However, certain adverse occurrences recorded in relation with herbal products are due to quality issues. Ongoing improvements in sub-atomic plant ID utilizing DNA arrangement information empower exact recognizable proof of plant species from herbal prescriptions utilizing characterized DNA markers for identification of species by the application of short sequence of genome by DNA barcoding. It depends on three unique cornerstones of current scientific classification, including molecularization (i.e., the utilization of the fluctuation of sub-atomic markers as discriminator; computerization (i.e., the non-repetitive rendering of the information utilizing informatic; and normalization (i.e., the augmentation of a way to deal with general gatherings of not carefully related life forms). DNA meta-barcoding is a specific application of this field which includes several organisms. Both the techniques are highly demandable for the rapid authentication of herbal drugs. The implementations for a strong and exhaustive barcoding framework are advocated and promise a scope of potential advantages, both monetarily and environmentally. The age and curation of DNA reference information of natural medications should turn into a public opinion need and perceived as a basic science foundation of the pharma world.

Keywords: DNA barcoding, metabarcoding, herbal drugs, authentication, herbavigilance

INTRODUCTION

The word pharmacovigilance is derived from the Greek word “pharmakon” and “vigilare”, which means keep an eye on the drug, and the term herbavigilance is a hybrid word that means keep an eye on herbal medicines or herbal derived products¹. It is a branch of research that focuses on acquiring, identifying, evaluating, tracking and preventing adverse drug outcomes in pharmaceutical goods. In addition to this, it also deals with detecting the hazards associated with pharmaceutical products and reporting any harm that has happened due to the pharmaceutical products and services². Numerous patients utilize natural enhancements as another option as well as subordinate to their recommended medication (Fig. 1). The populace

favors natural items since they are characteristic, and they are accepted because they assumed that they are safe and have fewer side effects than synthetic drugs³. Then again, plants contain various secondary metabolites that produce physiological effect in the body. On the off chance that a natural item is professed to have a beneficial effect on a specific wellbeing condition, at that point, it must be fit to change the physiological framework, i.e., apply a pharmacological reaction^{4,5}.

Consequently, it might probably have adverse effects also. In contrast to a single isolated active chemical moiety, the herbal products/medicine are the complexes of secondary metabolites that are affected by several factors like genotype, plant part (leaves, stems, root, root bark etc.), geographical origin (climate, soil), harvesting, extraction and storage time. In addition to these factors,

^a Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Research, GLA University, Mathura - 281 406, Uttar Pradesh, India

^b Department of Pharmacognosy, Institute of Pharmaceutical Research, GLA University, Mathura - 281 406, Uttar Pradesh, India

^c Department of Pharmaceutics, School of Pharmaceutical Sciences, Maharishi University of Information Technology, Lucknow - 226 013, Uttar Pradesh, India

^d Department of Pharmacology, Faculty of Pharmacy, R. B. S. Engineering Technical Campus, Bichpuri, Agra - 283 105, Uttar Pradesh, India

*For Correspondence: E-mail: krishn.singhal88@gmail.com

<https://doi.org/10.53879/id.60.01.12824>

combinations of herbs with other herbs and medicines also affect the quality of the herbal product^{6,7}.

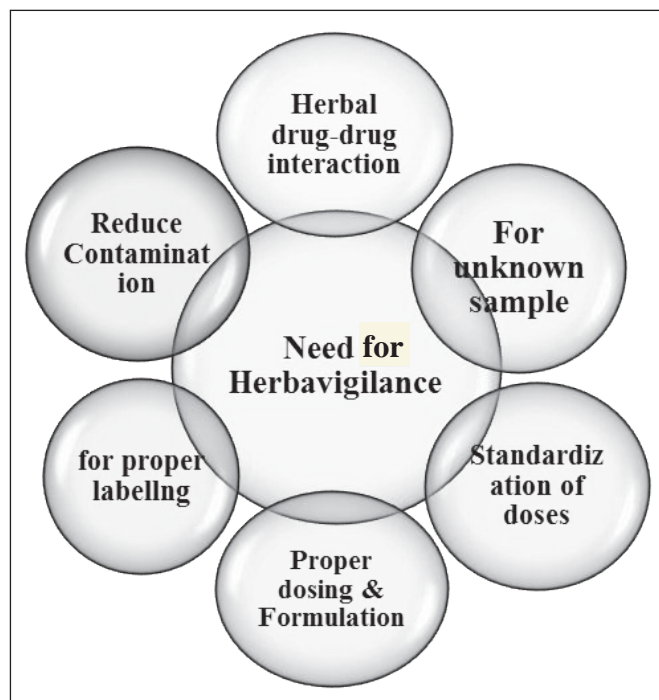


Fig. 1: Thrust areas of herbavigilance⁸

DNA barcoding is a strategy, which gives recognizable proof of various unidentified species that utilize a slightly normalized DNA part to characterize or find an animal species⁸. It uses small contrasts of base pairs in the specific quality part of various species to rectify segregation^{9, 10}. The gene is arranged to detect the base-pair contrasts along with stored in the standardized marker information base that is named as DNA barcoding markers. These genetic sequences might be obtained by an advanced library and utilized to distinguish the rare strains by researchers worldwide¹¹. The absolute DNA standardized tag ought to be ordinarily a constant tiny (400-800bp) succession of DNA that is simply generated and used to describe each one of the livelihood beings¹². Presently this is effectively applicable to animals. A gigantic on-line computerized library of scanner tags for obscured DNA standardized tag arrangement can be coordinated for the ID. The critical cycle in this process identifies new competitor quality that can be utilized all around. It ought to permit the clients productively to acknowledge the specific strain and quicken the strain revelation. It uses different data areas of the gene sequence to perceive maximum strain quickly¹³.

In the current study, we have studied and perused the relevant studies carried out on DNA barcoding and

herbavigilance through electronic database of Pubmed, Google Scholar, Scopus and Research Gate through the years of 1981 to 2020. The search includes key words like 'DNA barcoding', 'herbavigilance', 'DNA barcoding + protocol', 'DNA barcoding + Gene', 'BOLD' and 'NCBI' etc. Based on the literature survey, the gene and protocol involved in the DNA barcoding are discussed in this review.

DNA barcodes and herbavigilance

Herbavigilance is a hybrid term of pharmacovigilance that deals with the assessment, monitoring and prevention of harmful adverse effects of herbal derived products¹³. The safety parameters are still the point of discussion in the case of herbal products because of incorrect or misidentified herbs, adulterants, contaminated herbal products and wrong processing methods of herbal products¹⁴. Due to the increasing use of herbal products as adjuvant therapy and supplement therapy, the WHO laid down the regulation and utilization of herbal products for usage safely and effectively. As the standardization and comparative studies for the herbal products occasionally playing this natural changeability brings about aspects that might be unique and not really bioequivalent in any event joining reports of unfavorable impacts (or adequacy) demands careful consideration of the scientific or biological activity differences; notwithstanding, if searching for security signals for further investigation, at that point a conceivably practical methodology may be to consider collections of spices containing comparable mixes¹⁵. DNA barcoding can be used as a parameter for the herbal drug standardization for assessing the misidentified species in herbavigilance process. The plant species have a huge variety of members. They comprise various angiospermic and gymnospermic plants alongside the greeneries, plants, and greenery partners. Appraisals of complete strain identification differ incredibly between the creators; however, an ongoing evaluation has proposed that there are roughly 380,000 types of plant species, containing 352,000 types of angiosperms, 1,300 species of gymnosperms, and 13,000 strains of bryophytes and ferns¹³.

The DNA barcoding gaps are referred to as the distance distributed among the intra and intraspecific gaps that mirror the DNA barcoding variations¹⁶. The agreement for the tag of survival arranges DNA barcoding improvement and execution all around. It is a very much required tool in herbavigilance for the identification of already described plant species and uncovering of newly discovered plant species. There are three main characteristic features of plant DNA barcoding, i.e., molecularization, computerization and standardization

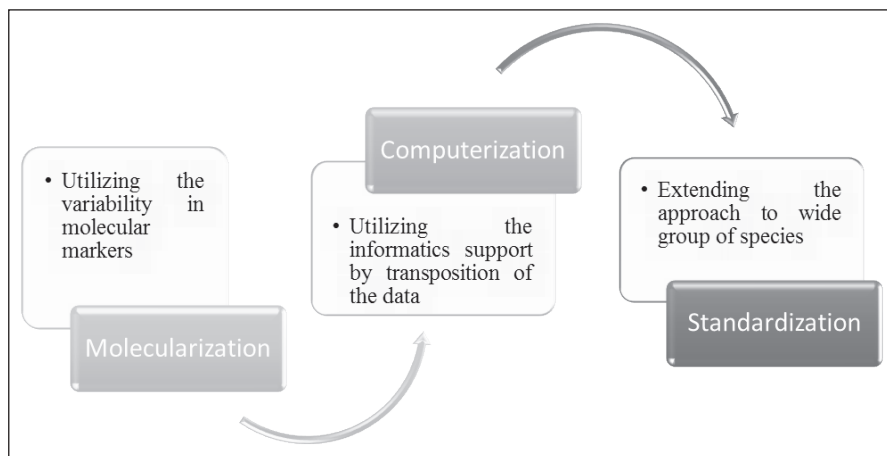


Fig. 2: Characteristics of DNA barcoding¹⁷

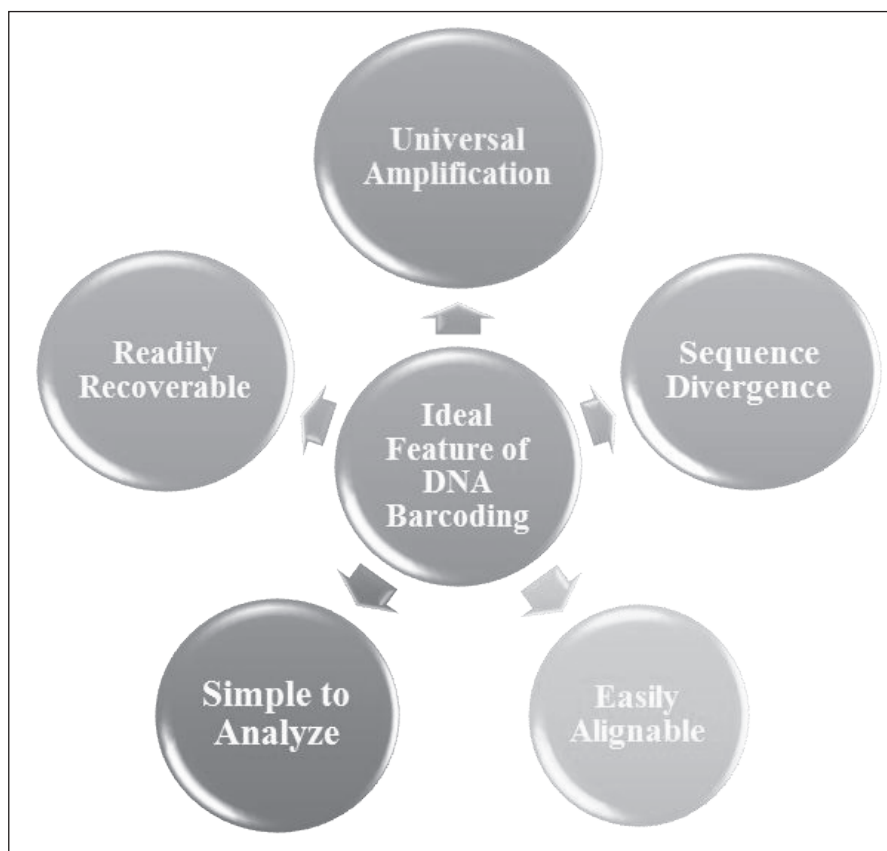


Fig. 3: Ideal feature of DNA barcoding²¹

(Fig. 2). These features comprise the basis of DNA barcoding¹⁷.

A definitive point of DNA barcoding is to segregate the strain utilizing a robotized framework, with the goal that undiscovered living species will be identified as fast as conceivable before it gets terminated¹⁸. DNA barcoding tag ends up being an excellent device to recognize the species over entire types of life, including animals, plants

and microorganisms quickly together with reliability. The considerable lot of the distributed work utilizes a detailed separation grid investigation¹⁹. The main aim of DNA barcoding includes identifying the molecular structure of species before it gets extinct²⁰. The classical feature of DNA barcoding consists of the universal amplification with the standard primers with high inter- and low intra-specific variation and easily recoverable and alignable (Fig. 3). The DNA barcode must be technically easy for insertion and deletion of base pairs²¹.

Methods and protocols for DNA barcodes

The DNA barcoding cycle involves two fundamental advances: (1) making the barcoding database for identified strains and (2) coordinating, or doling out the standardized tag arrangement of the obscure example against the barcoding database for recognition²². The initial step needs ordered skill in choosing one or ideally a few sample for every species to fill in as reference tests in the standardized identification library^{23,24}. All taxonomists generate DNA standardized tags and verify them with their monograph and store them at a central DNA barcoding organization. In some cases the specimens are obtained from previously housed herbaria but in some case it will be taken from the field. These specimens, at that point, fill in as the perpetual record that interfaces the DNA standardized identification to specific types of plant, organism, or

on the other hand, creature²⁵. When the reference scanner tag library is completed for the living beings under examination, regardless of whether they contain a geographic locale, a scientific classification, or an objective gathering (e.g., therapeutic plants, lumber trees, and so on), at that point, the DNA standardized tags produced for the unidentified tests are contrasted with the known scanner tags utilizing some kind of coordinating calculation. Most pragmatic estimates for strain task initiated by determining two DNA grouping which produces a sepa-

Table I: DNA barcoding profiling of medicinal plants⁷⁹

Sr. No.	Plant Name	Genome	Locus	Nucleotides	Gene Sequence	Depository
1	<i>Calotropis procera</i>	Chloroplast	RuBPCase large subunit	607 bp	rbcL	Sri Ramaswamy Memorial University
		Chloroplast	RuBPCase large subunit	1617 bp	rbcL	Mined from GenBank, NCBI
		Chloroplast	RuBPCase large subunit	1779 bp	rbcL	Mined from GenBank, NCBI
		Chloroplast	Maturase K	700 bp	matK	Mined from GenBank, NCBI
2	<i>Calotropis gigantea</i>	Mitochondrial	trnL-F intergenic spacer	494 bp	trnL-F	Bharathiar University
		Chloroplast	Maturase K	846 bp	matK	Mined from GenBank, NCBI
		Chloroplast	RuBPCase large subunit	1617 bp	rbcL	Mined from GenBank, NCBI
		Chloroplast	RuBPCase large subunit	527 bp	rbcL	Gujarat Biodiversity Gene Bank
3	<i>Ocimum basilicum</i>	Chloroplast	Maturase K	801 bp	matK	Mined from GenBank, NCBI
		Chloroplast	RuBPCase large subunit	687 bp	rbcL	Mined from GenBank, NCBI
		Chloroplast	RuBPCase large subunit	551 bp	rbcL	Mined from GenBank, NCBI
		Chloroplast	Maturase K	1518 bp	matK	Mined from GenBank, NCBI
4	<i>Capsicum annuum</i>	Chloroplast	Maturase K	935 bp	matK	Mined from GenBank, NCBI
		Chloroplast	RuBPCase large subunit	657 bp	rbcL	Mined from GenBank, NCBI
		Chloroplast	-	587 bp	rbcLa	University of Hawaii at Manoa
		Nuclear	-	221 bp	ITS2	Mined from GenBank, NCBI
5	<i>Matricaria chamomilla</i>	Chloroplast	-	552 bp	rbcLa	University of Guelph, OAC Herbarium
		Nuclear	-	330 bp	ITS2	University of Guelph, OAC Herbarium
		Nuclear	Internal transcribed spacer 1	258 bp	ITS1	Tromso University Museum
		Nuclear	-	327 bp	ITS2	University of Guelph, OAC Herbarium

6	<i>Allium sativum</i>	Chloroplast	RuBPCase large subunit	607 bp	rbcL	Sri Ramaswamy Memorial University
		Chloroplast	Maturase K	848 bp	matK	Mined from GenBank, NCBI
		Nuclear	-	242 bp	ITS2	Mined from GenBank, NCBI
		Chloroplast	-	548 bp	rbcLa	Canadian Museum of Nature, National Herbarium of Canada
7	<i>Melissa officinalis</i>	Chloroplast	Maturase K	813 bp	matK	Mined from GenBank, NCBI
		Chloroplast	RuBPCase large subunit	1743 bp	rbcL	Mined from GenBank, NCBI
		Chloroplast	Maturase K	841 bp	matK	Smithsonian Institution
		Nuclear	-	612 bp	ITS2	San Diego Natural History Museum, Herbarium
8	<i>Petroselinum crispum</i>	Chloroplast	Maturase K	810 bp	matK	Mined from GenBank, NCBI
		Chloroplast	RuBPCase large subunit	1743 bp	rbcL	Mined from GenBank, NCBI
		Chloroplast	Maturase K	1135 bp	matK	Mined from GenBank, NCBI
		Nuclear	-	222 bp	ITS2	Mined from GenBank, NCBI
9	<i>Mentha viridis</i>	Chloroplast	RuBPCase large subunit	607 bp	rbcL	Sri Ramaswamy Memorial University
		Nuclear	-	420 bp	ITS2	Research Collection of B. A. Bennett
		Chloroplast	-	520 bp	rbcLa	B.A. Bennett Herbarium
		Chloroplast	Maturase K	814 bp	matK	Finnish Museum of Natural History
10	<i>Rosmarinus officinalis</i>	Chloroplast	Maturase K	804 bp	matK	Mined from GenBank, NCBI
		Chloroplast	RuBPCase large subunit	687 bp	rbcL	Mined from GenBank, NCBI
		Chloroplast	RuBPCase large subunit	551 bp	rbcL	Mined from GenBank, NCBI
		Chloroplast	RuBPCase large subunit	660 bp	rbcL	Mined from GenBank, NCBI
11	<i>Salvia officinalis</i>	Chloroplast	RuBPCase large subunit	1737 bp	rbcL	Mined from GenBank, NCBI
		Chloroplast	Maturase K	810 bp	matK	Mined from GenBank, NCBI

		Chloroplast	RuBPCase large subunit	687 bp	rbcL	Mined from GenBank, NCBI
		Chloroplast	RuBPCase large subunit	924 bp	rbcL	Mined from GenBank, NCBI
12	<i>Thymus vulgaris</i>	Chloroplast	RuBPCase large subunit	607 bp	rbcL	Sri Ramaswamy Memorial University
		Chloroplast	Maturase K	1521bp	matK	Mined from GenBank, NCBI
		Chloroplast	Maturase K	810bp	matK	Mined from GenBank, NCBI
		Chloroplast	RuBPCase large subunit	687 bp	rbcL	Mined from GenBank, NCBI

rate calculation among the groups. In DNA barcoding, a grouping arrangement calculation is generally utilized to dole out an obscure example to known species varieties by finding the nearest information base succession to the example arrangement. GenBank is given by BLAST that maintains the succession library by utilizing the K2P distance and algorithm of Smith-Waterman²⁶. For some clients of DNA standardized tags, the cycle closes after the obscure example is effectively identified. In any case, scanner tags can likewise be applied as instruments for responding to essential organic inquiries, like networking of species²⁷.

Resources of DNA barcode

DNA barcoding authentication depends on the reference particulars and the exploration of genetic variation within the data²⁸. This data is stored and maintained by various publically funded databases like BOLD, NCBI GenBank and MMDBD. BOLD (Barcode of Life Data Systems) contains the information regarding over 58,510 plant species (Fig. 4). The information included is barcode, images, maps, collection coordinates and vouchers^{29,30}. The iBOL, CBOL and GBIF are the partners of BOLD. NCBI GenBank stands for National Center for Biotechnology Information, which is an extensive database containing a system that works on BLAST (Basic Local Alignment Search Tool) algorithm³¹. This system is based on the nucleotide sequence database. MMDBD is a Medicinal Materials DNA Barcode Database system that contains more than 15,375 sequences for nuclear, mitochondrial and chloroplast regions³². It also includes the database regarding the adulterants, photographs, primers and references for the sequences³³⁻³⁵.

DNA barcoding gene in plant

It has a colossal role in the protection science,

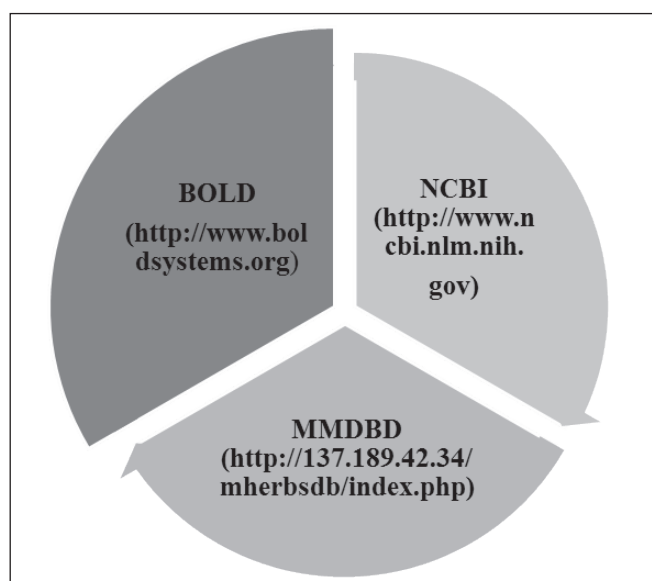


Fig. 4: Resources database of DNA barcoding^{29, 30}

particularly in the evaluation of biologically diversified active spots and furthermore to screen the global exchange of the uncommon species separated from the ordinary recognizable proof^{36, 37}. Plants have not been given a lot of significance in the beginning phases of DNA barcoding because of the inability of cytochrome oxidase (COX1) to function as a standardized identification³⁸. It must be practical in a broad scope of experts, and the system must be available and effortlessly usable by different clients³⁹. The quality of DNA barcoding was legitimately identified with the information accessible in the standardized identification database that assembled a total DNA barcoding data set^{40, 41}.

The plant DNA barcoding includes the arrangements of various gene sequences which are discussed here.

***matK* (Maturase K) gene sequence**

The gene *matK* is associated with group-II introns that responsible for maturase like protein in the chloroplast. *trnK*'s introns contains roughly 1500 base sets⁴². The gene contains high replacement rates inside species, consequently developing as one of the likely gene, and it is being utilized in the investigations of sub-atomic systematic and advancement⁴³. A similar exploration determines one hundred and two amino acids at the carboxyl end which are basically identified with segments of maturase-like polypeptide and this may be involved in grafting of Group II introns. From the various accessible genes for chloroplast, the *matK* has maximum replacement rate, that makes it as one of the perfect standardized coding competitors however, just to a smaller sub gathering^{44, 45}.

***rbcL* (Ribulose biphosphate carboxylase) gene sequence**

The gene *rbcL* is available as a single gene for every chloroplast genome; however, numerous duplicates of the gene sequence are available in every plastid; subsequently, the number duplicate per chloroplast for real *rbcL* can be high⁴⁶. It encodes the large subunit of ribulose-1,5-biphosphate carboxylase/oxygenase. It contains just exons, a polypeptide with approximately 475 amino acids⁴⁷. The *rbcL* promoter impacts the transcriptional inception rates by succession as well as changed by the close by *atpB* advertiser. The two promoters are situated 400 bp separated in inverse directions, bringing about dissimilar records⁴⁸⁻⁵⁰. Evacuation of a promoter or expanding the space between them kills this shared obstruction, which might be a control component to direct the various degrees of articulation in chloroplasts⁵¹⁻⁵³.

***psbA-trnH* (D1 protein of photosystem II) gene sequence**

The abundance of data is embedded in intergenic cp DNA regions for requests in population hereditary genes and low-level systematics⁵⁴. Segments of these non-coding regions whose capacities were obscure; however, a significant part of the variety may result from the spread of transformations that isn't limited by choice^{55,56}. In the population hereditary examinations, hereditary examinations, *psbA* was monitored in depth and uncovers enormous reversal toward the end closest to *trnH*, which seems, by all accounts, to be excess factors⁵⁷. In progressing the DNA barcoding endeavor, the role of intergenic spacers has been more significant⁵⁸. The angiosperms and gymnosperms strains contain a little spacer (200-500bp) of *psbA-trnH* gene, which were enhanced utilizing the general preliminaries.

Because of wide nucleotide variety, it is hard to locate the monitored locales among the exceptionally veered scientific classifications. Shockingly, a small sequence of around 6-30bp was preserved amongst the angiosperms. Regrettably, its curtness restricts its possibilities for phylogenetic deduction over exceptionally distributed taxa⁵⁹.

The *trnH-psbA* domain relicsa significant applicant in the plant barcoding⁶⁰. In a few plant's ancestry, *trnH-psbA* doesn't show better enhancement or numerous groups, and it is unpredictably more. It additionally contains mononucleotide rehashes, which are hard for succession accuracy and inclusion occasions. Inside specific gatherings the intra-explicit type was discovered to be high⁶¹.

***atpB* (Adenosine triphosphate β -subunit) gene sequence**

The spinach *atpB* gene includes the blend of ATP synthase (β subunit), and the proportion of the gene is assessed to be around 1497bp. The five subunits, i.e., alpha, beta, gamma delta and epsilon, make the chloroplast ATPase⁶². By contrast, the amino acid arrangements of concluded interpretation items were broken down. Additionally, the coding areas relegated to the beta and epsilon subunit share four nucleotides for all purposes. Additionally, the chloroplast genome was interpreted uniquely by *atpB* and *rbcL* gene⁶³.

***ITS* (Internal transcribed spacer) gene sequence**

The non-utilitarian RNA grouping known as the ITS location, which is present in the atomic genome, is located between the 18S and 25S rRNA coding regions. The range of the ITS1 and ITS2 is between 18S and 5.8S rRNA and 5.8S and 25S rRNA, respectively. The ITS spacers are taken from the transcriptional unit that is positioned in the centre of the auxiliary ribosomal RNAs during rRNA formation, and any unneeded development components are swiftly debased⁶⁴. The development of small and large subunit rRNAs was inhibited by cancellations of particular regions within ITS1, according to mutational analyses of the yeast ITS region. Several erasures or point mutations in ITS2 at the same time prevented or reduced the processing of colossal subunit rRNAs⁶⁵. The complete length of ITS locale is around 700bp, including the 5.8S rRNA region, which has a consistent length of 163 or 164bp. The size of the ITS district fluctuates because of point change. The ITS district of the atomic DNA (nr DNA) happens as pair rehashes at numerous chromosomal loci. The high duplicate number of the ITS locale advances recognition, intensification, cloning and

sequencing of nr DNA⁶⁶. The PCR proficiency of the ITS area is high when contrasted with scanner tag up-and-comers. Subsequently, it very well may be additionally exposed to limitation processing, which creates particular analytic groups that could adequately separate and recognize the plants at their species level^{67, 68}.

Application of DNA barcode data

1. Recognizable proof of various stages of plant life: To recognize seed and seedlings and finding the progressions of transformation⁶⁹⁻⁷¹.
2. It gives the recognizable proof of parts of plant material⁷².
3. DNA barcoding can be utilized in scientific examination⁷³.
4. Check of natural prescriptions/staples: DNA barcoding helps in recognizing contaminated items from unique parts⁷⁴.
5. Biosecurity and exchange of the controlled species: if there should arise an occurrence of illegal import and fare of financially important species, DNA barcoding help as a prompt to advance approved exchange⁷⁵.
6. DNA barcoding may be utilized to survey the systematically broadened species, both local and threatened species⁷⁶.
7. DNA barcoding can be utilized for biodiversity monitoring, conservation and management of the ecosystem⁷⁷⁻⁷⁸.
8. DNA barcoding can be utilized to confirm the distinguishing proof of exchanged decorative and farming plants. It can also be used to distinguish the importation or development of intrusive species and determine the metabolites of traditional medicine⁸².

Medicinal plants with DNA barcodes profile⁷⁹

The data for the given Table I was collected from the BOLD system database for the public that showed the DNA barcode profiling of various medicinal plants.

Detection of adulteration of botanicals' ingredients using DNA barcoding

Despite the fact that Ayurveda is among the oldest medicinal systems in the world, increasing economic demand for goods with Ayurveda roots has ramped up the motive for adulteration and substitution in the herbal market^{80, 81}. Fraudulent methods like the use of unregistered fillers and other species of lower quality are made possible through expanding additionally constrained stock volume of particularly feral species of plants⁸²⁻⁸⁶.

The challenge in plant pharmacovigilance is to find novel approaches to rigorously test and evaluate the quality of the raw ingredients and the final marketed herbal products^{87, 88}. When choosing an analytical technique for quality control, it is important to carefully analyze the several important elements that have a major influence on the quality of ayurvedic herbal products⁸⁹. Questions about the dependability and safety of natural supplements are raised through intentional adulteration by using sub-standard pharmaceutical aids of plant origin for producing desired effect^{90, 91}. To reduce these constrain and monitoring of this field barcoding technique is applied to all products from herbal origin^{92, 93}. DNA barcoding, which was initially created as an identification technique, is now used in the pharmaceutical sector to certify a variety of herbal products⁹⁴⁻⁹⁶.

Mosa *et al.*, 2018 examined at the veracity of herbal product trading in the UAE. Thirty samples were tested, representing six distinct herbal items (thyme, cardamom, anise, basil, turmeric and ginger), both fresh and dried. Three barcode loci were amplified, sequenced, and evaluated using BLAST, including *rbcl*, *matK* and *ITS*. In terms of amplification effectiveness, the data indicate that *rbcl* is the best marker for species identification, with 75 percent successful amplification, followed by *ITS* with 66.67 percent, and *matK* with 18.52 percent. Two samples, ginger powder and dried thyme leaves, were found to be adulterated. The adulterants came from the genera *Triticum* and *Oryza*⁹⁷.

Parvathy *et al.* employed three barcoding loci, namely *ITS*, *rbcl*, and *matK*, for PCR amplification of the reference samples and commercial samples from ten different firms to detect plant-based adulterants in sold turmeric powder. The potential of the barcoding loci in authenticating traded turmeric samples was assessed using PCR success rate, sequencing efficiency, the incidence of SNPs and BLAST analysis. The PCR and sequencing success of the loci *rbcl* and *ITS* was determined to be 100%, whereas *matK* revealed no amplification. The *rbcl* and *ITS* loci had 100% PCR and sequencing success, but *matK* had no amplification⁹⁸.

In the work of Parvathy *et al.*, three barcoding loci—*psbA-trnH*, *rbcl*, and *rpoC1*—were used to detect bio adulteration of commercial black pepper powder. PCR amplification of *P. nigrum* and commercial black pepper powder was applied to all three loci. Sequence analysis and BLAST results revealed that two out of nine market samples included adulterated chilli, which most likely originated from used black pepper powder that had been spiced up with chilli. With amplicons of 600 and 350 bp,

respectively, the *psbA-trnH* locus proved to be the best and most appropriate for identifying chilli adulteration in black pepper⁹⁹.

Kumar *et al.*, used DNA barcoding to investigate the adulterants in olive oil. SNP variation in the noncoding spacer region between *psbA-trnH* and the partial coding region of *matK* of the plastid genome was investigated using whole genomic DNA extracted from olive oil contaminated with canola and sunflower. For determining contaminations in olive oil samples, these DNA areas were amplified by PCR using particular primers, and the obtained DNA sequences were compared to preset consensus DNA barcode sequences of canola and sunflower. The mixture of canola and sunflower oil with olive oil was discovered after an adulterant DNA sequence was matched with their respective DNA barcodes¹⁰⁰.

The species identification approach used by Nithaniyal *et al.*, was DNA barcoding. After developing a reference DNA barcode library with 1452 *rbcL* and *matK* barcodes from 521 medicinal plant species, adulteration in 112 sold raw pharmaceuticals was investigated. For *rbcL*, *matK*, and *rbcL + matK*, the library's species resolution was 74.4 percent, 90.2 percent, and 93.0 percent, respectively. About 20% of the raw medications were found to be adulterated, and at least 6% of them were derived from plants with radically different therapeutic or hazardous qualities¹⁰¹⁻¹⁰³.

CONCLUSION

DNA barcoding is an excellent instrument for the discovery of species constituents in different kinds of natural items. It ought to be supported while expansion to the stockpile of present explanatory strategies. Regular DNA barcoding validation of natural products as a significant aspect of the assembling cycle could increase standard, credibility and responsibility in the natural product industry, and encourage herbavigilance checking and prompt discovery. Meanwhile, DNA barcoding to set up strain arrangement in groups of herbal accounts that have driven to suspect ADRs could help in refining putative reasons for impact. Close collaboration between middle clinical organizations and educational or business facilities specialized in plant DNA barcoding should be encouraged in order to pilot such DNA barcoding-based herbavigilance.

ACKNOWLEDGEMENT

The authors are thankful to Central Library, GLA University, Mathura for providing literature through DELNET service.

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