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REVIEW ARTICLE

Application of Molecular Markers in Forensic Botany

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ABSTRACT: Molecular markers based on DNA sequence have become a remarkable tool in the Forensic Sciences for the identification of culprits. Now a day's majority of criminal cases are being solved based on DNA evidence from different biological materials like blood, boon, semen, nails with skin piece, hair with hair follicle, spores and any plant part etc. available at the scene of crime. Presently, DNA evidence from plants have also played an important role in solving forensic cases and DNA from any plant part found at the site of incidence can be used to locate the murderers, kidnapers, victims or in arresting drug traffickers. All molecular markers are not useful in Forensic Botany, only some molecular markers are used for plant DNA evidence which includes DNA barcoding, RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), SNP (Single Nucleotide Polymorphism) and Microsatellites, but the most widely used molecular marker for plant evident is SSR (Simple Sequence Repeats) due to its high reproducibility with great discrimination power and error free results from small piece of evidence.

Keywords: Molecular markers, Forensic Sciences, Forensic Botany, RAPD, AFLP, SNP, SSR

INTRODUCTION

Molecular techniques allowed us to trace the dynamics of speciation and to determine the relatedness of species and the genetic diversity within populations (Gillan et al., 1995). At the present time molecular markers based on mitochondrial (mt) DNA are used in wildlife forensics because mt DNA have high exponential rate and less recombination ratio as compare to nuclear DNA (Bhaskar, 2011). Mitochondrial DNA is also transfer from parents to offspring in the haploid form and their nucleotide sequence is also important in identification and reorganization of species (Hsieh et al., 2001). Another gene used in species detection, finding phylogenetic relationship and forensic studies is Cytochrome b gene (Hsieh et al., 2005). There is also a wide use of large ribosomal 16S rRNA gene for species detection and phylogenetic tree formation (Stubbs et al., 2000). Molecular markers which are usually used in nucleotide sequencing of plant DNA comprise RAPD (randomly amplified polymorphic DNA), AFLP (amplified fragment length polymorphisms) SSR (simple sequence repeats or microsatellites) (Wan and Fang, 2003). On the base of Polymerase Chain Reaction (PCR) molecular markers are divided into non PCR based markers and PCR based markers (Omondi et al., 2016).

FORENSIC SCIENCE

The word Forensic originated from a Latin word 'forensis' imply "of or for the forum". So Forensic Science is explained as appliance of scientific techniques for providing justice and fair dealing (Koopman et al., 2012). Forensic Science is further divided into different fields of science: forensic biology, forensic anthropology (deals with the study of human remains for forensic investigation), forensic chemistry, forensic physics, forensic botany, forensic entomology (utilizing insects

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and arthropods in forensic science), forensic serology (using body fluids such as blood, semen for forensic analysis) etc. (Chandra and Sharma, 2014). So, Forensic science is a set of different disciplines like pathology, anthropology, genetics, toxicology deontology and chemistry which is use for official investigation systems (Edlund, 2010).

FORENSIC BOTANY

The Branch of botany in which plants are use as forensic evidence to solve a case is called Forensic Botany (Coyle et al., 2001). Putting this differently we can say that assessment of plant bits and pieces or residue gathered from crime scene which are then used to resolve criminal and other legal issues is defined as forensic botany (Chandra and Sharma, 2014). Forensic botany is helpful in different ways like telling us about what the victim had eaten?, where? and when? And sometimes in that situation when the criminal moved the dead body from the crime spot and plant evidence clear that victim is killed elsewhere and then body is moved from that place (Chandra and Sharma, 2014). The initial appliance of molecular technique of plant DNA evident analysis was done in 1992 to solve a murder case by molecular analysis of Palo Verde tree's seed pods which ensure the presence of suspect at crime spot (Yoon, 1993). When a crime is conducted in forest or in semi urban area, bryophytes can be a good source of evidence because bryophytes DNA typing have ability to remain stable in fluctuating environment (Virtanen et al., 2007).

FORENSIC GENETICS

Forensic genetics is the branch of forensic science which deals with the molecular analysis of forensic evidences which contain DNA samples at the place of crime or we can do DNA analysis of individuals to find the identity of a person after bomb blast or airplane crash and also for paternity testing (Phillips, 2008).

FORENSIC PLANT BIOTECHNOLOGY

Plant Biotechnology is utilization of a range of biological treatments for elucidation of different areas of plant sciences (Chawla, 2002). In the field of forensic science plant biotechnology techniques such as DNA fingerprinting, DNA bar-coding and use of Molecular markers is done to reveal the truth or solve the mystery of case by spotting and categorizing species and finding locality of a focused plant (Shukla, 2016). Plant DNA bar-coding has limited scope in contrast to animal DNA bar coding because in animals mitochondrial, cytochrome c, oxidase I can be used as universal barcode system. As there is no gene for bar-coding in plants that's why in this situations, molecular markers like Short Tandem Repeats (STRs), Single Nucleotide Polymorphisms (SNPs), Simple Sequence Repeats (SSRs), Variable Number Tandem Repeats (VNTRs) can be used in Forensic Botany (Zaya and Ashley, 2012).

RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD)

RAPD markers can be used in paternity analysis, taxonomic-based identification and genetic diversity (Van de Ven and McNicol, 1995). Random Amplified Polymorphic DNA analysis is also being used in population genetics studies like genetic diversity, divergence within and among populations based on assumption of Hardy-weinberg equilibrium (Brown, 2003). It detects the genetic variations in the genome of an organism in terms of sequence variation at the priming regions (Magalhães et al., 2007). Similarities in banding profiles among strains (i.e. the number and sizes, but not the intensity of amplified bands) can be calculated and used to infer strain relationships (Dutra et al., 2008). RAPD can be used to evaluate the genetic similarity within a population and it is also very helpful in characterization and identification of organism (Bennett, 1997). Random amplified polymorphic DNA analysis can be helpful in

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identifying genomic modifications among organisms of even same species due to high variability of the markers (Sunnucks, 2000). A women murdered case was solved by the RAPD analysis of seed pods of the Palo Verde tree (*Cercidium floridum*) in the vicinity of the murder place (Mestel, 1993). RAPD analysis was used for the identification of the suspect of strawberry which farmer was growing against the law and the court also considered this a clear evidence (Congiu et al., 2000). To stop the illegal international trade of Rauvolfia sp. forensic department use DNA bar-coding method (Eurlings et al., 2013).

INTER SIMPLE SEQUENCE REPEATS (ISSR)

ISSR molecular markers are also known as new generation markers which is latest method of DNA fingerprinting (Kumar et al., 2001), its specialty is that only one primer is use in PCR reaction which bind to SSR regions and a amplify ISSR region as ISSR is Inter simple sequence repeats of 100-3000 bp in length which are present between the flanking regions of SSR (Omondi et al., 2016). It has capacity to amplify large numbers of DNA fragments in each reaction cycle and is a principle method for DNA fingerprinting among different varieties (Singh et al., 2014).

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

RFLP technique first gave the concept of detection of DNA variation in different DNA samples, RFLP markers were used for the first time in the construction of genetic maps (Huys et al., 1996). In RFLP DNA is restricted into different sized fragments which can be visualized by southern hybridization with labeled probes. They are moderately polymorphic and highly reproducible (Kumar et al., 2009). RELP analysis which detects length mutation and alteration in base sequence has been used for constructing DNA fingerprinting (Lemke et al., 1991).

AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP)

AFLP is a multiplex PCR based method use to sequence DNA which is ligated to each end, where a chosen subset of restriction fragments are amplify with oligonucleotide primers (Corsini et al., 1999). AFLP reliability is of over 50 loci per reaction (Coyle et al., 2001; Miller Coyle et al., 2001). AFLP is helpful in producing DNA fingerprints and have no need of previous information about DNA sequence and results of AFLP are highly reproducible (Coyle et al., 2003).

SIMPLE SEQUENCE REPEATS (SSR)

Microsatellites are polymorphic loci present in nuclear and organellar DNA that consist of repeating units of 1-6 base pairs in length (Slavov et al., 2004). SSR markers are known as second generation markers. They are Simple sequence repeats (SSR) or Short tandem repeats (STR) or Sequence Tagged Micro satellite Sites (STMS) and it is defined as Micro satellites- arrays of tandomly repeated di-, tri-, tetra- and penta nucleotide DNA sequences (Maheswaran, 2014). These repeated sequences are on flanking regions of single copy sequences which give us grip for PCR amplification. SSR have high rate of polymorphism and high reproducibility (Kumar et al., 2009).

CRIMINAL CASES SOLVED BY USING PLANT DNA AS EVIDENCE

POLLEN DNA ANALYSIS AS FORENSIC EVIDENT

Pollens are very persistent and have thick wall which help them to face harsh environment that's why their DNA typing is helpful in forensic science and researchers also evaluate that pine pollen are good source of DNA evident in forensic cases because DNA typing of pollens are

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feasible till after two weeks which may stick to cloths and in result it will be helpful to track down the criminal or victim (Coyle et al., 2005; Schield et al., 2016).

FORENSIC DNA ANALYSIS OF WHEAT FLOUR FOR WHITE POWDER CASES

White powder is often used in terrorist attacks so this white powder (wheat flour) DNA fingerprinting is supportive in criminal cases. The specific regions which are sequenced in DNA analysis are ribulose bisphosphate carboxylase large subunit gene (*rbcL*) by using STR (Kikkawa et al., 2015).

A GRASS MOLECULAR IDENTIFICATION SYSTEM FOR FORENSIC CASES

Grass material is a good source of forensic evidence and is helpful in solving criminal cases which are done in the areas having grass (forest, lawn, garden etc.). Australian scientists used a series of PCR reaction assay to develop a system which is worthwhile in identification and discrimination of grasses at their taxonomic levels. They used the four chloroplast and two mitochondrial loci sequence to identify the specie of grass forensic cases (Ward et al., 2005).

QUERCUS EVIDENCE LEAVES TO CRIME SCENE TREES BY USING MICROSATELLITES

In a female murder case Quercus (Oak) leaves were collected as evidence from suspect's car and at the spot having victim, both were identified by using DNA microsatellites which in result declared that suspect is the murderer of that female (Craft et al., 2007).

DNA ANALYSIS HELPFUL IN DRUG ENFORCEMENT

Now a day's Molecular biological techniques are widely used in drug enforcement. There is a difficulty in reorganization of grabbed drugs most importantly when plant material is in dried. So molecular DNA analysis is helpful to overcome this problem, as all drugs are form by different plant material processing (Coyle et al., 2001).

OPIUM POPPY IDENTIFICATION BY USING SPECIFIC GENETIC MARKERS IN DRUG ENFORCEMENT

Opium Poppy (*Papaver somniferum*) is used to form drug and to stop its illegal use, law enforcement agencies use molecular genetic markers to identify these drugs. They use 10 markers which are universal for all plants but specific to some poppy plant species and 3 markers out of 10 produced amplicons only in poppy and 3 for genus specificity and all these markers are helpful in forensic cases related to poppy (Lee et al., 2010).

CASES INVOLVING THE CROSS-BORDER TRAFFICKING OF CANNABIS

DNA profiling of 93 Cannabis plants has been performed by using STRs in U.S (Gilmore et al., 2003). department of forensic science accounted that they designed a test to separate each entity of marijuana samples for which they test 11 cases, containing 199 samples (Alghanim and Almirall, 2003). Cannabis species were identified by doing cloning and amplifying nuclear ribosomal DNA internal transcribed spacer region (ITS 1 and ITS 2) (Gigliano et al., 1997).

CONCLUSION

Non-human DNA fingerprinting techniques based on plant parts have become a remarkable tool in resolving criminal investigations and civil cases. Forensic botany has a wide application in the vegetable matter identification the in stomach, to identify specific locations of kidnapping/murder and tracking drug by different molecular markers. Among the molecular markers, Microsatellites are the best markers because its results are error free and have high reproducibility.

CONFLICT OF INTEREST

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