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Review on use of recent molecular techniques to access biodiversity in plants

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Abstract

Molecular tools developed in the past few years provide easy, less laborious means for assigning known and unknown plant taxa. These techniques answer many new evolutionary and taxonomic questions, which were not previously possible with only phenotypic methods. Molecular techniques such as DNA barcoding, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites and single nucleotide polymorphisms (SNP) have recently been used for plant diversity studies. This review presents a basic description of different molecular techniques that can be utilized for DNA fingerprinting and molecular diversity analysis of plant species. DNA barcoding uses particular regions of DNA making helping in categorization and recognize unknown species. Researchers now interested to generate DNA barcodes designed for all living organisms and to build up data accessible to public to help in understanding of natural biodiversity of world. Cyclotides are peptides derived from plants with particular head to tail cyclic backbone that have three disulphide bonds by forming a cystine knot. Recent information about DNA barcoding can be used for detection of unidentified biological specimens to a taxonomic group, accurate detection of phytomedicinals, and in the biodiversity of living organisms.

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Introduction

Molecular techniques such as DNA barcoding, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites and single nucleotide polymorphisms (SNP) have recently been used for plant diversity. DNA barcoding is especially used to determine the data base that identifies different species. Random Amplified Polymorphic DNA (RAPD) technique is PCR based procedure which is uses the random primers which bind to the nonspecific positions on the DNA and amplification of DNA and some molecular markers are also utilizes PCR primers comprising of nonspecific sequences in different length and size of nucleotides. Microsatellites and single nucleotide polymorphisms (SNP) Assortment arises through reproduction slippage, inadequate crossing over, modifications enhancement or disturbing the sequences of replications, although single nucleotide polymorphisms SNPs originate through point mutations. Simple series replications are tandem repeats of small as 10 base pairs and DNA sequences that are useful markers for genomic mapping and Loss of heterozygosity of well-defined chromosomal loci. Single nucleotide polymorphisms SNPs is type of genetic variations of different plant population in biodiversity. The nucleotides are associated at specific position but in SNPs the substitution of a single nucleotide to another nucleotide that distinguish the diversity of plants on genetic bases (Altschul *et al.*, 1990).

The conservation and sustainable use of plant genetic resources require accurate identification of their accession. The emergence of DNA-based markers has changed the practice of species identification techniques(Ahmad *et al.*, 2019).The dramatic advances in molecular genetics over the last few years have provided workers involved in the conservation of plant genetic resources with a range of new techniques for easy and reliable identification of plant species(Armstrong *et al.*, 2005). Many of these techniques have been successfully used to study the extent and distribution of variation in species gene-pools and to answer typical evolutionary and taxonomic questions.

Many of these techniques have been successfully used to study the extent and distribution of variation in species gene-pools and to answer typical evolutionary and taxonomic questions. DNA barcoding is a practice with the purpose to discover the varieties based on species specific differences in short sequences of their DNA (Hebert *et al.*, 2003). DNA barcoding utilizes principles of biochemistry, microbiology and biotechnology to recognize plant species in most efficiently detection method that is faster and accurate as compared to other traditional methods. This skill is now adopted in morphological characteristics, physiological conditions and allows species discovery without individual taxonomic information (Erickson *et al.,* 2014). This has enabled research scientists especially in field of molecular biology to put efforts on DNA barcoding technique to estimate the herbal plant and related biological products accuracy (Hebert *et al.*, 2003).

Sequencing based molecular techniques provide better resolution at intra-genus and above level, while frequency data from markers such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and microsatellites provide the means to classify individuals into nominal genotypic categories and are mostly suitable for intra-species genotypic variation study**.** This distinction is important to grasp for population studies, particularly when the diversity data are used as a basis for making decisions about conservation of plant resources. For instance, a recent study on Napier grass has showed that AFLP is incompatible with RAPD and morphological data; re-registration of all accessions of Napier grass based on DNA barcoding is suggested as a means to resolve the lingering problems regarding the identity of accessions. The main objective of this review is to provide a basic understanding of the recently developed molecular tools and their potential application in the conservation of plant resources (Olsvik *et al.*,1993).

The aims of the research are to access the biodiversity of different plants using molecular techniques and for the identification of different organisms. Molecular techniques such as Amplified Fragment Length

Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), DNA barcoding, Microsatellites and single nucleotide polymorphisms (SNP) have recently been used for plant diversity studies. To access the different verities of plants and microorganisms these techniques evaluate the biodiversity between them. As in case of diseased plant to access the pathogen attack on susceptible plant and change in growth pattern of normal plant.These techniques are most necessity part of research on biodiversity and genetics that distinguish the next verities in future. Resistant verities of different plants having R gene that have high potential to resist the pathogen attack. These techniques play a significant role in RNA, DNA and Protein synthesis etc.

Molecular Techniques in Plant Biodiversity

Conventional Sequencing Technique

Currently, dye-terminator sequencing technique is the standard method in automated sequencing analysis(Olsvik *et al.*,1993).The dye-terminator sequencing method, along with automated high-throughput DNA sequence analyzers, is now being used for the vast majority of sequencing work. The basic technique related with dye terminator sequencing and phylogenetic analysis is illustrated in Fig. 1. shows that Dye-terminator sequencing utilizes labeling of the chain terminator ddNTPs, which allows sequencing in a single reaction, rather than four reactions as in the previously used.

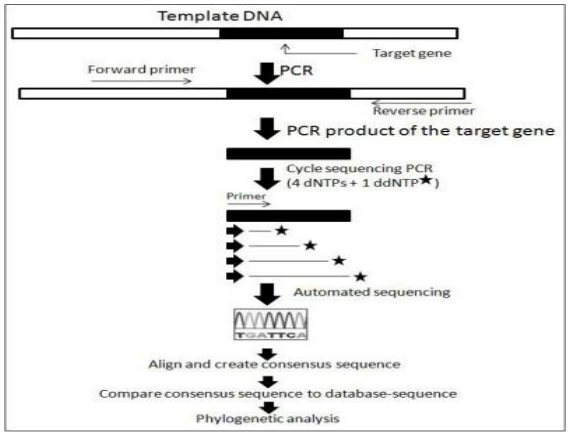

Fig. 1. Schematic diagram summarizing the sequencing of a target gene for application in phylogenetic analysis.

Current interest is in the DNA barcoding of plants with the aim to identify an unknown plant in terms of a known classification. DNA barcoding is a technique for characterizing species of organisms using a short DNA sequence from a standard and agreed-upon position in the genome(Hamilton *et al.*, 2012).

Next Generation Sequencing Techniques

Next generation platforms do not rely on Sanger chemistry as did the first generation machines used for the last 30 years (Schuster *et al.*, 2008). The first of this kind of 2nd generation of sequencing technique appeared in 2005 with the landmark publication of the sequencing-by-synthesis technology developed by 454 Life Sciences based on pyro sequencing.Commercial 2nd generation sequencing methods can be distinguished by the role of PCR in library preparation. There are four main platforms; all being amplification-based: (i) Roche 454 GS FLX, (ii) Illumina Genome Analyzer IIx, (iii) ABI SOLiD 3 Plus System and (iv) Polonator G.007. Common principles of these 2nd generation sequencing techniques are illustrated in Fig. 2.

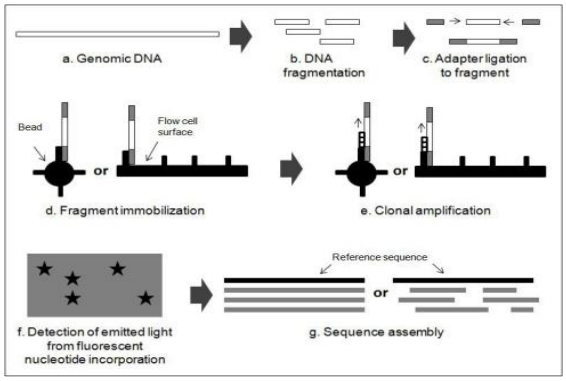

Fig. 2. A common workflow of next-generation sequencing methods.

Random Amplified Polymorphic DNA (RAPD)

Random Amplified Polymorphic DNA principle basis on DNA amplification that uses single primerwith correct nucleotides sequences. Then next these primers become aware of polymorphism due to nonappearance of correct nucleotide sequence in orderand polymorphism purpose as a genetic DNA marker and that purpose to build genetic map using biotechnological tools. In view of the fact that most of

Random Amplified Polymorphic DNA (RAPD) markers are predominant that becomes possible to make a distinction whether the amplified segment of DNA is heterozygousand homozygous at aexactingposition of locus. In exceptional cases, co-dominant Random Amplified Polymorphic DNA markers experientially observed as different-sized DNA segments that can be amplified from similar locusmight be detect (Williams *et al*., 1990).

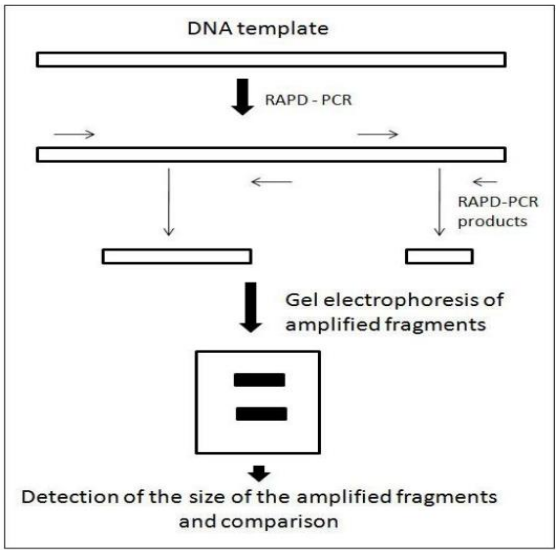

Fig. 3. The principle of RAPD-PCR technique.

Molecular DNA Barcoding

Tissue samples obtained starting specimens by now housed in herbaria or be able to be in use directly from live specimens in field with properly pressed, tagging, and put into voucher specimens. These specimens can give out as a vital permanent documentation that can matched with the DNA barcode to a selective species of plant (Kress *et al*., 2005). The method of generating and to apply DNA barcodes for the principle of recognition of biological specifies involve two steps one is the creating of the DNA barcode library of identified species and second one is the matching of DNA barcode sequence of an unidentified sample against the DNA barcode library(Coissacet., 2016).

The primary step is to select single to a number of individuals per species to provide as reference sample in library of DNA barcode that is called DNA barcode library.

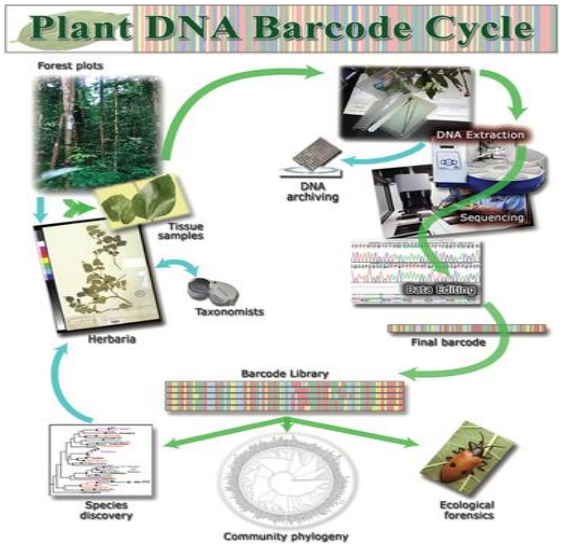

Fig. 4. Principles stepsin DNA barcoding.

Amplified Fragment Length Polymorphism

The Amplified Fragment Length Polymorphismtechnique used in molecular biology is based on discriminatory PCR amplification by polymerase chain reaction of restriction fragments from a total digest of genomic DNA. The technique involves stages of followed by extraction of highly purified DNA, restriction endonuclease digestion of DNA enzyme mixture, usually Eco RI and MseI, ligation of adapters. The electrophoretograms can be analyzed using programs like Gene Mapper.AFLP is applicable to all species, and unlike RAPD, this technique is highly reproducible as it combines restriction digestion and PCR. However, AFLP requires more DNA (300–1000ng per reaction) and is more technically demanding than RAPD, however the automation and recent availability of kits means that this technology can be brought to a higher level (Webb *et al*., 2005). Metals toxicity also leads to death of certain plants (Shafiq *et al*., 2019). Nuclear and chloroplast sequences sometimes fail to revealain plants variability when plant species are closely related. However, AFLP distributed throughout the whole genome provides a robust solution to overcome the hurdles in plants fingerprinting (Vos *et al*.1995).

Identification of Species in Biological Diversity

In additional cases, morphological and natural variants that activated to generate sequence records (Swenson *et al*., 2012) to set up whether nearby is at

the substructure of genetic substantiation for recognizing of different taxa and subfamilies of ecologically important species. Discoveries of biological species is an important and revolutionary step towards all related information getting of the filled spectrum of class from comparatively small (Kress *et al*., 2009) in addition to natural poor groups such as bryophytes all the way through to striking ecologically as well as ethnically important vegetation (Liu *et al*., 2013).

Plant DNA barcodes are especially important applications in biological discovery of supplementary in order to detect new taxa (Swenson *et al*., 2013) and biodiversity new groups related to plants. In several research studies, surprising sequence differences led to reconsideration of morphological and environmental dissimilarity (Lahaye *et al*., 2008).

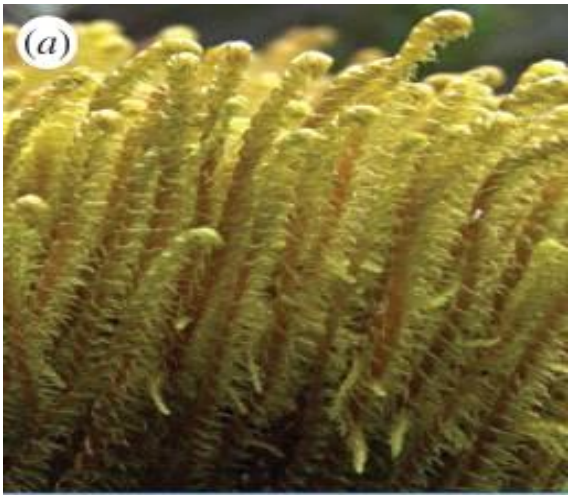

Fig. 5(a). Species Dicovery of Bryophyte (Herbertus).

Fig. 5(b). National DNA barcode of flora of Wales.

Fig. 5(c). Floristic barcoding of Cape Flora.

Fig. 5(d). DNA barcode of flora of China.

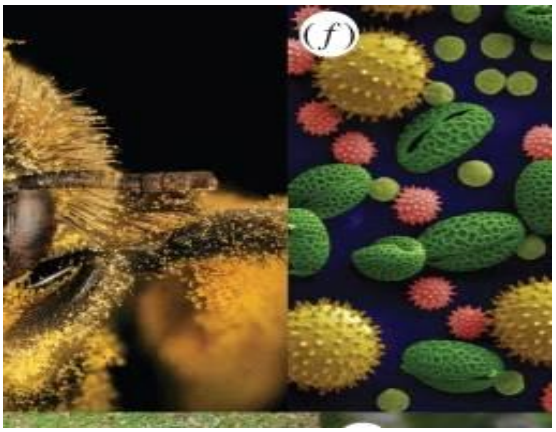

Fig. 6(e). Study of pollen Movement.

Role of DNA Barcoding in Plants

Many of these investigations in which DNA barcodes have been applied to commercial medicinal (Nicole *et al*., 2008) and herbal supplements have concluded that in some cases the genetic markers used, which varied quite widely among studies, were not able to discriminate among species States (Newmaster *et al*., 2013). Timber is not the only commercial plant product in need of accurate species identifications by regulators and quality control specialists. Traditional medicines, teas, and herbal supplements together are an important and large component of the commercial

market in biodiversity, locally, nationally, and internationally. It is estimated that medicinal plants account for over US$60 billion in annual revenues in the United States (Techen *et al.*, 2014), for a review of statistics on markets and use. From the early development of plant DNA barcodes, applications to monitor this market have been in development (Costion *et al.*, 2016).

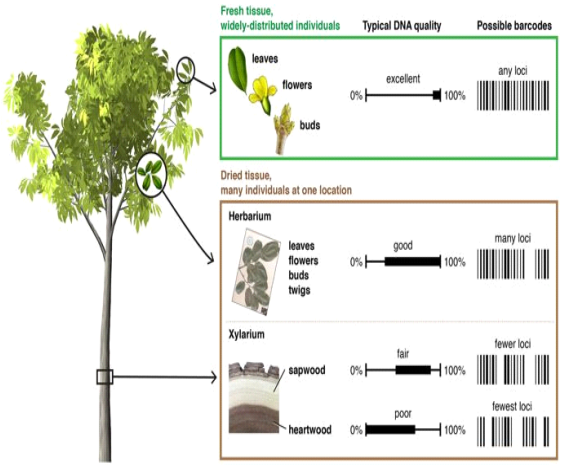

Fig. 6. Plant Forensic Tools in DNA barcoding technique.

Currently known distribution of cyclotides in five major families of angiosperms plants, illustrating that they are found in five major families of angiosperms, namely the *Rubiaceae, Violaceae, Cucurbitaceae, Solanaceae*, and *Fabaceae* (Newmaster *et al.*, 2013). Cyclotides are ubiquitous in the *Violaceae*, having been found so far in the >35 species in this family that have been screened (Craik *et al.*, 2015).

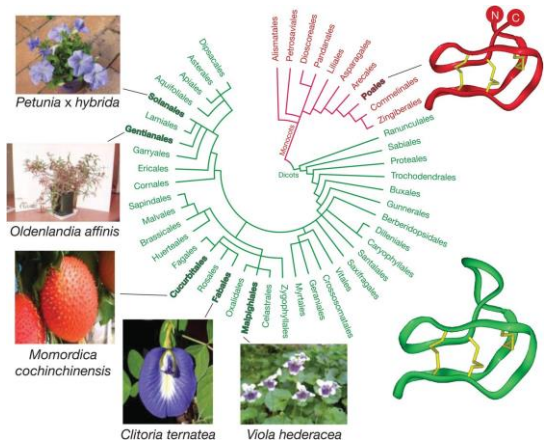

Fig. 6. shows the currently known distribution of cyclotides in five major families of angiosperms plants.

Phylogenetic tree showing the distribution of known cyclotides in the orders Solanales (Solanaceae family), Gentianales (Rubiaceae family), Cucurbitales (Cucurbitaceae family), Fabales (Fabaceae family), and Malpighiales (Violaceae family) (Kress *et al.*, 2005). Thus far, cyclotides have been only show in dicotyledons (green), as illustrated by the green cyclotidestructure (Kress *et al.*, 2011). In monocots (red), linear cyclotide analogues (red structure) with the same fold (cystine knot) but lacking the head-to-tail cyclic backbone have been described. Typical cyclotide-containing representatives of each family are shown for each order (Newmaster *et al.*, 2012).

Conclusion

There are many diseases that affected the most plants and finally fills them.Therefore, it is important to carry out research on more techniques at molecular level potentially against viruses and bacterial diseases. The achievement goals of sequencing technology such as operation of microfluidic PCR basis with improvement in traditional techniques with the intention to offer a quicker and less exclusive option at huge scale multi locus on plant DNA barcoding are diagnostic tool of current state of discoveries in genomics.

References

Ahmad I, Khan S, Naeem M, Hayat M, Azmi UR, Ahmed S, Murtaza G, Irfan M. 2019. Molecular Identification of Ten Palm Species using DNA Fingerprinting, Int. J. Pure App. Biosci **7(1),** 46-51.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J. Mol. Biol **215,** 403-410.

Armstrong KF, Ball SL. 2005. DNA barcodes for biosecurity: invasive species identification. Philos Trans R Soc B BiolSci **360,**1813-1823.

Baldwin BG.1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. Mol. Phylogenet. Evol **1**, 3-16.

Coissac E, Hollingsworth PM, Lavergne S, Taberlet P. 2016. From barcodes to genomes: extending the concept of DNA barcoding. Molecular Ecology **25,** 1423-1428.

Costion CM, Kress WJ, Crayn DM. 2016. DNA barcodes confirm the taxonomic and conservation status of a species of tree on the brink of extinction in the Pacific. PLOS ONE **11,** e0155118.

Craik DJ. 2015. Plant cyclotides. Advances in Botanical Research, Volume **76**, *London: Academic Press.*

Erickson DL, Jones FA, Swenson NG, Pei N, Bourg N, Chen W, Davies SJ, Ge X, Hao Z, Howe RW, Huang CL, Larson A, Lum S, Lutz JA, Ma K, Meegaskumbura M, Mi X, Parker JD, Fang Sun I, Wright J, Wolf AT, Ye W, Xing D, Zimmerman JK, Kress WJ. 2014. Comparative evolutionary diversity and phylogenetic structure across multiple forest dynamics plots: A megaphy logeny approach. Frontiers in Genetics **5,** 358.

Hamilton JP, Buell CR. 2012. Advances in plant genome sequencing. Plant J, **70,** 177-190.

Hebert PD, Cywinska A, Ball SL, de Waard JR. 2003. Biological identifications through DNA barcodes. Proc Bio lSci, **270,** 313-321.

Kress WJ, Erickson DL, Jones FA, Swenson NG, Perez R, Sanjur O, Bermingham E. 2009. Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. Proceedings of the National Academy of Sciences USA **106,** 1862-18626.

Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH. 2005. Use of DNA barcodes to identify flowering plants. Proceedings of the National Academy of Sciences USA **102,** 8369-8374.

Kress WJ. 2011. DNA barcoding and systematic monographs. In: T Stuessy, W Lack eds. Monographic plant systematics: Fundamental assessment of plant biodiversity. Berlin: Regnum Vegetabile 153. A.R. G. Gantner Verlag K.G. 49-71.

Lahaye R, Bank M, Bogarin D. 2008. DNA barcoding the floras of biodiversity hotspots. Proc Natl Acad Sci USA, **105,** 2923-2928.

Liu YJ, Newmaster SG, Wu XJ, Liu Y, Ragupathy S, Motley T, Long CL. 2013. Pinelliahunanensis (Araceae), a new species supported by morphometric analysis and DNA barcoding. Phytotaxa **130,** 1-13.

Newmaster SG, Fazekas AJ, Steeves RAD, Janovec J. 2008. Testing candidate plant barcode regions in the Myristicaceae.Molecular Ecology Resources **8,** 480-490.

Newmaster SG, Grguric M, Shanmughanandhan D, Ramalingam S, Ragupathy S. 2013. DNA barcoding detects contamination and substitution in North American herbal products. BMC Medicine **11,** 222.

Nicole S, Negrisolo E, Eccher G, Mantovani R, Patarnello T, Erickson DL, Kress WJ, Barcaccia G. 2012. DNA barcoding as a reliable method for the authentication of commercial seafood products. Food Technology and Biotechnology **50,** 387-398.

Olsvik O, Wahlberg J, Petterson B, Uhlén M, Popovic T, Wachsmuth IK, Fields PI. 1993. Use of automated sequencing of polymerase chain reaction-generated amplicons to identify three types of cholera toxin subunit B in Vibrio cholerae O1 strains. J. Clin. Microbiol. **31,** 22-25.

Schuster SC. 2008. Next-generation sequencing transforms today's biology. Nat. Methods **5,**16-18.

Shafiq S, Adeel M, Raza H, Iqbal R, Ahmad Z, Naeem M, Sheraz M, Ahmed U, Azmi UR. 2019. Effects of Foliar Application of Selenium in Maize (*Zea Mays* L.) under Cadmium Toxicity. Biological Forum-An International Journal **11(2),** 27-3.

Swenson NG. 2012. Phylogenetic analyses of ecological communities using DNA barcode data. In: WJ Kress, DL Erickson eds. DNA barcodes: Methods and protocols. New York: Humana Press, Springer Science+Publishing Media, LLC. 409-419.

Swenson NG. 2013. The assembly of tropical tree communities the advances and shortcomings of phylogenetic and functional trait analyses. Ecography **36,** 264-276.

Techen N, Parveen I, Pan Z, Khan IA. 2014. DNA barcoding of medicinal plant material for identification. Curr Opin Biotechnol **25,** 103-110.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M. 1995. AFLP**:** A new technique for DNA fingerprinting. Nucl. Acids Res **23,** 4407-4414.

Webb CO, Donoghue MJ. 2005. Phylomatic: Tree assembly for applied phylogenetics. Molecular Ecology Notes **5,** 181-183.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. **18,** 6531-6535.