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The Green Revolution: Botanical Contributions to Forensics and Drug Enforcement

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Forensic botany encompasses many sub-disciplines, including plant anatomy, plant ecology, plant systematics, plant molecular biology, palynology, and limnology. Although the field of forensic botany has been recognized since the mid-1900's, the use of trace plant material as physical evidence in criminal casework is still novel. A review of published forensic casework that used plant evidence is presented here. Cases include the analysis of wood evidence in the Charles Lindbergh baby kidnapping, the use of pollen in establishing the location of a sexual assault, and pollen analysis to determine the time of year for burial in a mass grave. Additional cases discuss the use of plant growth rates to determine the time of a body deposit in a field, the use of diatoms to link individuals to a crime scene, and plant DNA typing to match seedpods to a tree under which a body was discovered. New DNA methods in development for plant species identification and individualization for forensic applications are also discussed. These DNA methods may be useful for linking an individual to a crime scene or physical evidence to a geographic location, or tracking marijuana distribution patterns.

Key words: *botany; DNA, plant; criminology; gene probes, DNA; jurisprudence; kidnapping; laboratories, forensic; marijuana; narcotic control; plant structures*

Forensic botany is defined as the use of plant evidence in court. Forensic botany is subdivided into several botanical subspecialties, including plant anatomy (the study of cellular features), plant systematics (taxonomy and species identification), palynology (the study of pollen), plant ecology (plant succession patterns), and limnology (the study of freshwater ecology) (1). In the past decade, molecular biology has been an important tool to further the research of these disciplines (2-4).

Non-human DNA typing methods have been used on occasion for solving criminal and civil casework (5-9). Although most forensic scientists are familiar with methods for human identity testing, the use of plant, animal, and insect evidence is not yet routine. This is due in part to a lack of awareness by evidence collection teams, who do not necessarily see the value in collecting botanical trace evidence. Prosecutors are also frequently unaware of the potential of botanical evidence to provide linkages between crime scenes and individuals or they may simply not know who to contact for assistance. In addition, significant resources are required to construct population databases from the large number of plant species that may be encountered in forensic casework. Forensic botany has a wide range of potential applications, including the identification of vegetable matter in stomach contents to verify an alibi (1), identification of specific locations of kidnapping victims (2,3), and tracking drug distribution networks (4). The goal of this article is to present a review of traditional forensic botany methods (ie, species identification by their morphological characteristics) and casework. In addition, this article will discuss some of the new molecular methods (DNA) that are being developed for forensic casework, investigative leads, and drug enforcement.

Plant Anatomy and Systematics

Plant systematics is a broad discipline that includes the study of evolutionary relationships between plant species and taxonomy (the identification of plant species) (1,10,11). Species identification is a typical first step in analyzing botanical evidence for casework (1,11,12). Plant anatomy uses features, such as leaf morphology and tree growth ring patterns, to aid in species identification and in performing physical matches of evidence, respectively. The kidnapping and death of Charles Lindbergh's young son in 1932 was the first modern era case to use such botanical evidence in court (1,13). A wooden ladder was used to gain access to the second-story nursery to kidnap Lindbergh's son (Fig. 1). Arthur Koehler, a wood identification expert for the Forest Products Laboratory of the United States Forest Service in Wisconsin, was able to provide critical evidence against Bruno Richard Hauptman, who was later convicted of the crime (13). Koehler had an excellent academic record and had provided evidence in several cases before the famous Lindbergh trial. His testimony is noteworthy, since the use of scientific experts in the mid-1930's was generally limited to fingerprints, handwriting, bullet comparisons, and analyses of stomach contents (1,13). Koehler first identified the four tree species used to construct the ladder as yellow pine, ponderosa pine, Douglas fir, and birch by microscopic analysis of wood grain patterns. Next, Koehler analyzed the tool marks left on the wood from both the commercial planing mill and the hand plane used by Hauptman during the construction of the ladder. Koehler used oblique light in a darkened room to observe the plane patterns left on the wood. Amazingly, he was able to trace the wood by the mill plane marks to a shipment of yellow pine delivered to the National Lumber and Millwork Company in Bronx, New York. The hand plane marks on the ladder exactly matched those made by a hand plane found in Hauptman's possession. Finally, Koehler compared the annual growth rings and knot patterns on rail 16 of the ladder to a section of wood in Hauptman's attic. The pattern of knots and growth rings on rail 16 exactly matched the exposed end of wood in the attic supporting the prosecution's position that a section had been removed to construct the ladder. This case exemplifies the use of plant anatomy and plant systematics in providing critical links to Hauptman's involvement in the Lindbergh kidnapping (13).

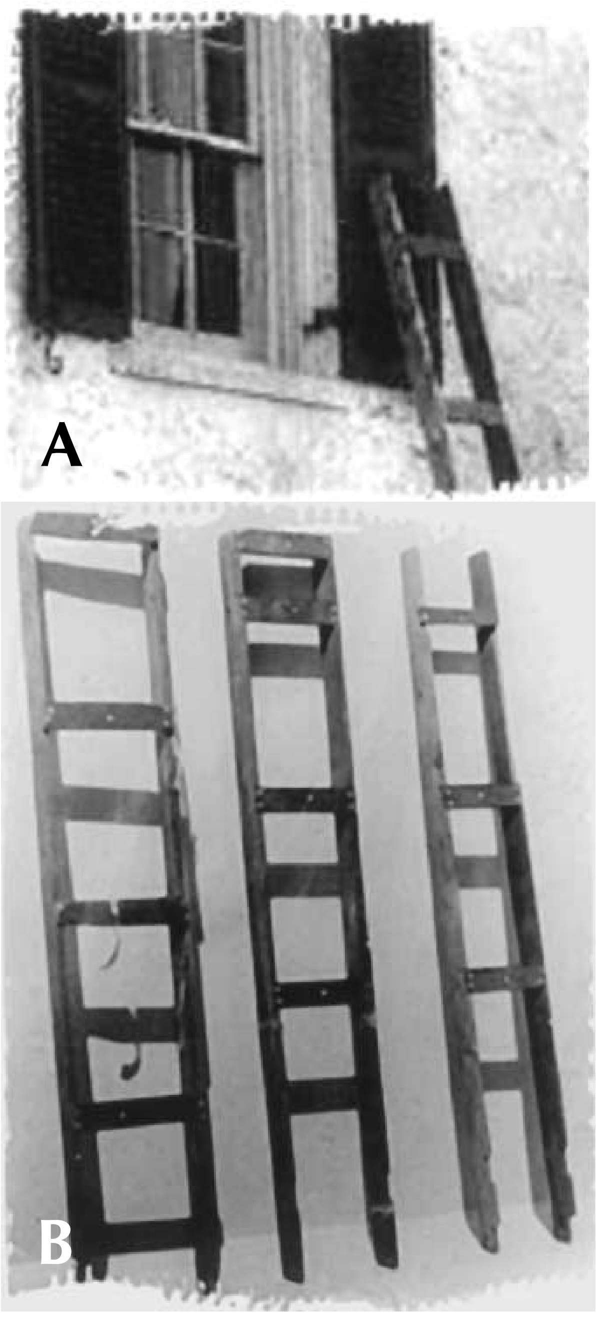

Figure 1. A) The second-story nursery window used in the kidnapping of Charles Lindbergh's son. B) The hand-constructed wooden ladder used to access the nursery window. Photos courtesy of New Jersey State Police.

Palynology

Forensic palynology refers to the use of pollen in criminal investigations (Fig. 2) (1). The major plant groups identified as pollen sources include flowering plants, conifers, and ferns. Ferns technically produce spores instead of pollen but are included in pollen types (14). Pollen is microscopic and not visually obvious trace evidence during crime scene collection but is retained on clothing, embedded in carpets and pervasive in soil. Pollen grain morphology can be used to identify a plant genus and often the species (1). Crime scenes that are restricted to a few square meters, such as a rape scene or the entry point of a burglary, are good choices for pollen evidence (14-16). Localized areas have a specific pollen distribution pattern representing the combination of plant species found in the surrounding vegetation. Common pollen types from plants that use wind for distribution will be less useful than pollen from uncommon, poorly distributed species. Insect distributed pollen is typically deposited within a few feet of the source plant. Pollen analysis consists of species identification and an estimation of the percentage that each plant species represents in an evidentiary sample. A similar pollen composition from shoeprints and from the shoes that made the prints indicate a strong match correlation (14). Pollen evidence collected from a burglary entrance and a suspect's shoes, for example, could provide a linkage in a case.

A case that exemplifies the use of pollen in criminal casework is described by Horrocks et al (16). In Auckland, New Zealand, a prostitute alleged that the defendant had raped her in an alleyway approximately seven meters from his car, after failing to pay her in advance for her services. The defendant claimed that he had never been more than one meter away from the car and had not entered the alleyway. Furthermore, he claimed that he had not had sex with the victim and the soil on his clothing was from the driveway area. An examination of the crime scene and the evidence showed no footprints and no seminal fluid stains. A soil sample was collected from the defendant's clothing, the disturbed area of ground in the alleyway, and from the driveway area near the defen-

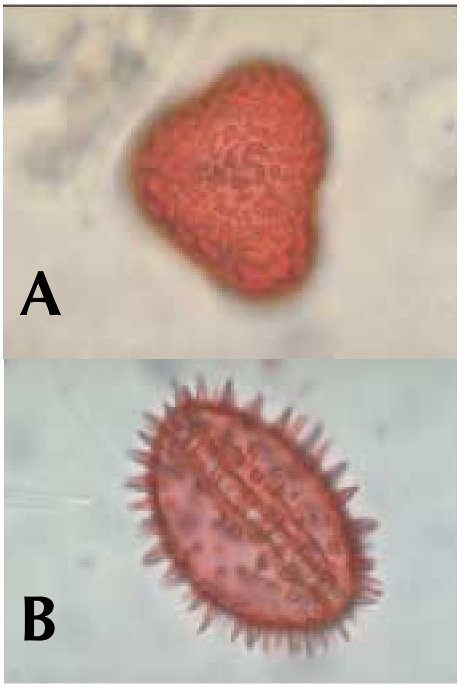

Figure 2. Plant species have pollen grains of specific size and shape. Light micrographs (40× magnification) of an *Ophioglossum vulgatum* pollen grain (A) and a *Nuphar lutea* pollen grain (B). Micrographs courtesy of Uppsala University and Keith Barnett.

dant's car. All the soil samples were prepared for pollen analysis by deflocculation with potassium hydroxide, acetylation to remove cellulose and organic matter, and a silicate removal step with hydrofluoric acid (16). Samples were bleached to remove additional organic matter and analyzed under a microscope for pollen identification and counting. The types of pollens were similar between the two locations, but the amounts of each type were different in each sample. The alleyway sample contained 76% *Coprosma* (an evergreen shrub) pollen, but the driveway sample contained only 8%. The defendant's clothing contained approximately 80% *Coprosma* and only small amounts of other pollen species. These results support the victim's account of the sexual assault taking place in the alleyway.

Pollen analysis has also been used to establish time of death (12). In Magdeburg, Germany, a mass grave containing 32 male skeletons was discovered in February of 1994. The identity of both the victims and the murderers was unknown. Two hypotheses were proposed: 1) the victims were killed in the spring of 1945 by the Gestapo at the end of World War II, or 2) the victims were Soviet soldiers killed by the secret police after the German Democratic Republic revolt in June of 1953. The ability to differentiate between the spring and summer was critical to solving the case. Pollen analysis was performed on 21 skulls. Seven of the skull nasal cavities contained high amounts of pollen from plantain, lime tree, and rye (12). All of these plant species release pollen during the months of June and July. Pollen analysis supported the hypothesis that the remains were of Soviet soldiers killed by the Soviet secret police after the June 1953 revolt.

Plant Ecology

Plant ecology involves studying the growth patterns of vegetation in areas that have been disturbed (1). These patterns and the vegetative (non-flowering) portion of plants can be useful in estimating time of death (1,10,11). For example, when a body is discovered lying on top of a weed plant with a broken top, useful information can be obtained to define time windows for when the death occurred (10). A certain amount of shading will eventually kill a plant, so if the weed plant is lacking chlorophyll, a minimum amount of time must have already elapsed. If new shoots are present at the base of the plant, this may establish a second time window. Agricultural research on many plant species has defined the time for new shoot initiation after the top of a plant has been removed. The length of the new shoot can sometimes establish a third time window. Forensic anthropology sometimes uses plant anatomy to determine an approximate time of death (1,11). In one case, the brain cavity of a skull was filled with plant roots (11). The anatomy and developmental stage of the roots indicated that the plant was approximately one year old and the plant was putatively identified as *Ranunculus ficaria* L (buttercup family) (11). The predictable stages of plant development were useful in estimating the time that the skeletal remains had been in their present location. They were able to determine the skeleton had been there for at least one year; however, a maximum time could not be established. The plant could have developed secondarily sometime after the body had lain in its present location, so a maximum time estimate was not possible.

Limnology

Limnology is the study of freshwater ecology and can be applied to a subset of forensic cases (8). In particular, aquatic plants (e.g., algae, diatoms) have been useful to link suspects to a crime scene or to establish that drowning occurred in freshwater (8,17). Diatom populations vary seasonally in lakes, rivers, and ponds (17). In early spring, diatom populations expand in freshwater. Following this expansion, the live diatoms decline but a large number of dead diatoms remain in summer water. In the fall, a second diatom expansion occurs and then progressively declines through the winter months. When a person drowns in freshwater, diatoms are taken in along with water into the lungs. The diatoms are dispersed to the internal organs of the body. The diatom test is performed by extraction of bone marrow from an intact femur, heating the marrow in a nitric acid solution and centrifuging to pellet the solids (17). The solids are examined on slides by phase contrast microscopy for the presence of diatom species. Each species has a characteristic shape and refractive pattern from the silica in the cell wall (Fig. 3), which can be used for identification. In a study of 771 cases, the diatom test was positive for 28% of presumed freshwater drowning cases but was rarely positive for domestic water drowning (17). The low rate of diatoms observed in domestic drowning could be traced back to cleaning agents containing crushed diatoms for abrasives.

In 1991, two young boys were brutally attacked by teenage assailants while fishing at a suburban pond in Connecticut (8). The boys were held at knifepoint, bound with duct tape, savagely beaten, and dragged into the pond to drown. One boy managed to get free, save himself, and rescue his friend. After many hours of criminal investigation, three suspects were apprehended. To link the suspects to the crime scene, investigators seized the sediment-encrusted sneakers of both the victims and the assailants and analyzed them for algal and diatom species. A microscopic analysis of samples from each pair of sneakers plus reference samples from the pond showed the same species and distribution pattern of each species (8). These results supported the position that the samples all originated from a common freshwater location.

Figure 3. Diatoms have a characteristic shape and refractive index as illustrated here. Figure adopted from Bodies of Evidence by Brian Innes.

Plant Molecular Biology and DNA

The previously discussed cases have relied on traditional botanical methods for species identification (1,11-13,16,17). In the age of DNA analysis, forensic botany is using molecular biology to aid in criminal and civil investigations. The first criminal case that used plant DNA typing to gain legal acceptance was a homicide that occurred in 1992 in Arizona's Maricopa County (9). A woman's body was found under a Palo Verde tree in the Arizona desert. Near the body was a beeper, eventually traced to a suspect, Mark Bogan. A few seed pods from a Palo Verde tree were found in the back of Bogan's truck. Officials wanted to know if DNA analysis could match those seed pods to the tree where the body was discovered. Dr T. Helentjaris from the University of Arizona used a technique of Randomly Amplified Polymorphic DNA (RAPD) analysis to generate a band pattern from the evidence in question (9). He also surveyed a small population of other Palo Verde trees to determine if the band patterns were unique to each individual. His convincing testimony on plant evidence helped convict Mark Bogan of murder. RAPD marker analysis has also been used in civil court cases to identify patent infringements. In Italy, RAPD analysis of a patented strawberry variety "Marmolada" helped settle a lawsuit involving the unauthorized commercialization of the plant (6).

Molecular methods can be used to identify a plant species from minute leaf fragments and pollen grains. Forensic botanists have used DNA technology because often botanical trace evidence does not contain the necessary morphological or histological features that would allow one to identify a plant at the genus or species level. This is particularly true for fragmented and deteriorated plant material. The Bode Technology Group, Inc. (Dr Robert Bever; Springfield, VA, USA) is developing and using molecular methods to analyze botanical trace evidence (2,3). This type of analysis is a valuable tool for potentially linking an individual to a crime scene or physical evidence to a geographic location. One useful application for the molecular analysis of botanical trace evidence is the identification of a geographic region where a kidnapped individual may be located. Based on flowering times and the plant species represented in the trace pollen evidence found with a ransom note, a geographic region may be identified and would provide the police with an investigative lead. Plant systematists have characterized many loci that are useful for the identification of plants, including several nuclear (18S, ITS1, ITS2) and chloroplast (rbcL, atpB, ndhF) genes (2,3). Using some of those genes, the Bode Technology Group, Inc. has identified a DNA extraction, cloning, and sequencing procedure to identify plants. With these methods, they have identified numerous species of plants from physical evidence. These include species of algae, evergreens, and many flowering herbs, shrubs, and trees. Many plants have a limited geographic distribution or grow in specific habitats (1-3). Some of these locations will be general areas, such as roadsides or areas of new construction. Other locations will be more specific, like the Mojave Desert or southern Florida for plant species that have a severely restricted geographic range. Linking botanical trace evidence to a geographic region could provide law enforcement and investigators with valuable information.

Drug Enforcement and DNA

Drug enforcement is taking advantage of new plant molecular biology techniques, too. Often in drug seizures, identification of the seized substance is a problem, especially if the plant material is fragmented and dried. A variety of methods are currently employed to identify *Cannabis sativa* L (marijuana). Marijuana can be identified by classical botanical characterization, especially if the type of cystolith hairs present on the leaves are used. However, presence of cystolith hairs is not a conclusive identification, since more than 80 plant species have similar cystolith hair morphology (18). A chemical screening test called the Duquenois-Levine color test (19) is frequently used in combination with cystolith hair observation as a method to identify *Cannabis*. A positive Duquenois-Levine test for marijuana exhibits a purple color in the chloroform layer of the extracted plant material (19). As with many chemical screening tests, a faint color can be subjectively interpreted. Marijuana can also be identified by chromatographic methods that test for the presence of tetrahydrocannabinol (THC) and other cannabinoids (20,21). Unfortunately, not all *Cannabis* samples contain detectable levels of THC. An alternative strategy uses molecular genetics to identify *Cannabis*. *Cannabis* species identification has been achieved by cloning and sequencing the nuclear ribosomal DNA internal transcribed spacer regions (ITS 1 and ITS 2) (22).

In addition to the identification of marijuana samples, it is desirable but difficult to link individual growers and distributors to specific illicit field and greenhouse operations. Molecular genetics may offer a solution to this problem (Fig. 4). In certain regions of Canada and the United States, marijuana is propagated clonally by taking cuttings from a high-THC content "mother" plant and rooting them directly in the soil (Dr G. Shutler, personal communication). This form of propagation results in large numbers of plants having identical DNA analogous to identical twins in humans. DNA typing of marijuana in this situation would allow one to link common growing operations and assess distribution patterns by tracking clonal material. Other growers start their marijuana plants from seed (Fig. 4). Each seed has it's own unique genetic composition. DNA typing of marijuana grown from seed would allow one to link a leaf found in an individual's vehicle back to a plant from a growing area near the suspect's home, for example.

The Connecticut State Forensic Science Laboratory is developing a molecular strategy for creating unique band patterns from marijuana samples, which uses a technique called amplified fragment length polymorphism (AFLP) analysis (4). AFLP analysis is based on the selective PCR amplification of restriction fragments from a total digest of plant DNA to generate a fluorescent band pattern (23). The AFLP technique has been used on some plants (24-26), insects (27), and bacteria (28) but has not been applied to forensically relevant plant species until recently (4). Validation of the AFLP technique on marijuana samples and the construction of a marijuana AFLP database for comparative purposes is in progress at the Connecticut State Forensic Science Laboratory.

Clonal propagation — identical DNA

Propagation by seed — unique DNA

AFLP analysis

Link an individual to a sample

Link growers

Track distribution networks

Figure 4. Amplified fragment length polymorphism (AFLP) analysis is a plant DNA method that can be useful to provide linkages between marijuana growers, distributors, and users.

Summary

Forensic botany has been useful for solving criminal and civil cases but is still a very under-used resource. Traditional botany uses simple, inexpensive methods for the identification of a plant species. Identification of pollen or stomach contents can be useful in verifying a person's alibi, a victim's location or the time of death. A wide variety of plant species exist and many are restricted to specific geographic locations. These features make plant evidence useful to forensic scientists. In addition, forensic scientists are turning to academic research to identify molecular methods that can be applied to trace botanical evidence. The development of DNA typing methods for plant species may allow forensic scientists to take advantage of the enormous genetic diversity that exists in plant populations.

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References

1 Bock JH, Norris DO. Forensic botany: an under-utilized resource. J Forensic Sci 1997;42:364-7.

2 Bever R, Golenberg E, Barnes L, Brinkac L, Jones E, Yoshida K. Molecular analysis of botanical trace evidence: development of techniques. Proceedings of the American Academy of Forensic Sciences 52nd Annual Meeting; 2000 Feb 21-26; Reno, Navada, USA. p. 23-4.

3 Brinkac L, Cimino M, Gross N, Hopkins E, Jones E, Bever R. Analysis of botanical trace evidence. Proceedings of the Promega 11th International Symposium on Human Identification; 2000 Oct 10-13; Biloxi, Mississippi, USA. p. 31.

4 Miller Coyle H, Divakaran K, Jachimowicz E, Ladd C, Lee HC. Individualization of marijuana (Cannabis sativa) samples for forensics applications and narcotics enforcement. Proceedings of the American Academy of Forensic Sciences 53rd Annual Meeting; 2001 Feb 19-24; Seattle, Washington, USA. p. 30-1.

5 Basten CJ. Implications of canine population structure for DNA forensics. Proceedings of Cambridge Health-

tech Institute's 3rd Annual DNA Forensics Meeting; 1999 June 13-15; McLean, Virginia, USA. p. 1-13.

6 Congiu L, Chicca M, Cella R, Rossi R, Bernacchia, G. The use of random amplified polymorphic DNA (RAPD) markers to identify strawberry varieties: a forensic application. Mol Ecol 2000;9:229-32.

7 Menotti-Raymond MA, David VA, O'Brien SJ. Pet cat hair implicates murder suspect. Nature 1997;386:774.

8 Siver PA, Lord WD, McCarthy DJ. Forensic limnology: the use of freshwater algal community ecology to link suspects to an aquatic crime scene in southern New England. J Forensic Sci 1994;39:847-53.

9 Yoon, CK. Botanical witness for the prosecution. Science 1993;260:894-5.

10 Hall, DW. The contributions of the forensic botanist to crime scene investigations. The Prosecutor 1988; 22:35-8.

11 Quatrehomme G, Lacoste A, Bailet P, Grevin G, Ollier A. Contribution of microscopic plant anatomy to postmortem bone dating. J Forensic Sci 1997;42:140-3.

12 Szibor R, Schubert C, Schoning R, Krause D, Wendt U. Pollen analysis reveals murder season. Nature 1998; 395:449-50.

13 Graham, SA. Anatomy of the Lindbergh kidnapping. J Forensic Sci 1997;42:368-77.

14 Horrocks M, Coulson SA, Walsh KAJ. Forensic palynology: variation in the pollen content of soil on shoes and in shoeprints in soil. J Forensic Sci 1999;44: 119-22.

15 Horrocks M, Coulson SA, Walsh KAJ. Forensic palynology: variation in the pollen content of soil surface samples. J Forensic Sci 1998;43:320-3.

16 Horrocks M, Walsh KAJ. Fine resolution of pollen patterns in limited space: differentiating a crime scene and alibi scene seven meters apart. J Forensic Sci 1999;44:417-20.

17 Pollanen MS, Cheung C, Chiasson DA. The diagnostic value of the diatom test for drowning, I. Utility: a retrospective analysis of 771 cases of drowning in Ontario, Canada. J Forensic Sci 1997;42:281-5.

18 Nakamura GR. Forensic aspects of cystolith hairs of *Cannabis* and other plants. J Assoc Off Anal Chem 1969;52:5-11.

19 Butler, WP. Duquenois-Levine test for marijuana. J Assoc Off Anal Chem 1962;45:597-600.

20 Barni Comparini I, Centini F. Packed column chromatography, high resolution gas chromatography and high pressure liquid chromatography in comparison for the analysis of Cannabis constituents. Forensic Sci Int 1983;21:129-37.

21 Gillan R, Cole M, Linacre A, Thorpe J, Watson N. Comparison of *Cannabis sativa* by random amplification of polymorphic DNA (RAPD) and HPLC of cannabinoids: a preliminary study. Science and Justice 1995;35: 169-77.

22 Siniscalco Gigliano G, Caputo P. Ribosomal DNA analysis as a tool for the identification of *Cannabis sativa* L. specimens of forensic interest. Science and Justice 1997;37:171-4.

23 Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, et al. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 1995;23:4407-14.

24 Becker J, Vos P, Kuiper M, Salamini F, Heun M. Combined mapping of AFLP and RFLP markers in barley. Mol Gen Genet 1995;249:65-73.

25 Lin J-J, Kuo J, Ma J, Saunders JA, Beard HS, MacDonald MH, et al. Identification of molecular markers in soybean-comparing RFLP. RAPD, and AFLP DNA mapping techniques. Plant Mol Biol Rep 1996;14:156-69.

26 van Eck HJ, van der Voort JR, Draaistra J, van Zandvoort P, van Enckevort E, Segers B, et al. The inheritance and chromosomal localization of AFLP markers in a non-inbred potato offspring. Molecular Breeding 1995;1: 397-410.

27 Corsini G, Manubens A, Lladser M, Lobos S, Seelenfreund D, Lobos C. AFLP analysis of the fruit fly *Ceratitis capitata*. Focus 1999;21:72-3.

28 Huys G, Coopman R, Janssen P, Kersters K. High-resolution genotypic analysis of the genus Aeromonas by AFLP fingerprinting. Int J Syst Bacteriol 1996; 46:572-80.

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