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Review Article

DNA Barcoding of Herbal Medicinal Products: A Challenging Task

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Abstract: There is a global resurgence of traditional and complementary medicine, specifically the herbal products have been booming for the last few decades. However, the events of substitution and adulteration of herbal drugs/ medicinal products is an increasing concern for consumer safety. The prevailing situation of adulteration highlights the dire need of an effective scientific method for improved precision while carrying out the correct identity of the medicinal flora and their herbal products. DNA barcoding has come out as a solution for correct identification of herbs and to find the adulterants in herbal products. There are challenges involved in the barcoding method for medicinal plants in terms of developing barcodes and the analysis of data to measure the distinguishing power. Though, the solution to these problems is available and DNA barcoding can help to formulate a system to ensure the quality of herbal drugs which will help the pharma industry of herbs to regain the lost confidence of consumers.

Keywords: Herbal products, DNA barcodes, mini-barcodes, meta-barcoding, barcoding challenges.

1. INTRODUCTION

The herbal commodity market is expanding globally due to increased confidence in traditional healthcare system. The exponential rise in the business of herbals through past decade confirms the global attention towards the herbals and the associated traditional healthcare [1]. However, it has also been observed that consumer faith is damaging due to adulteration and substitution of herbal drugs. The consumer health is at risk due to the substitution events where the original herbal species is replaced by a non-medicinal plant. Similarly, the addition of fillers which are not labeled decreases the therapeutic potential of the herbal pharmaceuticals [2]. The traditional system for identification of plants is based on morphological characters which cannot typically be used for processed plant material or powdered form. The commercially available technologies employed for validation of herbal products are based on physical, chemical, biochemical analysis and recently developed molecular analysis and tools (DNA dependent) [3]. DNA is found in all tissues, less degradable and more resistant to external factors therefore, the DNA based identification is more reliable in contrast to RNA and proteins [4]. Hebert et al., [5] proposed DNA barcoding for correct identification of existing species and to unearth the new species. A standardized region of DNA (<1000 bp) called as DNA barcode is used in this method. DNA barcoding provides a simplified solution to the complex problem of correct identification of raw herbal material/medicinal products and assures a significant quality check within the market of herbal products [6]. More recently DNA barcodes have been included in pharmacopoeias, providing tools for regulatory purposes [7]. The study highlights the necessity for quality control of the marketed herbal products and shows that DNA metabarcoding is an effective analytical approach to authenticate complex multi ingredient herbal products [8]. However, there are challenges being faced in generating the barcodes and the analysis of data for estimating the distinguishing power of these barcodes [9].

2. LIMITATIONS AND CHALLENGES OF DNA BARCODING IN HERBAL PRODUCTS

2.1 DNA Extraction

There is a minimum requirement of quantity and quality of DNA to be found in herbal sample to

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carry out successful DNA barcoding. Several studies showed a relatively low barcode success in their work [9-12]. The diverse manufacturing methods of herbal products and the part of plant used or type of material used in the products may be the reasons of low barcode success. Naturally there are many secondary metabolites, polysaccharides, polyphenols, glycol-proteins are found in plants. Their presence can obstruct the process of DNA isolation, gene amplification and sequencing [13]. Under good laboratory practices isolation of DNA from herbals should be carried out shortly after collection of material to stay away from such storage conditions which damages DNA and where the cross-contamination of samples can occur [14]. The widely used methods of DNA extraction are cetyl trimethyl ammonium bromide (CTAB) method [15] and commercially available DNA extraction kits [16]. However, these methods and kits are not helpful in extracting DNA from those plant tissues (roots, tubers etc.) where secondary metabolites are found in high concentration. Through early stages of DNA isolation, the high amounts of polysaccharides and polyphenols must be eliminated by utilizing methods having increased concentration of CTAB, polyvinylpyrrolidone (PVP), and β-mercaptoethanol (β-Me) [17-20].

Largely the herbal products are available in the form of tablets, capsules and liquid extracts and the DNA of plant species used is either degraded or removed during the process of manufacturing, therefore, the isolated DNA from these products is either fragmented or absent. It could also hint towards the possibility of presence of excipients (fillers, binders, lubricants, diluents, pigments, stabilizers etc.) that may affect the extracted DNA or hinder the amplification of the targeted region by the primers [13]. The manufacturing processes through which extracts and tinctures are prepared involve extensive heat treatments, filtration and distillation resulting in complete removal or degradation of DNA which make these materials unsuitable for DNA barcoding [21-22].

2.2 Selection of DNA barcoding loci

In animals, the mitochondrial cytochrome c oxidase 1 (*CO1*) is considered as a universal barcode but it cannot be employed for plants based on its slow rate of evolution and limited divergence [5, 23]. The Consortium for the Barcode of Life (CBOL) suggested the combination of two locus *mat*K-*rbc*L as the universal DNA barcode for plants in 2009 as they belong to the relatively fast- evolving plastid genome. The other commonly used regions of nuclear and plastid genome are *ITS, ITS*2*, psbA-trnH, atpF-atpH, ycf*5*, psb*K-I, *psbM, trnD, nad*1*, trnL-F, rpoB, rpoC*1*, and rps*16 [2, 24-26]. Though, none of these individual plant barcodes have both discriminating regions and the regions of attachment of universal primers simultaneously. Hence, a multi-locus barcode with two or three loci in combination was proposed [24-25]. The two locus barcode of *rbc*L-*mat*K also posed some difficulties as *mat*K is problematic in amplification in some plants because of the non-conserved primer binding site of universal primers. The other recommended two locus combination was *rbc*L + *trnHpsbA* which failed to work for some of the plants due to highly variable *trnH-psbA*. High variability of *trnH-psbA* poses difficulty in the alignment of this combination. To resolve this issue, a tiered approach was put forth by Newmaster et al. [2]. The method utilizes the easily amplifiable and alignable *rbc*L region as a scaffold on which data from highly variable non-coding regions such as *ITS*2 or the *trnH-psbA* region are employed for identification of plant species.

The short length *i.e.* 200-230 bp of *ITS*2 serves as an advantage for the identification of herbal supplements. The fragmented DNA of herbal supplements may not be able to amplify 600-800 bp long barcodes. Despite of this advantage, there are disadvantages of *ITS*2 as a plant barcode includes occurrence of multiple *ITS*2 copies in the same individual, which resulted in the inaccurate identity of species based on their resemblance to the copies of the sister species. There are also technical issues in the amplification and sequencing of *ITS*2 that can happen due to occurrence of DNA from other co-existing species of plants [27-28]. The concept of "mini-barcodes" was introduced for barcoding of herbal dietary supplements through short-barcodes (< 200 bp) of standardized *mat*K and *rbc*L regions [29-30]. Mini-barcodes provide the ease of amplification for processed dietary materials along with their ability to discriminate closely similar species.

2.3 Amplification and Sequencing

The ease of amplification and use of universal primers has been a pre-requisite of DNA barcoding

method. The inherent biases in the amplification step can result in false negative or false positive results [31]. The commercially available kits for DNA isolation utilized in the initial preparation of samples, efficacy of amplification reaction itself, differences in the melting temperatures of the primers are the factors affecting the amplification success [32-33]. The balanced melting temperature of the primer pairs and the affinity between the template DNA and universal primers are both the significant factors to carry out robust amplification [34].

The presence of inhibitory secondary compounds, inactive ingredients and excipients in the herbal supplements in the form of tablets, capsules and pills hamper the PCR reactions and may result in multiple nucleotide sequences indicating the mixed DNA sample [35].

In majority of the studies, Sanger's di-deoxy sequencing [36] is the commonly used sequencing method for DNA barcoding. It generates up to 1000 bp reads of sequences, however, the limiting factors of this method includes requirement of high conc. of DNA (100-150 ng) and its low throughput [37]. The other challenge is formation of two sequencing signal patterns (electropherograms) for each sequence generated, making it un suitable for those herbal samples that contain more than one species or excipients. Presence of multiple species in a sample results in the formation of multiple or overlapping sequence peaks causing the sequencing to be failed and making the accurate determination of barcode impossible [13]. Similarly, the fungal *ITS* barcodes in multiple copies causes problems for direct method of Sanger sequencing. Molecular cloning in an appropriate microbial/bacterial host is one of the solutions for improving the poor read quality, however, cloning introduces biases against extreme base composition *e.g.*, stretches with high guanine and cytosine contents), inverted repeats, and genes not accepted by the bacterial cloning host [38].

The recently developed high throughput sequencing technique called as the Next-Generation Sequencing (NGS) has been used as an answer to issues of the Sanger's sequencing. In this method parallel sequencing of multiple DNA fragments from various DNA templates can be performed in a single reaction [39]. NGS can generate up to one million DNA sequences, 700 bp long in a single run of sequencing. The NGS is comparatively cost effective; however, the cost of bioinformatics is additional based on the huge amount of obtained data in this technique. The next-generation sequencing "meta-barcoding" method is a combination of high throughput DNA sequencing and low-throughput DNA barcoding to conduct the analysis of DNA barcodes from environmental sediments, ancient or processed samples at a mass level [40-42].

3. CONCLUSION

DNA barcoding has both the advantages and challenges. Despite the limitations, this method has its benefits when utilized in herbal industry correctly. DNA barcoding and metabarcoding have greater prospective for quality assurance of herbal products.

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