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Reviewing DNA barcoding as a means of identifying rare and endangered plant species

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Abstract

Genamic barcoding is a relatively new concept. The system was developed to automate the identification of species with high precision and speed by using tag DNA sequences that are already widely used in biology. DNA barcoding may be helpful to for taxonomists and environmentalists. The initial goal of DNA barcoding is to create a database of all species' barcode sequences that can be accessed online. These databases will be used as a reference point against which DNA barcodes from recognised and unidentified samples may be compared. Because of this, it may be possible to solve several problems with conventional structural taxonomy, such as misinterpreting species because of phenotypic variation and genotypic fluctuation of the characters, failing to recognise cryptic taxa, and having trouble locating reliable characters because of lengthy maturation times (CBOL Plant Working Group, 2009). Locations where damage or a lack of development of morphological features makes it difficult to accurately identify species using just morphological qualities might benefit greatly from this method. DNA barcoding is a helpful tool since it provides information about undetected species, but it should not be mistaken for classification. Methods for selecting and redefining plant barcodes are discussed, and the authors share their thoughts on and recommendations for future research and practise.

Keywords : barcoding, species, cyperaceae, endangered, specimens, phylogenies

INTRODUCTION

DNA barcoding is a technique that has the potential to be used for the accurate identification of species. For the most effective preservation and use of plants, particular species identification via plant DNA barcoding is essential. Barcoding based on plant DNA is already being used in a number of different contexts. Regrettably, in many parts of the globe, a deficiency of taxonomical competence may cause this process to go more slowly (Chase et al., 2009). When trying to determine the species of a plant, it is possible that some plant parts, such as seeds, pollen, roots, or even samples of air, soil, and water, may be just as valuable as the whole plant itself (De vere et al., 2012). DNA barcoding has been used to gather genetic sequences, which have been used to create phylogenetic trees, which are utilized in ecological, phylogenetic communities. Phylogenetic plants are used to classify organisms according to their evolutionary relationships (Krees et al.,2009; Krees et al., 2010). The Plants Working Group of the Collaboration for the Bar codes of Life suggested adopting portions of the plastid genes rbcL and matK as the standard plant DNA barcodes, despite the fact that they recognized the possibility that other markers would be necessary. DNA barcoding is an efficient method of identification, but only if we are able to build sequence reference databases that can be trusted (Hollingsworth et al., 2011). Each DNA sequence has to be linked to the particular plant specimen from which it was extracted, as well as the date, location, and identity of the researcher who made the first discovery of the sequence. Customers of the information require access not just to the

DNA sequence itself but also to the primers used to make it, trace files, and character statistics (De vere et al., 2012). Because the Gene Bank is where DNA sequences are kept, any pertinent information must be made accessible to the general public there. Last but not least, give some thought to including your information in the Barcode of Life Information system (BOLD). BOLD is a system for managing tasks that also maintain relevant content, such as scanned herbarium specimens, trace files, and images.

FUTURE PERSPECTIVES AND PERSPECTIVES FOR PLANT DNA BARCODING:

Since its introduction to the botanical world more than a decade ago, DNA barcodes have been used in many studies spanning fundamental and applied plant research.

In order to better serve the botanical community, plant DNA barcoding is expected to progress in two main areas shortly.

- The expansion of a universally usable database of DNA barcodes for plants across the world.
- Creating novel markers and using cutting-edge sequencing methods.

matK gene

MatK is one of the genes with a high mutation rate because of how rapidly it evolves. It is around 1550 base pairs in length and encodes the maturase enzyme, which is required for the excision of type-2 introns in RNA transcripts (Neuhaus et al., 1987). Because it is situated in group 2 of trnK's introns, matK may be amplified by PCR using a primer set constructed from conserved areas in trnK, rps16, and psbA because matK is a component of trnK. Due to the rapid pace at which it is evolving, some people have investigated the idea that matK may be utilized as a plant barcode. Separately and in tandem with other locations, this idea has been explored. Examples include a study that found rpoC1 + rpoB + matK to be the most influential trio for land plant barcoding out of 98 examined cpDNA loci (Ford et al., 2009).

The matK gene was found to be the most promising candidate for use as a universal barcode for terrestrial plants after experiments were conducted using matK, rbcL, rpoB, rpoC1, and trnH-psbA in the Cyperaceae family (Starr et al., 2009).

Due to its fast development and extensive distribution in plant species, the matK gene has been utilized as a marker in building phylogenetic trees (Hilu et al., 1997; Kelchner et al., 2000). (Hilu et al., 1997; Kelchner et al., 2000). Nevertheless, it was also shown that PCR-based attempts to amplify matK in a few taxonomic classes failed. To get around this issue, researchers have created primer sets that work well across a wide range of taxonomic groupings (Cuenoud et al., 2002). This primer set amplifies a 930-bp DNA fragment beginning at position 429 in the matK sequence and ending at position 1313. For example, (Schmitz-Linneweber et al., 2001; Cuenoud et al., 2002). The evolutionary links of many angiosperms have been resolved to owe to the revelation that matK evolved at a pace that is adequate for differentiating between diverse genera and species (Soltis et al., 1998; Johnson et al., 1995). (Soltis et al., 1998; Johnson et al., 1995). Tests conducted by the CBOL Plant Working Group found that over 87% of angiosperm matK samples could be amplified and sequenced with only one set of primers. Compared to angiosperms, the success rate was much lower for cryptogams (15%) and gymnosperms (86%).

The CBOL Plant Working Group recommends using matK and rbcL as the standard two-locus barcode for plants thanks to the large variety of uses for these barcodes and their ability to identify between various species.

rbcL gene

Compared to other gene sequences, the rbcL gene has been the subject of the most crucial study and analysis. Since this was discovered, most of the research group looked into its potential uses in

barcoding. Enzyme rubilose 1,5-bisphosphate carboxylase/oxygenase has a unique gene that codes for its most significant component (RUBISCO). RUBISCO plays a crucial role in photosynthesis. The rbcL gene was the first plant gene ever sequenced (Zurawski et al., 1981). One approach to the issue of achieving sufficient species discrimination to justify its usage as a barcoding sequence was to amplify short sequences with enough diversity. This was one answer to the problem of attaining acceptable species discrimination to allow its usage; the optimum Molecular genetic region should be small enough to amplify from degrading DNA, and it should be assessed using single pass sequencing (Chase et al., 2007). Most taxa have primers for PCR amplification and sequencing of this small region of the rbcL gene. This was made feasible by the ease with which PCR amplification may be done on many plant groups. For example, (Krees et al., 2007; Fay et al., 1997). Most phylogenetic analyses find that rbcL works best for reconstructing generic relationships, but provides little help when working at the species level (Soltis et al., 1998).

Agarose Gel Electrophoresis

Electrophoresis is well-proven as a reliable and systematic method for separating nucleic acids. Due to their high gel strength, low-percentage agarose gels can successfully separate even considerable DNA fragments. The pore size created by the agarose filaments is controlled by molecular sieving in the gel matrix (Devor, 2010). Centrifugation on polyacrylamide gels is the best method for isolating DNA particles with fewer than 110 pairs. As agarose concentration increases, the pore size tends to shrink. It is possible to create low-melting agarose by adding structural modifications to ordinary agarose. Low-melting agarose is used as a precipitant to separate the DNA fragments. The pore size of agarose may be a significant achievement by hydrooxyethylation, which declines the compressibility of the agarose packages. As a result, the pores get smaller. Since the bundles in an agarose gel interact through non-covalent interactions, the gel may be re-melted after it has been set. This means that a DNA fragment of the same size will take longer to move across a low-melting agarose gel than a regular agarose gel (Dea et al., 1972). In some scenarios, however, such as when it is difficult to dispose of hazardous material or when young children are conducting an inquiry, a less harmful dye may be used.

Loading dyes have three main functions during gel electrophoresis:

- The sample will have to be made denser so that it can be deposited into the gel.
- Dye additives give foods their hue and make transportation more convenient. In conclusion, since the dyes migrate at constant rates across the gel, the amount that the Dna molecules have traveled may be calculated.

Polymerase Chain Reaction (PCR)

The Polymerase Chain Reaction, often known as PCR, is a method that may be used to calculate the length of a brief DNA or RNA sequence by using components with a deficient copy number. The objective of creating a high number of copies of a specific area of DNA or RNA may be achieved via the use of PCR to accomplish this goal. In the past, amplifying DNA would take several weeks, and even more, time would be necessary to clone the relevant sections into vectors for expression in bacteria. This was because the procedure was performed manually. However, as a result of the invention of PCR, which can be performed in test tubes, we can now complete this process in a few hours. The polymerase chain reaction, often known as PCR, is a technology that has shown to be very successful since it can eliminate an infinite number of DNA copies.

In contrast to this, the substances that are used in the PCR process are the same molecules that are required by nature to duplicate DNA:

1. Primers are two brief sequences of solitary Genes that, when placed together, seem to be the final stage of the Segment of DNA that is being copied. Primers are used in the process of DNA cloning. Primers are used in the process of DNA cloning. To make the process of copying go more quickly, you may employ primers.

2. As it moves along a strand of DNA, a polymerase enzyme reads the code as it goes and gathers the DNA building blocks it will need to construct a duplicate of the strand. This process occurs as the enzyme moves along the strand.

The PCR method may be separated into three individual stages that are carried out in order. It is recommended that you do thirty to forty repetitions of each of these three procedures in order to maximise your results. It is necessary to utilise an automated cycler in order to get the cycles ready. This gadget swiftly warms and cools the test tubes that contain the reaction mixture. It does this by transferring heat from one line to another.

1. Denaturation: When DNA is heated to 95 degrees Centigrade, the typical DNA molecule's double strand of DNA will melt and separate into two single strands. This process is known as denatured proteins. Denaturation is the term that describes this process.
2. At temperatures in the middle range, around 54 degrees Celsius, primers may polymerize to the single-stranded "pattern" (The template is the sequence of DNA to be copied). After the primer and template have been joined by the polymerase, there will be a brief stretch of double-stranded DNA. The polymerase will then begin the process of duplicating the template after it has completed this step.
3. When the temperature is brought up to 72 degrees Celsius, the DNA polymerase reaches its maximum active state, and it is then possible for DNA building blocks that are complementary to the template to be attached to the primer. As a consequence of this, a molecule of DNA with two strands will be generated.

A given individual copy of a two-stranded DNA template can be amplified into 2 genes in a single cycle via the amplification process. In the next cycle, these two aspects could get more attention than they did in the previous one. The growth rate of template copies produced throughout each cycle iteration is exponential. This results in an ever-increasing number of documents being made. The response has very little work to do to carry out the instructions for this answer. The only necessary things are heat, a test tube, and some chemicals that are not very expensive.

Sanger sequencing for DNA

A single individual copy of a double-stranded DNA template can be amplified into two copies in a single cycle via the amplification process. Additional focus may be placed on these two aspects throughout the next cycle. The growth rate of the number of template copies produced throughout each cycle iteration is exponential. This results in an ever-increasing number of documents being made. The response has a minimal amount of work to do to carry out the instructions for this answer. You will need a test tube, some inexpensive chemicals, and heat to do this experiment.

1. The number of nucleotide bases that are present in the target DNA sequence is used to calculate the appropriate number of DNA fragments to create, and each fragment is completed by adding a nucleotide that has been tagged at the end of the process. You will need a DNA primer, nucleotide (dATP, dCTP, dGTP, and dTTP), DNA polymerase, the DNA sequence that you wish to target, and some tagged dideoxynucleotides in order to be successful in completing this work (ddATP, ddCTP, ddGTP, ddTTP). It is not feasible to add any more nucleotides to the DNA chain after a dideoxynucleotide has been added to it. As a consequence of this, the very last nucleotide in each fragment will be a tagged one. Dideoxynucleotides are only used in very minute quantities in compared to the vast quantities of ordinary nucleotides that are called for.
2. With the use of capillary gel electrophoresis, the n distinct DNA sequences may be arranged in increasing order of their length after being separated from one another. When compared to moving smaller parts, moving bigger ones takes much less time. After the conclusion of the second stage, the DNA fragments that were produced are transferred to the third stage in the procedure in the reverse order of the length of the sequence they contain.

3. After the conclusion of each sequence, a laser is used in order to activate the label that has been attached to the nucleotide. This allows the nucleotide to be identified. In order to correctly attribute the light produced by each stimulated nucleotide, a one-of-a-kind label is placed to each base. This allows for the light to be appropriately assigned. With the help of the laser, we are able to pinpoint the exact position of the fluorescent peak that is caused by each nucleotide in a chromatogram. This is something that was previously impossible. The use of electrophoresis served the purpose of demonstrating that the DNA fragments shown in the chromatogram were ordered in the appropriate sequence.

If there is only one copy of the template and the target sequence is 1,000 nucleotides long, it will take much more time to create the 900 tagged fragments. On the other hand, if there are many copies of the template, it should be able to produce all 1,000 of the labelled segments in a shorter period of time, at least according to the theory (sigma Aldrich Sanger sequencing).

CONCLUSION

DNA barcoding has made it possible to obtain standard DNA barcodes from even the rarest and most endangered plant species. Methods that contextually enhance search efficiency utilising frequently used plant names and character-based keys for differentiating closely related species when using plant DNA identification will need to be invented in the not-too-distant future. In cases when the flowers or fruit of the desired plant cannot be obtained, this method may be used to accurately identify commercial products like herbal medicine compounds, rice kinds, and timber wood.

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