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Selection and Evaluation of Loci for DNA Barcoding in Plants

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Abstract: The methodical cataloging of biosphere species by traditional taxonomic practices is insufficient due to huge diversity. To facilitate rapid identification researchers have to use altered methods with the growing need of accurate and accessible taxonomic information. DNA barcoding is one of them, which utilize a unique pattern of DNA sequence for identification of sample. In DNA barcoding a short gene sequence from a standardized region of the genome or a unique pattern of DNA sequence is used as a tool for identification of sample. In this review paper we will discuss about the basic properties of DNA barcode and strength or weakness of barcode regions explored by different researches in reference to plants.

Keywords: *DNA barcoding, DNA sequence, gene sequence, genome, plants.*

1. INTRODUCTION

Taxonomy explains the identity of organisms and their relationships (Savolainen *et al.*, 2005). Linnaeus (1758) introduced his binomial system to classify the world's biological fauna and flora under the field of taxonomy. The diversity of biosphere is so large that the systematic cataloging of species by traditional taxonomic practices is insufficient to cope with the growing need for accurate and accessible taxonomic information (Godfray, 2002; Godfray and Knapp, 2004; Newmaster *et al.*, 2006). There are two main problems with classical taxonomy; first samples were collected and handled with high precautions to preserve their distinguished characteristics. The second one is that there should be a highly trained specialist for differentiating closely related species. Now a days in order to facilitate rapid and accurate identification altered method called as DNA Barcoding being used. The concept of DNA barcoding was started 30 years before by Carl Woese who first showed that DNA sequences could be used to reconstruct the tree of species. However, it was first used by Hebert *et al.*, (2003). DNA Barcoding has been proved as a novel system designed to provide rapid, accurate, and automatable species identifications (Hebert and Regory, 2005). In DNA Barcoding a short gene sequence from a standardized region of the genome or a unique pattern of DNA sequence is used as a tool for identification of sample (Hebert *et al.*, 2003). DNA Barcoding is a potentially powerful heuristic in identifying samples of described species and generating hypothesis of new ones. Its power has been amplified when its limitations are recognized and its utility not overstated or over-sold in the name of novelty (Goldstein and DeSalle, 2010). In 2004, the Consortium for the Barcode of Life (CBOL) was founded as an international initiative to promote and develop DNA Barcoding, funded from the Alfred P. Sloan Foundation.

2. PROCESS OF DNA BARCODING

The complete process of DNA Barcoding divided into four segments. First segment belongs to the sampling and isolation of their DNA. In second segment the obtained DNA amplifie using primer of specific DNA loci (Barcode) and the product of PCR separated by electrophoresis. In third segment the DNA obtained after second

segment will be sequenced. In fourth segment DNA sequence of the tested or unknown sample barcode align to the reference barcodes.

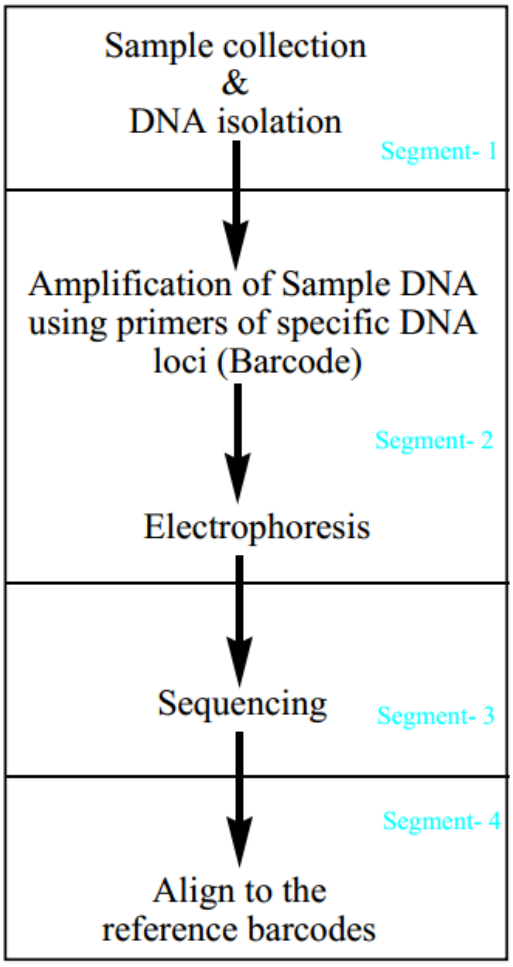

Figure 1: Flow diagram of four segments in DNA Barcoding

3. APPLICATIONS OF DNA BARCODING

In forensic contexts DNA Barcoding has been used for large-scale biodiversity survey through identification of a single fragment of material. It has also being used for species discovery and identifications (Kress and Erickson, 2007; Fazekas *et al.*, 2008; Seberg and Petersen, 2009; Costion *et al.*, 2011; de Vere *et al.*, 2012), application to phylogenetics (Chase *et al.*, 2005; Starr and Ford, 2009), taxonomic revision (Lara *et al.*, 2009), unraveling of food webs and predator prey relationships (Kaartinen *et al.*, 2010), Quarantine (Bonants *et al.*, 2010), fight against illegal trade of endangered species (Eaton at al., 2010), illegally logged timber (Lowe and Cross, 2011; Muellner *et al.*, 2011), large scale floristic inventories and monitoring changes over time, authentication of food and herbal medicines, elucidation of historical patterns of distribution and environmental sampling (Taberlet *et al.*, 2007

4. CHARACTERISTICS OF DNA BARCODE LOCI

Potential of DNA Barcoding became more applicable with change a number of the characteristics which are necessary particularly in a readily accessible and cost effective manner. i.e., the DNA barcode system has to be universally applicable, universal amplificationable, technically simple sequenced in one reaction, exhibit sufficient variability for species-level identification, high interspecific and low intraspecific sequence divergence. Together, these allow easy referencing and species delimitation, easily alignable, contain few insertions /deletions, difficult to interpret, readily recoverable from herbarium samples and other degraded samples (Chase *et al.*, 2007) or at least highly desirable in a DNA barcode. To fulfill these visions a universal, relatively cheap, scalable system needs to be in place for plant DNA Barcoding (Cowan and Fay, 2012).

In animals' cox1 gene of mitochondria, fulfill all of these characteristics, has been used as a tool for DNA barcoding due to a high base-substitution rate, but the gene content and order are highly conserved. The choice of a barcode marker specific for plants and fungi has been more problematic (Sbordoni, 2010).

Plants cox1 gene holds a little potential as a suitable DNA barcode region (Chase *et al.*, 2005; Cowan *et al.*, 2006; Fazekas *et al.*, 2008; Kress and Erickson, 2008) because during evolution from aquatic to the terrestrial habitats coincide there mitochondrial genome behave like a different way than animals. In the plant mitochondrial genome rate of evolution is much slower than in animals (Cho *et al.*, 2004; Jing *et al.*, 2011) due to lower base substitution rates, frequent genome rearrangements, transfers of genes between different genomes (plastid, mitochondrial, and nuclear) in individual and across species (Wolfe *et al.*, 1987, 1989; Palmer and Herbon, 1988; Laroche *et al.*, 1995, 1997; Yang *et al.*, 1999; Adams and Palmer, 2003; Cho *et al.*, 2004).

5. LOCI SUGGESTED AS PLANT DNA BARCODES

Volume of available data and current understanding of genome structure shows that a single gene has not been utilized for DNA barcoding in plants (Newmaster *et al.,* 2006) because the plant genome has evolved quite differently. Also, an inability for two groups to mate productively with each other commonly defines animals as separate species, but many plant species can hybridize, which blurs their genetic boundaries. So scientists around the world tested several highly promising gene segments that might serve as a barcode for all plant life (see table. 1). One of promising gene segment is large subunit of the ribulose-bisphosphate carboxylase gene (rbcL) of plastid DNA and nuclear genome sequence have been used to study for DNA Barcoding across the angiospermic plants with the invention of the polymerase chain reaction (PCR) (Chase *et al.,* 1993). The first major challenge for plants was the identification of a DNA region or regions that showed above explained characteristics of Barcode. A Plant Working Group was established at the inaugural meeting of the Consortium for the Barcode of Life (CBOL). A large number of multiple coding and non-coding plastid loci, alone or combined, have been tested for the identification of plants and potential plant DNA barcodes have been proposed (table 1) by Consortium for the Barcode of Life (CBOL) and different researchers.

Kress *et al.*, (2005) tested atpB-rbcL, ITS, psbM-trnD, trnC-ycf6, trnH-psbA, trnL-F, trnk-rps16, trnV-atpE, rpl36-rps8 and ycf6-psbM regions of DNA in 19 plants species from 7 families of angiosperm, They also evaluated ITS, rbcL, trnH-psbA regions in 83 plant species belonging to 50 families. After analysis they recommended the nuclear internal transcribed spacer (ITS) region and trnH-psbA as a plant DNA Barcode. ITS, a potential barcode region, could be easily recoverable for sequencing from herbarium samples or other degraded samples than plastid DNA but paralogues and multiple uncorrected copies making it impossible to sequence the region without cloning. Therefore other coding and non-coding regions of plastid genome concentrated for a potential DNA barcode. Kress and Erickson (2007) tested ITS, accD, ndhJ, matK, trnH-psbA, rbcLa, rpoB, rpoC1, ycf5 regions of DNA on 48 phylogenetically diverse genera of 43 families (from 39 orders) and proposed the gene loci rbcLa and the non-coding spacer region trnH-psbA of plastid as a Barcode. Kim and Lee (2007) proposed two non-coding spacer regions, atpF-atpH and trnH-psbA based on whole plastid genome sequencing of vascular plants. After number of studies (see table. 1) a final evaluation of the resulting seven leading candidate regions atpF-atpH, matK, rbcL, rpoB, rpoC1, psbK-psbI, trnH-psbA under the Plant Working Group (PWG) led to the proposal that partial (single-read) regions of rbcL and maturase K (matK) could be used as a two-locus plant DNA barcode. PWG's first proposal for a standardized protocol to barcode all land plants was published in 2007 followed by a proposal of standard DNA barcodes for land plants after 2 years (CBOL- Plant Working Group, 2007; 2009). Other non-coding regions are likely to be useful to enhance the discriminatory power of the barcode in particular taxa. A large number of studies conducted through RAPD, SCAR markers have been developed for the identification of several plant materials (Sun *et al.,* 2011; Yu *et al.,* 2011; Doh and Oh, 2012; Li and Park, 2012; Abdin *et al.,* 2012). Heubl (2010) and Techen *et al.*, (2014) suggested that RAPD, RFLP, ARMS, CAPS, AFLP, DAF, ISSR, SSR markers, sequencing, hybridization, microarrays and DNA chip technology could be used in DNA barcoding or identification of traditional medicinal materials.

rbcl and matK combination has been approved by Consortium for the Barcode of Life (CBOL) as a global DNA barcode for land plants, while trnH-psbA remains under scrutiny as a backup barcoding locus (Biswal *et al.,* 2012). However rbcL and maturase K (matK) failed to completely fulfill the desired characteristics of Barcode. Several studies were conducted on closely related species or single lineages in which the discriminatory power of the core barcodes has been questioned (Hollingsworth *et al.,* 2009; Pettengill and Neel, 2010; Roy *et al.,* 2010; Wang *et al.,* 2010; Clement and Donoghue, 2012; Liu *et al.,* 2012). The China Plant Barcode of Life Group (2011) proposed the

addition of nuclear ITS (Internal Transcribed Spacer) to the matK + rbcL combination as plant barcode in order to achieve maximum identification rates even in closely related species (Techen *et al.*, 2011).

Table – 1: The loci tested and suggested by different groups for DNA Barcoding of plants

S. No	DNA segment tested for barcodes	Proposed regions	References
1	ITS, atpB-rbcL, psbM-trnD, trnC-ycf6, trnH-psbA, trnL-F, trnK-rps16, trnV-atpE, rpl36-rps8, ycf6-psbM	ITS and trnH-psbA	Kress *et al.*, 2005
2	ITS, rbcL, trnH-psbA	ITS and trnH-psbA	Kress *et al.*, 2005
3	ITS, accD, ndhJ, matK, trnH-psbA, rbcLa, rpoB, rpoC1, ycf5	rbcLa and trnH-psbA	Kress & Erickson, 2007
4	atpF-atpH, atpH-atpI, rps15-ycf1, ndhG-ndhI, psbK-psbI, petA-psbJ, trnH-psbA	atpF-atpH+ psbK-psbI	Kim & Lee, 2007
5	rpoC1, rpoB, matK, trnH-psbA, ITS, trnL-F	rpoC1+ rpoB+ matK and rpoC1 + matK + trnH-psbA	Chase *et al.*, 2007
6	ITS, accD, ndhJ, matK, trnH-psbA, rpoB, rpoC1, ycf5	ITS	Sass *et al.*, 2007
7	trnL (UAA) intron	trnL9UAA intron	Taberlet *et al.*, 2007
8	accD, matK, trnH-psbA, rbcL, rpoB, rpoC1, UPA	matK and trnH-psbA	Newmaster *et al.*, 2008
9	matK, trnH-psbA, psbK-psbI, atpF-atpH	matK or matK + trnH-psbA + psbK-psbI	Lahaye *et al.*, 2008
10	accD, matK, trnH-psbA, rbcL, rpoB, rpoC1, ycf5, ndhJ	matK or matK + trnH-psbA	Lahaye *et al.*, 2008
11	Cox1, 23SrDNA, rpoB, rpoC1, rbcL, matK, trnH-psbA, atpF-atpH, psbK-psbI	rbcL, rpoB, matK, trnH-psbA, atpF-atpH	Fazekas *et al.*, 2008
12	CO1, rpoC, rpoB, rbcL-a, matK, trnH-psbA	TrnH-psbA + rbcL-a	Erickson *et al.*, 2008
13	AtpF-atpH, rpoB, rpoC1, rbcL, matK, psbK-psbI, trnH-psbA	rbcL + rpoC1 + matK + trnH-psbA	Hollingsworth *et al.*, 2009
14	accD, matK, ndhA, ndhJ, ndhK, rpl22, rpoB, rpoC1, rpoC2, ycj2, ycf5, ycf9	matK, rpoB, rpoC1, ndhJ, ycf5 and accD	Ford *et al.*, 2009
15	matK, rbcL, rpoB, rpoC1, trnH-psbA	MatK	Starr *et al.*, 2009
16	atpF-atpH, matK, rbcL, rpoB, rpoC1, psbK-psbI, trnH-psbA	rbcL + matK	CBOL- Plant Working Group, 2009

A potentially negative factor with ITS is presence of long poly-G, poly-C, and poly-A repeats that are difficult to sequence (Sass *et al.*, 2007). One of the loci rbcL of Global Plant DNA Barcode was easily retrievable across the land plants (Jeanson *et al.*, 2011; Kuzmina *et al.*, 2012; Burgess *et al.*, 2011; Pang *et al.*, 2011; Plant China BOL Group., 2011; Fazekas *et al.*, 2008; CBOL Plant Working Group. 2009) but the other matK was not so easily retrievable using universal primers particularly in the non-angiospermic samples (Ford *et al.*, 2009; CBOL Plant Working Group. 2009; de Vere *et al.*, 2012; China Plant BOL Group *et al.*, 2011; Sass *et al.*, 2007; Liu *et al.*, 2011).

Successfully recovery of the matK Loci from gymnosperms, taxon-specific primers are required (Fazekas *et al.,* 2008; Ran *et al.,* 2011). Li *et al.*, (2011) recommended a new set of matK primers for barcoding, with high PCR universality, high sequence quality, and high coverage across gymnosperms. Jing *et al.,* (2011) proposed a New universal matK primers for DNA barcoding matK472F (5-CCCRTYCATCTGGAAATCTTGGTTC-3) and matK1248R (5GCTRTRATAATGAGAAAGATT TCTGC-3) for angiosperms. Riaz *et al.*, (2011) presented ecoPrimers (http://www.grenoble.prabi.fr/trac/ecoPrimers) software which fulfills all requirements for designing new barcode regions suitable for metabarcoding studies. Tanabe and Toju proposed two new computational methods of DNA Barcoding and showed a benchmark for bacterial/archeal 16S, animal cox1, fungal internal transcribed spacer, and three plant chloroplast barcode loci (rbcL, matK, and trnH-psbA) that can be used to compare the performance of existing and new methods (Tanabe and Toju, 2013).

6. REFERENCE DATABASE

A freely accessible, single barcode library for all plants is desirable so that one could align the tested or unknown sample barcode to the reference barcodes. However several barcode libraries are there.

6.1 BOLD (The barcode of life data system, http://www.barcodinglife.com) was created and is maintained by the University of Guelph in Ontario. It offers researchers a way to collect, manage, and analyze DNA barcode data.

6.2 CBOL (Consortium for the barcode of life, http:// www.barcodeoflife.org/) is a public reference library of species identifiers which could be used to assign unknown specimens to known species. CBOL was founded in 2004 through support from the Alfred P. Sloan Foundation and promotes barcoding through working groups, networks, workshops, conferences, outreach, and training. CBOL has 200 member organizations from 50 countries and operates from a Secretariat Office located in the Smithsonian Institution's National Museum of Natural History in Washington, DC.

6.3 iBOL (International Barcode of Life project, http://www.ibol.org/) consists of a group of hundreds of scientists from 26 nations working together to gathering barcode records from all multi-cellular life and building the informatics platform needed to store these records and to enable their use in species identification.

6.4 MMDBD (Medicinal Materials DNA Barcode Database, http://137.189.42.34/mherbsdb/) this DNA barcode database for users to retrieve and analyze DNA sequences of medicinal materials. This website includes DNA sequences and information and key references of the medicinal materials recorded in the Pharmacopoeia of the People's Republic of China, American Herbal Pharmacopoeia and other related references (Lou *et al.,* 2010).

6.5 BioBarcode (Asian BioBarcode, an exemplary DNA Barcode System, http://www.webcitation.org/6DkLfe69s) used for promoting international collaboration for building an Asian biodiversity system aiming to be the Asian biodiversity database server (Lim *et al.,* 2009).

6.6 NCBI (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/)

7. CONCLUSION

matK, rbcL, ITS, and trnH-psbA have been studied extensively as the barcode loci for plants (Gonzalez *et al.,* 2009; CBOL Plant Working Group. 2009; Roy *et al.,* 2010; Starr *et al.,* 2009; de Groot *et al.,* 2011; de Vere *et al.,* 2012; Ebihara *et al.,* 2010; Gao *et al.,* 2010; Kress *et al.,* 2009; Li *et al.,* 2011; Wang *et al.,* 2010). But each locus has its own strength and weakness, therefore yet the screening for single or multiple Loci from plastid and nucleus, appropriate for DNA barcoding in different plant groups, has been an important research focus around the globe (Tripathi *et al.,* 2013). Overall it was considered more likely that it would be possible to improve the recoverability of matK through the improvement of primer universality than to overcome the sequence quality issues of the tested non-coding regions.

8. FUTURE PROSPECTS

After completing a decade of DNA Barcoding still there are not single loci which can work universally all over the plant kingdom. There is a need to work in the

development of DNA Barcoding primers and an automated system to predict which primers will work well in a given taxonomic group so that laboratory success rate could be improved. Work should also be undertaken for improvement of PCR and sequencing protocols for regions rich in mononucleotide repeats so that the ITS regions can be sequenced. Robust multiplex PCR protocols can be developed for routinely amplifying the core rbcL+matK barcode or supplementary markers, simultaneously. Thus we could reduce laboratory expanses and the potential for laboratory error during Barcoding of plants. Work has to be done for enhancement of mini-barcodes for degraded DNAs to facilitate the identification of processed plant material and degraded samples. The computational tools also shall be developed which help us to obtain a universal primer from the stored barcodes data.

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