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Review Article

DNA Barcoding in Plants and Current Molecular Issues

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Abstract

DNA barcoding especially used in the field of molecular biology that uses primers with 500-700 segments to classify various species at molecular and genetic level. This review focuses on the role of DNA barcoding in conservation of biodiversity and current applications for the discovery of species. Some species of plants and animals remain unidentified at molecular level. DNA barcoding uses molecular tools for the discovery of species found in natural ecosystem and playing important role in biodiversity conservation. Genetic barcoding mainly focuses on conservation strategies on populations due to differences found in the mitochondrial DNA. The most commonly used DNA barcodes for the plant species are ITS, rbcL, psbA-trnH and matK. DNA barcodes has several applications for the detection of plants species by providing specific information about the taxa. There is need to use DNA barcoding in combinations with other sciences such as ecology and taxonomy methods for conservation of biodiversity. There is also need to use databases for appropriate storage of data about species to conserve biodiversity.

Keywords: DNA barcoding, Biodiversity, mitochondrial DNA, Ecology, phylogenetic diversity.

INTRODUCTION

Plant DNA barcoding plays an important role in the evolution of biodiversity and to keep an eye on the international trades in many rare species[1]. In the early stages of barcoding, plants did not get much importance because the cytochrome oxidase (COXI) is incapable to act as barcode in plants. To find an appropriate marker there was a contest between the botanists. Numerous gene regions were suggested as feasible candidates for the plant barcoding, but still no any universal barcode is present[2] . As in case of animals the COX1 act as a universal barcode but in plants due to shortage of consensus region, the universal barcode is still unknown. While for the smaller taxa, many groups suggested successful barcode candidates. The potency of DNA barcoding was directly linked to availability of data in the libraries of barcode which was also helpful in the formation of absolute DNA barcoding database[3]. To fulfill all the requirements of barcoding there is the need of PCR conditions with stranded rang and also the set of PCR primers for every gene that act as a marker of barcode. That can be utilized for every wildest type of taxa and also for the users. There should also be some focus on the barcoding of plants from degraded samples[4].

The plants mitochondrial gene COXI region was not suitable for the barcode to distinct different species as the plant mitochondrial evolves slowly as compared to the animals. Recently many combinations of DNA region have been proposed by[5]. Possibly in future the combination of DNA region for barcode surely will contain trnH-psbA that is noncoding intergenic spacer and also matK which is a plastidial coding sequence but at present there is no any concurrency on a certain gene candidate that is best for the plant barcoding[6].The certain species that are related to each other cannot be differentiated morpholigicaly at the early stages of life[7] (young leaves, seeds and seed coats).

But these species can be distinguished at the molecular level with the help of DNA barcoding. In forensic examination any adulterated product can be differentiated by the original product through DNA barcoding[8]. It can also act as biosecurity in trafficking of economically precious things or controlled species. DNA barcode also utilized to assess those species that are taxonomically different both the native and invaded species[9]. The purpose of DNA barcode to build up a

diagnostic tool that is based on the taxonomic data collected through the DNA barcode reference library [10].

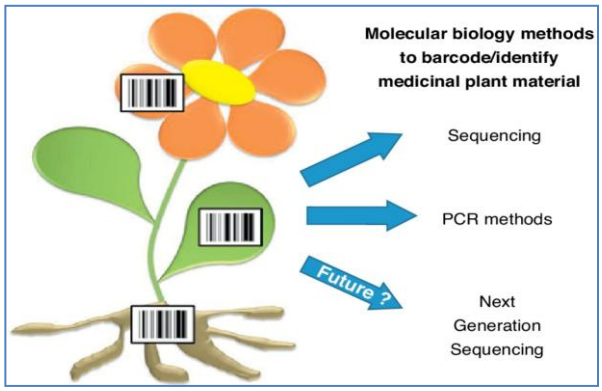

Fig-1: Plant DNA barcodes detection by molecular methods

The purpose of this review article included the current applications of DNA barcoding in biodiversity conservation as well as discussing the molecular issues for the species discovery in a particular habitat. This review also explains the special types of DNA barcodes in plants and their role in biochemical identification of particular species.

DNA barcoding in Plants

DNA barcoding has important applications in the discovery of plant species. There are various steps involved in DNA barcoding that follows appropriate collection of samples from the plant tissues and passes through DNA extraction then sequencing, data editing and final barcode compare to barcode library. It finally leads to discovery of many species[11].

Plant DNA barcodes

The studies on the plant barcoding were initially limited to the genome of chloroplast to understand the variation of its gene sequence of non-coding (ITS, psbA-trnH) and coding (matK, rbcL, rpoC1 and psbA-trnH), which has been explained by. It was proven by the studies that the genome of chloroplast under goes a lot of variations and has discrimination ability[2]. The facts showed that the species lineage that was reconstructed through chloroplast DNA regions illustrate many noteworthy errors, when hybridization. Introgression or linear sorting events take place[12].

matK

The maturase like protein was encoded by the gene of chloroplast called matK , which is involved in the Group II splicing of introns. The intron region of the trnK accommodates about 1500 base pairs of matK. The two exon regions of trnK flanked by the matK were chopped down during splicing thus leaving the whole matK gene intact during splicing. Within species this gene has high substitution rates that's why it is promising as one of the capable gene that can be used in the studies of evolution and also molecular systematic[5].

For the family Zingiberaceae matK is approved DNA barcode. The analysis of matK gene was performed on the large scale by the Linnaean society of London in the year 2010. They compare the available primer to get the match of generated sequence of matK, to purpose the possible modification in barcode and also to simplify the barcode complications[13].

rbcL

There is only one copy of rbcL gene in each chloroplast genome, but many copies chloplast genome can be seen in every plastid. So the actual copy number of rbcL per chloroplast can be quite high. rbcL consists only on exons and encode the polypeptide of about 475 amino acids. The chloroplast genes including rbcL can be expressed in Escherichia coli because the resemblances between the two genomes were found at transcriptional and translational identified sequence. When one promoter was removed or the space between two promoters was increased it eradicate that mutual interference, that can be used as a control mechanism for the regulation of different levels of expression in genome of chloroplasts. While the non synonymous substation occur when substitution is done between rbcL of parent species. Even the CO_2 and O_2 specificity of ribulose 1, 5 bisphosphate carboxylase/oxygenase (RuBisCO) can altered by replacement of a single amino acid in rbcL[14] .

A contingency study was conducted to assess the rbcL+matK barcode between major taxonomic groups, including non-angiosperms vs. angiosperms and mono-vs. polytypic-genera a contingency study was conducted [15]. rbcL was proved a better barcode than matK for the conifers of welsh flora and the native flowering plants when the comparison of two DNA barcodes marker rbcL and matK was done for studies [16].

psbA-trnH

For the lower level systematic and also for the inquires in population the intergenic cp DNA region provide very useful information. The studies of population genetics explained that intergenic spacer (psbA-trnH) end that is near to the psba remain highly conserved but it showed a big inversion at the end near to the trnH, that appears to be inconsistent or variable[17]. In DNA barcoding these intergenic spacer are of much importance. In gymnosperm or angiosperm species psbA-trnH contain a very small region of 200-500 bp which can be amplified by using universal primer that are developed by. However, among the taxonomic groups that are highly diverged the nucleotide variation makes it difficult to identify the conserved regions[18].

The photosystem II reaction center protein D1 encoded by the psbA gene, the expression of this gene effect many process in plant including the physiological stage of plant, the light intensity pf photosystem and also the developmental stages of plant. This gene is present at very low level in the amyloplast and pile up in chloroplast. For more than 20 years the study on chloroplast psbA UTRs was conducted to study their role in regulation of gene expression. The recurrent inversion in the portion of trnH-psbA contain flanking region of inverted repeats. In many plant lineages an inversion was noted in trnH-psbA region. Several traits have been shown by the trnH-psbA region; contain a short length of about 500bp. This region also contain flanking primers that are involved in the smooth amplification and also sequencing through high molecular weight. It is also more effective to analyze the degraded DNA which is suspected to contain the desirable barcode[3].

But in some plant lineages the trnH-pasbA region was not effective in the multiple band formation or amplification. As compared to the possible barcode it was irregularly longer. Within some different groups to differentiate among related species trnH-psbA was not proved effectively variable while in other species the intra-specific variations were found vey high. In about 21 genera and also 18 families to study the meta-analysis of DNA barcode in plants trnH-psbA was more significant in identification as compared to the other matK+rbcL[19].

ITS

The ITS or internal transcribed spacer belong to the nuclear genome. It is a non functional RNA sequence that is located between the coding region of 18S and 25S rRNA coding region. The ITS1 located between the 18S and 5.8S rRNA and ITS2 present between 5.8S and 25S rRNA. During rRNA maturation the ITS that is a transcriptional subunit present between the structural ribosomal RNA. As these ITS spacers are actually the product of maturation that are non functional so they are readily degraded. When studies was conducted on yeast it was shown that if deletion in certain regions of ITS1 was promoted it cause the inhibition of production of mature small and large subunits of rRNAs, while on the other hand the mutations in the ITS2 effect the processing of larger subunit of Rrna. In all flowering plants the length of ITS1 and ITS2 is variable but it is always less than 300bp for ITS1 and about 250 bp for ITS2. While the total length of ITS region is about 700 bp that includes the region of 5.8S rRNA, which has the costant length of 163 or164 bp[20].

In multiple chromosomal loci the nuclear region of ITS, occurs as tandem repeats[21]. The high copy number of the region of ITS encourage the cloning, detection, sequencing and amplification of nr DNA. As compared to other barcode candidate the ITS region gives better and clear result in PCR, that's why it can further be put into restriction digestion which results into diagnostic bands. These bands are helpful in identification of plants at their specie level[22].

The purpose of DNA barcoding research was to identify the better candidate genes to identify all the species of plants by utilizing both the coding and non-coding regions[23]. With the help of DNA barcoding a person who even don't have enough taxonomic training can easily identify the plant specimen[17]. DNA barcode also have important role in the evolutionary studies. In the differentiation of plant species of family fabaceae ITS2 was proven very helpful. By using ITS2 as a barcode gene about 893 species in 96 diverse genera from family Rosaceae were easily evaluated. ITS have specie discrimination success rate of 78% and 100% at the specie and genus level respectively. To know the authentication of Chinese herbal medicines ITS2 region is most commonly used barcode [24].

To discriminate between plant species of Asteraceae ITS2 was used as barcode and got the success rate of about 80%. ITS2 was also utilized in the identification of teo morphologically same species such as Swartzia grandifolia and S. longicarpa and also to classify the species Caranga rosea and C. sinica of the plant Fabaceae. The important feature of ITS2 is that it can also be used for the identification of system Pan-eukaryote and Eukaryota, that's why it is used as a universal barcode for both plants and animals. The chloroplast inter-generic region psbA-trnH was also reported as the excellent candidate of DNA barcode because it is utilized for the identification of Dendrobium species[25]. DNA barcoding was applied to identify the distribution of criptic species in India Velliangiri hills, this provide useful information in both traditional and scientific feilds. Similar technique was utilized to identify the Berberis species in India and also for the endangered species of Paphiopedilum [26].The morphologically similar species has also been distinguished by using ITS2 barcode. For the identification of contaminants present in the North American herbal products was done through the ITS2 when it is used in combination with rbcL[9].

The phylogenetic analysis and also studies of molecular evolution of species of Panax was done by using chloroplast intergenic spacer (IGS) like trnE-trnT, trnT-psbD, ndhF-rpl32 and rpl14-rpl16. To discriminate the species of Korean Orchidaceae the IGS like atpF-atpH, psbK-psbI and trnH-psbA in addition to the coding region of rbcL and matK were used as barcodes[27].

Molecular issues in DNA barcoding

In recent years in operational terms DNA barcode was defined in a very well manner and it was explained as a genetic method for a better taxonomical research[1]. A single gene that was located at the 5' end

of mitochondrial DNA (mtDNA) consist on 600 base pairs and called cytochrome *c* oxidase I (COI). This gene was sequenced and used as a barcode. For the classification of closely linked species and their texa COI was used in very standardized manner, the gene was sequenced and provide information about species accordingly[28].

However as technologist came forward with modern technologies and new samples the concept of a stranded barcode is being revised. In the process to add suitable alternative barcode gene for organisms, plants and fungi the modern studies moved away from the original barcoding protocol[29]. In modern studies the classification of specimen also based on operational taxonomic units instead of the traditional species concepts [30].

As the 11 digit universal code identify a specific retail product same as the term of DNA barcode which is actually a standardized sequence of DNA that identify a texa[31]. The main central part of this concept of barcode is the identification and utilization of a specific homologous gene that can be easily amplified through PCR and easily distinguishes the two species even two morphologically similar species[8]. For this purpose the mitochondrial DNA was preferred over the nuclear genome because of the reason that mitochondrial genome under goes very rare recombination[5].

Another advantage of mitochondrial DNA is that it is haploid and easily inherited to the offspring this ability remove theproblem of sequencing a heterozygous organism[32].

The use of gene COI as a barcode was justified in two important aspects: first one is its primers that are very robust and involved in the analysis of different organisms and the second important point it has high evolution rate as compared to the other DNA sequences this ability allows to distinguish between two different species and even in those species that was very closely linked[33]. Therefore, it was explained in the study that mitochondria could proved a better tool to determine the relationship between two species and also to determine the endangered species which have divergent genetic patterns that are worthy of conservation attention[34]. Very small failure rate of only about 5% were noticed when the mitochondrial DNA was used in studies. while during the identification of Canadian freshwater fish the success rate of about 93%[35].

A problem related to mt DNA was experienced by the scientists: that was the failure rate of barcoding through mt DNA cannot be predicted because effects of maternal inheritance on the rate of molecular divergence cannot be precisely predicted. It was also seen that not all of the mtDNA is maternally inherited explanation of this point is that of bivalve mollusks which show the doubly uniparental inheritance. Furthermore, a different texa is used to register the parental inheritance of mtDNA. DNA barcoding does not come with desired result where mtDNA which is paternally inherited, was being used as barcode[9]. Therefore many evolutionary process that affect the genders differently cannot counted when mtDNA was used as a single source of information and also information tilted on the basis that from which parental side (maternal or parental) the selection of data should be done [36].

Heteroplasmy is another problem for DNA barcoding which is explained as the presence of difference in nucleotide even in the same sample[25]. The phenomenon of heteroplasmy was discovered in bats, fish, insects, and nematodes. Variability of nucleotides comes from the length of the sequence and also because of sequence variability. The proponents of barcoding have not fully explained the phenomenon in which heteroplasmies occur because of the insertions/deletions within the coding region of genes. Heteroplasmy also explained by the sample of alleles that are represented by the mitochondria of an individual, these alleles are within a population and these are like any other gene that's why they require another genetic marker [28].Another type of genes that are actually mitochondrial in origin called pseudogenes (numts) can be easily amplified and cause problems in DNA barcoding, theses genes can be found in different clades of eukaryotes[37].

These pseudogenes are actually the copies of mtDNA present in nucleus and are non functional. Incorrect overestimation of many unique species can be done through the barcoding in those species in which individuals contain the pseudogenes of the gene COI. Although the quantity of incorrect references of species can be reduced, if the numts were already removed before barcoding. It is a long procedure to remove the numts that require inspection of the sequence characters, its neucleotide composition and also indels. Another extra work is done in the violation of "cheap and quick" characteristic of DNA barcoding[38]. All the limitations setup a trend to utilize different sources of genetic data to offer more correct assessments of biodiversity[39].

CONCLUSION

Plant DNA barcoding plays an important role in the evolution of biodiversity and to keep an eye on the international trades in many rare species. There is more advancement needed for detection of genetic materials of plants in the field of molecular biology. There is more ideal DNA barcodes for plants needed that for the discovery of new species in a particular habitat.

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