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Review

Microsatellite Development and Potential Application in Authentication, Conservation, and Genetic Improvement of Chinese Medicinal Plants

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ABSTRACT

Medicinal plants are popular and widely used as a major source of herbal drugs and pharmaceutical compounds. Ever-growing demands make medicinal plants faced to several problems including efficacy and safety to meet business requirements, conservation, and artificially assisted breeding. As a powerful molecular tool, microsatellites offer the great potentials for various purposes in plants. This review provides a scenario of microsatellites in medicinal plants including development from genomic or expressed sequence tag libraries, cross-species transferability, genotyping, and potential applications. We emphasized on the authentication of medicinal plants by microsatellite markers.

Key words

authentication; cross-species transferability; medicinal plants; microsatellites; potential applications

1. Introduction

Chinese herbal medicines are becoming increasingly popular throughout the world. However, potentially harmful and ineffective substitutes and adulterants are often mixed into the raw herbal materials as a result of species confusion or misidentification and the lack of perfect quality control standards (Chan and Critchley, 1996). It is urgent for the authentication of Chinese herbal medicines by sophisticated and reliable methods to ensure the purity, quality, and safety of the drugs and prevent potentially toxic ingredients and adverse effects. There are a variety of traditional methods available to authenticate Chinese herbal medicines including morphological examination, phytochemical analysis, histological detection etc. (Tam et al, 2006; Zhao, 2010; Zhao et al, 2006). In practice, these methods depend on experiential and professional knowledge more or less. It is difficult to enhance the accuracy of authentication. For example, microscopic authentication relies on observing

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cell structure and internal features within herbal tissues, by which it is indistinguishable for close relative species because of similar features (Heubl, 2010). As an accurate, sensitive, and reliable method, DNA-based molecular authentication of the medicinal herbs provides a powerful tool to complement traditional methods as mentioned above (Al-Qurainy et al, 2011; Guo et al, 2011; Hon et al, 2003; Sui et al, 2011; Li et al, 2013).

Hypervariably repetitive DNA sequences such as microsatellites can be of great value in revealing polymorphisms even between closely related individuals. Thus, it is very useful for the authentication of Chinese medicinal plants. This review provides an overview of the current status of microsatellites development and cross-species transferability in herbal plants and presents the potential applications of microsatellites toward the safety and effectiveness of Chinese herbal medicines and conservation of medicinal plants.

2. Microsatellite and its applications

Two DNA bands were observed when purifying mouse DNA using cesium chloride (CsCl) density gradient centrifugation (Kit, 1961). The main band contains 92% of the total DNA and the minor, called satellite band contains 8% of the total DNA with a buoyant density of 1.690 g/cm^3. The term "satellite DNA" was originally defined by this density separation. Following research on the CsCl isopycnic density separation in land crab *Gecarcinus lateralis* and brown crab *Cancer pagurus* confirmed that the satellite DNA was rich in guanylate and cytidylate residues (the heavy satellite) or adenylate and thymidylate ones (the light satellite) (Skinner, 1967). Until 1974, these satellite DNA have been determined as $(TAGG)_n/(ATCC)_n$ repeat sequences for the first time in the hermit crab *Pagurus pollicaris* by Skinner et al (1974). Subsequently, more and more different tandem repeat motifs were widely identified in eukaryotic genomes (Gebhard and Zachau, 1983; Hamada et al, 1982; Tautz and Renz, 1984) but not detectable in the genomes of eubacteria, archaebacteria or mitochondria because of the limitations of technological conditions (Gross and Garrard, 1986). These repetitive sequences were defined for the first time as "Simple Sequence Repeats" by Tautz (1989) and "Microsatellites" by Litt et al (1989). Now microsatellites are known to be probably the most popular and powerful genetic markers in biological studies.

Microsatellites are ubiquitous in genomes and are highly variable. Microsatellites, also known as simple sequence repeats (SSRs), short tandem repeats (STRs) or simple sequence length polymorphisms (SSLPs), are consisted of motifs of one to six bases repeated several times, which are widespread in all eukaryotic and many prokaryotic genomes (Tautz and Renz, 1984; Toth et al, 2000; van Belkum et al, 1998). Microsatellites are also known as variable number of tandem repeats (VNTRs) due to their highly variability (Nakamura et al, 1987). Comparison to other parts of the genome, microsatellites are extremely unstable with mutation rates in the range from 10^{-5} to 10^{-2} per generation (Gemayel et al, 2010). Strand-slippage replication and unequal crossover generating addition or deletion of a number of full repeat motifs are considering as the major mechanisms for microsatellites expansions or contractions, which is the theoretical fundamental of microsatellites as genetic markers (Ellegren, 2004; Fan and Chu, 2007; Gemayel et al, 2010; Kruglyak et al, 1998; Levinson and Gutman, 1987; Morgante et al, 2002; Schlotterer and Tautz, 1992; Tautz and Renz, 1984). Thus, microsatellites loci in different individuals exhibits length polymorphisms due to repeat number differences, which is scored as different alleles.

Highly variable, multi-allelic, and co-dominant inheritance features make microsatellites very powerful molecular markers for assaying genetic variation. Microsatellites as a particularly valuable genetic tool can be utilized in various biological research areas, such as fingerprinting, genetic linkage mapping, pedigree analyses, population genetic structure analyses and so on (Ellegren, 2004; Guichoux et al, 2011; Mittal and Dubey, 2009; Zane et al, 2002). In addition, microsatellite markers are often used to screen BAC libraries to anchor the physical map onto the genetic map in many plant species, which provides an efficient tool to link genotypic and phenotypic variation (Beyer et al, 2007; Chen et al, 2002; Gupta and Varshney, 2000; Han et al, 2011; Wei et al, 2009; Yu et al, 2009). Moreover, interspecific transferability of microsatellites, especially among the cereal species, can be used as anchor markers for constructing comparative maps, which will facilitate to detect the common gene loci associated with traits of interest across species (Jiang et al, 2011; Lagercrantz, 1998; Sim et al, 2009; Varshney et al, 2005b; Yu et al, 2004).

Microsatellites are not only considered as molecular markers but also play an important role in accelerating the evolution of coding and regulatory sequences and regulating gene expression. Traditionally microsatellites have been regarded as nonfunctional "junk DNA" due to frequently be associated with non-coding regions (Cox and Mirkin, 1997; Tautz and Renz, 1984). However, the availability of complete genome sequences indicated that approximately 10% to 20% of genes and regulatory regions in eukaryotic genomes contain microsatellite sequences (Gemayel et al, 2010). Therefore, the microsatellite sequences within coding regions are believed not to be selectively neutral, but have specific biological functions. For example, there are many human genes (including ORFs and promoter) containing unstable microsatellite sequences, expansions of which trigger many neurological diseases known as trinucleotide repeat expansion diseases (TREDs), such as spinal and bulbar muscular atrophy, fragile X syndrome, myotonic dystrophy type 1, Huntington's disease and so on (Fondon et al, 2008; Gatchel and Zoghbi, 2005; Gemayel et al, 2010; Hannan, 2010; Karlin and Burge, 1996; Krzyzosiak et al, 2012; Nadir et al, 1996; Orr and Zoghbi, 2007). Another TTC/GAA triplet repeat-associated genetic defect in