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A Brief Review of Molecular Markers to Analyse Medicinally Important Plants

Sevgi Marakli[1]*

Abstracts: Suitable identification and characterisation of medicinal plants are necessary for conservation of plant resources, investigations of genes associated with desirable traits, and understanding of evolutionary relationships. Therefore, various molecular marker techniques such as RAPD, AFLP, SSR and ISSR, SNP, SCoT, ITS and SCAR have been improved to provide detail information about genomes, which were not previously possible with only phenotypic methods. This brief review represents usage of these markers for molecular diversity analyses of medicinally important plants.

Keywords: AFLP, ITS, RAPD, SSR, SNP, SCoT, SCAR

Introduction

Herbal medicines play a significant role as an alternative to synthetic pharmaceuticals, reaching $115 billion by 2020 [1]. For this reason, conservation of endemic, threatened and endangered medicinal species, improving high-quality cultivars with desirable traits and even knowledge of the germplasm diversity have gained importance in the past decades [2]. Hence, different molecular markers have been commonly used for these purposes to give detailed information about genomes which is not possible with phenotypic methods.

DNA barcoding provides species-level identifications using short standard DNA regions, known as DNA barcodes or markers [3]. Ideal DNA markers should be highly polymorphic in nature, codominant inheritance, frequent occurrence in the genome, fast and easy testing, high reproducibility, and even easy exchange of data among laboratories [4]. DNA barcoding has been widely applied to answer a broad range of questions related

[1]Amasya University, Faculty of Arts and Sciences, Department of Biology, 05100 Amasya, Turkey
*Corresponding author: sevgi.marakli@amasya.edu.tr

to taxonomy, molecular phylogenetic, population genetics, and biogeography [5, 6, 7], as well as trade control of flora, fauna and food products [8, 9, 10].

In this brief review, detail information of molecular marker techniques: Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) , Microsatellites, or Simple Sequence Repeats (SSR), Inter Simple Sequence Repeat (ISSR) Single Nucleotide Polymorphism (SNP), Start Codon Targeted Polymorphism (SCoT), ribosomal internal transcribed spacer (ITS) and Sequence-Characterised Amplified Region (SCAR) in medicinal plants is discussed.

RAPD

This technique is based on the amplification of genomic DNA with single primer of an arbitrary nucleotide sequence with no previous information about the genome. Since most of the RAPD markers are dominant, it is not possible to distinguish whether the amplified DNA segment is heterozygous (two different copies) or homozygous (two identical copies) at a specific locus. However, in some cases, codominant RAPD markers, obtained as different-sized DNA segments amplified from the same locus, could be detected [11]. There are different studies reporting various polymorphism rates in medicinal plants by using this technique. Baruah et al. [2] reported that RAPD could be effectively used for genetic diversity evaluation among *Cymbopogon* species, cultivated for its essential oil Similar to Baruah et al. [2], Gantait et al. [12] used both RAPD and ISSR markers in *Rauvolfia serpentina* (L.) Benth. ex Kurz., which is one of the most vital pharmaceutically important plant from *Apocynaceae* family [13] to investigate post-germination genetic constancy. Furthermore, genetic fidelity of *Amomum subulatum* Roxb. used in Ayurvedic medicine [14] was also analysed during tissue culture process by using this marker [15]. Different experiments in medicinally important plants have shown that RAPD could be easily applied to detect polymorphisms in plant organs, and genetic diversity investigations of both intra- and inter-species.

AFLP

AFLP technique depends on the PCR amplification of restriction fragments obtained as a result of digestion of genomic DNA [16]. Many studies have analysed genetic diversity for different plant species using AFLP molecular markers [17]. Among molecular markers, AFLP produces much more reproducible bands, hence this technique is an

important method to detect genetic variability of plants especially plants regenerated *in vitro* [18, 19]. One of these studies was performed by Ebrahimi et al. [20] which genetic stability of *in vitro* regenerated plantlets of an endangered medicinal plant, *Kelussia odorotissima* Mozaff. native to Iran, was analysed.. In another study, Ghosh and Mandi [21] studied genotypes of *Murraya koenigii* growing in eastern Asia, revealing genetic variability in plants collecting from different altitudinal regions.

SSR and ISSR

SSRs consist of repeating units with one to six bp in length [22]. SSR markers have been extensively used in parentage analysis, genetic variation, molecular evolution, systematic taxonomy, linkage and comparative mapping, and functional diversity studies in plant species [23, 24]. Medicinal plant species have also been investigated by using SSR molecular marker technique. Kherwar et al. [25] analysed 24 SSRs in 36 varieties of guava (*Psidium guajava* L.), in addition to wild species. Genetic diversity, genetic characterisation of genotypes, cultivar identification and linkage mapping in guava were also being investigated using RAPD and AFLP markers [26, 27].

Next-generation sequencing (NGS) technologies have also been used to detect new SSR markers. Development of cumin (*Cuminum cyminum* L.) SSR markers were performed using Illumina Miseq NGS (next-generation sequencing) platform [28]. Genome-wide identification of SSRs was also carried out in tea plant (*Camellia sinensis*) [29]. Not only land plants but also aquatic plant species have also been analysed by using SSRs [30].

ISSR, as a modification of SSRs, offers such advantages such as high reproducibility, high polymorphism, low DNA requirements, easy handling, and high genomic distribution [31]. Chombe and Bekele [32] investigated the genetic diversity in populations of *Aframomum corrorima* (an economically important medicinal plant in Ethiopia) via seven ISSR primers. Moreover, Hashemifar and Rahimmalek [33] also reported that ISSR markers showed high efficiency in the evaluation of population diversity in *Perovskia*. Similar findings were also published for *Thymus sibthorbii* by Abraham et al. [34].

SNP

SNP is a DNA sequence variation occurring when a single nucleotide (A, T, G or C) differs among members of a species. SNP is one of the most abundant marker systems,

and several computational methods, genotyping approaches and transcriptome resequencing have been performed to detect new SNPs [35, 36]. Generally, molecular markers are used for genetic diversity studies. In addition to these studies, researchers have also identified the differences between control and disease resistant and DNA regions associated with desirable traits by using these markers. Manivannan et al. [37] studied with pepper, reporting useful SNP markers related to pungency and disease resistance via NGS platform.

SCoT

SCoT markers, similar to others, are one of the reliable techniques due to lots of advantages such as efficient, informative, and even inexpensive. Primers used in this method are designed according to short conserved region surrounding the ATG translation start (or initiation) codon, showing the correlation between functional genes and their corresponding traits [38, 39, 40]. Hence, this method has been successfully applied in medicinal plants to explore their genetic variability [41, 42]. Some studies have used this marker alone or in combination with other markers. Mao et al. [42] suggested that combination of ISSR and SCoT markers showed more valuable and superior results than single analysis of ISSR and SCoT as a result of genetic diversity and population structure analyses in *Senna obtusifolia* L.

ITS

There are various reports for molecular systematic investigations of plants by using ITS regions. Many studies have been performed to identify *Dendrobium* species using ITS sequence analysis [43, 44]. Moreover, polymorphism ratios of *Swertia species* used in Indian medicine were also analysed by using different DNA barcodes including *matK* (megakaryocyte-associated tyrosine kinase), *rbcL* (ribulose-bisphosphate carboxylase), *psbAtrnH* (photosystem II protein D1-structural RNA-His tRNA) and *nrITS* [45] where the highest interspecific divergence was obtained as a result of ITS analysis. Lee et al. [46] also successfully applied ITS technique to study genetic diversity in tea plant.

SCAR

Every plant DNA barcode method has also advantages, including failed PCR amplification, insufficient sequence variability between species, and incomplete sequence information [47, 48]. Therefore, there could be three different ways to overcome

these problems namely 1. Usage of combination of at least two different markers; 2. Comparative analyses of genomic polymorphisms and 3. Investigation of new DNA barcode regions using plastid genome sequencing [49, 50, 51]. In the first method, RAPD technique, either alone or in combination with other techniques is extensively used for the genetic analyses of different medicinal plants, and even other organisms [52, 53]. SCAR markers derived from the molecular cloning of RAPD fragments in medicinal plants are commonly applied because of its stability, sensitivity, and reliability [54]. This method amplifies only target-containing samples by using specific primers, and differentiates positive or negative amplification of target regions, as well as length polymorphisms of target regions by gel electrophoresis of closely related samples [53].

Conclusion

Molecular markers with low assay cost, convenience and fast and easy application and automation are undoubtedly valuable tools for population genetics and plant breeding programs [55, 56]. Although each method has its benefits and limitations, suitable choice of one marker and/or combination of different markers could be easily used to overcome these disadvantages. Information provided in this brief review shows the basic description of different molecular techniques used in molecular diversity studies performed in medicinal plant species.

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