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The importance of plant DNA examination in forensic botany

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Abstract

Plants possess a natural genetic diversity that is used for various scientific research purposes. This diversity is useful in deoxyribonucleic acid (DNA) fingerprint and barcode studies, which have many applications in scientific research such as determining the relationships between plant species, determining parentage, creating genome maps, and determining genetic variations. It can also help understand evolutionary issues, identify species' subspecies or races, and prepare phylogenetic trees and cladograms. Moreover, genetic diversity can assist in population genetics, plant breeding, revision studies, and forensic medicine applications. The widespread use of plant material in human life has led to the formation of the concept of criminal botany in the field of forensic sciences. This study, it was aimed to determine the origin of plant findings which frequently encountered at crime scenes by conducting criminal botanical investigations so that the ribulose bisphosphate carboxylase/oxygenase large subunit (*rbcL*/RuBisCO) gene was used to identify various exotic/tropical plant species (*Ananas comosus* (L.) Merr, *Chamaedorea elegans* Mart., *Nolina recurvata* (Lem.) Hemsl. (*Beaucarnea recurvata* Lem.), *Pandanus veitchii* hort., *Phoenix dactylifera* L., *Phormium tenax* J.R.Forst. & G.Forst., *Sansevieria trifasciata* Prain, *Theobroma cacao* L., *Washingtonia filifera* (Linden ex André) H.Wendl.)) grown in the tropical greenhouses of Istanbul University Alfred Heilbronn Botanical Garden (AHBG). This gene is specific to plants and is obtained from chloroplast. Different restriction enzymes (*AccI, BamHI, DraI, PstI, and SacII*) were used for typing these plants. Their molecular characterizations were investigated by using Cleaved Amplified Polymorphic Sequence (CAPS) markers, also known as Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP), which is a technique based on the fragmentation of Polymerase Chain Reaction (PCR)-amplified DNA regions with restriction enzymes/endonucleases using appropriate primers, resulting in DNA particle length polymorphism. Exotic and tropical plant samples, which are also fibrous plants, were subjected to DNA analysis to contribute to forensic botany in solving illegal activities and increase the use of plants as evidence in crime scene investigations.

Keywords: Forensic botany, exotic and tropical plant species, ribulose bisphosphate carboxylase/oxygenase large subuni gene, restriction enzymes, cleaved amplified polymorphic sequence markers deoxyribonucleic acid fingerprint and barcode

Introduction

Criminal botany is a broad section of forensic sciences. It can be defined as a specialized field within biology where examinations and analyses are carried out in laboratories depending on the types of physical evidence [1]. Providing crucial supporting evidence for criminal investigations using the traditional classification of plant species or advanced biochemical and molecular methods, criminal botany combines many fields and ultimately results from their application to law. Plant materials obtained from crime scenes or suspects, which help to solve crimes during criminal investigations, can be examined in one or more subclasses. Physiology, which investigates the growth and development of plants; morphology, which studies the structure of plants; anatomy, which studies the internal structure of plants; systematic, which investigates the naming and classification of plants and the relationship between them; cytology, which studies the structure and function of plant cells; molecular biology, which studies the genetic structure and function of the plant; economic botany, which studies the past, present and future uses of plants; ecology, which studies the relationship of plants with the environment in which they live; palaeobotany, which investigates fossil biology and evolution, is among these sub-sciences [2]. Forensic pharmacy, forensic pharmacology, forensic entomology, and forensic microbiology

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are four branches of science that cooperate closely with criminal botany [3]. Especially in recent years, criminal botany has become a prominent field, because the vegetative (root, stem, and leaf) and generative (flower, fruit, and seed) organs of plants can reveal reliable information about both the victim and the suspect or the crime scene in forensic cases. Forensic molecular genetic analysis, which has made significant progress and has become more accurate, is used extensively in solving crimes worldwide. To ensure reliable results in criminal investigations, current molecular genetic techniques must be used in addition to traditional microscopic studies. Although plants used in criminal botany are examined and used as evidence to elucidate crimes, unfortunately, comparing them at the DNA level and using them as primary evidence in forensic cases is not yet a frequently used method in Türkiye [4]. Fibre plants consist of fibre cells, and when they mature, they no longer have a DNA-containing nucleus or other cytoplasmic organelle. However, cells of parenchyma, collenchyma, and epidermis containing nuclei or organelles attached to fibres may have DNA [5]. DNA analysis is highly reliable in detecting diversity and relationships between plant genotypes within a population [6]. This study aimed to lead the way in assessing plant-based materials in forensic labs and can be used as a reference guide for experts.

In botanical terms, fibre is a plant cell formed by structural thickening of the cell wall. Fibres have essential functions in plant physiology, such as strengthening plant tissues and giving flexibility, tensile strength, and elasticity to the plant or organ [7]. It is estimated that there are approximately 1000 fibre plant species worldwide that can be used economically [8]. Fibers are grouped into two main groups, natural and artificial, according to their origin. Natural fibres are divided into three groups, these are vegetable, animal, and mineral (asbestos) fibres. While sorting vegetable fibers we can group them into four categories which are seed (cotton) fibers, leaf (abaca, sisal, aloe) fibers, fruit (coconut, kapok) fibers, bast-stem (flax, hemp, jute, ramie) fibers. On the other hand, animal fibres are divided into two main groups which are counted as leather products (sheep and rabbit wool, goat hair, camel hair) wool hairs, and secretion products (genuine (mulberry) silk and wild (Tussah) silk) fibres. Artificial fibers can be divided into three groups; regenerated (artificial, semi-artificial) fibers, inorganic-based (metallic, glass, and carbon fiber) fibers, and synthetic (polyester, polyamide, acrylic, polypropylene, polyethylene, polyvinyl chloride, polyurethane) fibers. Regenerated fibers are divided into three types according to their source; cellulose source (nitrate, copper, viscose, acetate, triacetate), protein source (casein, zein, soybean, peanut), and algae source (alginate) [9]. Forensic fibre experts report that 60 % of the fibre findings in crime scene investigations are clothing, 25 % are household items, and 15 % are materials used in technology, and all these textile products we frequently use in daily life [10]. The main methods applied for fibre analysis are SEM (Scanning Electron Microscope), TEM (Transmission Electron Microscope), Infrared Spectroscopy (IR), Micro Spectrophotometry (MS) and Thin Layer Chromatography (ITK). The most common way to distinguish between different types of fibres is by their colour. While the number of fibres gives us important clues about active contact, their location is also of great importance in designing and reconstructing the way the event occurs [11]. Matching can be made by comparing the content of an examined piece of fabric, the shape, structure, pattern, colour, and traces of the fibres on the weave with another sample [12]. Microscopy is often used to match the cut ends of the fibre and analyze the cellular composition of fibres to determine plant species. Wool fibres are unique from other fabrics due to their rougher surface and thicker diameter [13]. However, there are similarities between the structures and properties of linen and cotton fibres, which are vegetable fabric fibres.

With the help of advanced technology, the use of botanical evidence has become increasingly popular in solving criminal cases. By analyzing plant material, pollen, and other botanical evidence, investigators can gain valuable insights into a crime scene and identify suspects. Therefore, law enforcement agencies must recognize the significance of botanical evidence and incorporate it into their investigations. In addition, the use of plants for poison and therapeutic purposes has been quite common since ancient times. Volatile compounds secreted by plants play a vital role in various industries such as shampoo, soap, cosmetics, and perfume. Plants are also widely used for landscaping parks and gardens, as well as in furniture and construction materials. The presence of these materials in a structure can provide valuable evidence for forensic investigations. Identifying botanical evidence in personal crimes is crucial, but it is also important to identify plants that are illegal to grow, use, and sell. For this reason, it becomes important to identify plant parts such as seeds and leaves at the entry points of countries [14].

Using Botanical Evidence in Legal Investigations

Forensic botany is a scientific discipline that utilizes plant-based evidence to assist in criminal investigations. It involves analysing the plant materials found at the crime scene to determine various properties such as the origin of the evidence, the time elapsed since the incident, whether the body was moved, and the manner of death (murder, suicide, or accident). To do this, experts in this area study the physiological, morphological, ecological, systematic, and genetic characteristics of the plants found at the crime scene. Items such as ropes, sacks, and fabrics made from plant and animal fibres or secretions like silkworms can involve DNA. By determining the exact properties of materials containing plant DNA, it can be found out where they are made, who produces and sells them, and even who buys them. Just as the fingerprint evidence found at the crime scene is compared with the fingerprints of suspects to clarify a crime, plant DNA can also be isolated from materials at the crime scene or the place associated with the suspects.

The ubiquity of plants allows them to be used as supporting evidence in forensic science. Identifying botanical evidence in a forensic case helps solve cases such as determining geographical origin, establishing connections between the crime scene and individuals, testing alibis, and identifying plant species whose possession and trade are prohibited. In this section, the use of plants as evidence in legal investigations is explained. Additionally, in addition to identifying a plant by its morphological characteristics, determination of species characteristics and population similarity by DNA barcoding and

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DNA marker methods is emphasized.

Plant evidence is an important factor in solving criminal cases. The unique habitats and life cycles of plants can help link objects or individuals to a particular location. In cases where biological evidence is scarce or insufficient, microscopic pollen grains or small overlooked seeds become important in solving the cases [1]. For instance, plant parts found on clothing or under a vehicle can reveal the location of a crime and even the movement of a vehicle or person. By combining evidence from various locations, it becomes easier to establish links between victims or suspects and a particular crime scene [14]. Plants can indicate when an event occurred. Plants complete their life cycle in two stages: vegetative (growth phase) and generative (flowering phase). The life cycles of plants are divided into three groups: annual, biannual and perennial. Plants also show morphological and anatomical differences according to seasonal and climate characteristics. Today, in many countries, pollen calendars and maps are created based on the pollination periods of plants. Crime scene detection becomes easier by using pollen calendars and maps. The anatomical structures of the age rings formed by the accumulation of the conduction system in the wood tissue of trees also provide information about the climate characteristics of the spring and autumn seasons. An age ring in the wood tissue corresponds to one year of the tree. Thus, the estimated time of death can be determined by counting the age rings on the grave or the branch growing from the skeleton (dendrochronology) [15].

The genus and species identification of plant materials collected as evidence from the crime scene can be made by using sources containing flora information of the country where the incident took place. This identification must be made by an expert botanist to give the most accurate result. In addition, the application areas of genetic information and technology are frequently used to identify plant evidence that has not preserved its integrity. In cases where all plant characters are not available, plant DNA helps determine the identity of the plant. Genetic tools constitute a large amount of data in the use of plant samples as evidence. DNA, which forms the genetic material in all organisms, is universal to all life (except RNA viruses). In this way, DNA connects all living things and also records the history of mutations that cause DNA changes. Because some parts of the genome change faster than others, scientists can make comparisons at different levels using different regions of the genome. The origin of the material can be determined by identifying the species through genetic analysis and genetic identification can be made between two samples [14].

When it is not possible to identify a plant species morphologically and anatomically, it is possible to identify the species using DNA sequences. Similarities and differences in the sequence of DNA bases in a particular part of the plant genome are used to identify species. DNA barcoding is a rapidly developing field in which a standardized set of DNA regions is sequenced and an unknown sample is identified (usually by species) by comparing known samples in a database. Although Internal Transcribed Spacer (ITS) and sequences in the chloroplast genome, which encode ribosomal genes, are commonly preferred in DNA barcoding studies in plants, gene regions such as *rbc*L*, matK, trnH-psbA* are also used [16]. A DNA barcoding process; includes the extraction of DNA from the sample, PCR amplification of the regions containing the protected regions with relevant primers, sequencing of these regions with Sanger sequencing, and identification of the obtained sequences in international databases such as National Center for Biotechnology Information GenBank Database (NCBI).

Case Studies in Forensic Botany

Case studies where plants can be used as biological evidence:

- Creating a geographical profile from a plant sample using the plant's growing environment as a reference,
- Identification of a secondary crime scene where the victim's body was disposed of by using plant parts such as leaves, seeds, pollens or fibres collected from a person or vehicle,
- In murder, theft, and hit-and-run cases leave/take fibre on vehicles or grass stains on clothes after sexual assault (contact traces),
- Pollination process indicating burial season of skeletal remains in a mass grave,
- Use of plant contents taken from gastric lavage during autopsy to determine the time of death.

In forensic botany, determining the population from which plant species come is as important as identifying the species of plant samples used as evidence [16]. With genetic analyses based on DNA sequence polymorphism, the population origin of the plant sample and the genetic similarity between two plant samples can be identified. DNA marker methods such as RFLP, Amplified Fragment Length Polymorphism (AFLP), Single Nucleotide Polymorphism (SNP) and microsatellite or Simple Sequence Repeat (SSR) are frequently used to determine the population of origin of a plant sample and the region of distribution. Which marker system to use varies depending on the genetic characteristics of the plant species.

Molecular marker techniques are a method that facilitates the determination of genetic diversity based on the PCR used to reveal differences in a certain DNA fragment within the genome [17]. In the selection of any DNA marker system, it is critical to select markers that will be sufficiently variable within a population to distinguish individuals, even if they are closely related. In forensic medicine applications, plant DNA is used to identify *Cannabis sativa* samples, which are widely used in drug production [1]. Other applications include using plant DNA profiling to produce physical evidence to connect a suspect to a crime scene. For example, the seed pods of the *Palo verde* tree (Cercidium sp.) found at the murder scene and in the suspect's vehicle were used as evidence by matching the genetic profile with the RAPD marker technique [18]. Considering the effectiveness of botany in forensic cases, it should be encouraged to be used more widely.

Case Study 1

On March 1, 1932, botanical evidence was used for the first time in a New Jersey court investigation. In this incident, airman

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Charles Lindberg's son was kidnapped from his own home when he was only 20 months old. It was determined that the cause of death of the baby, who was found very close to the house where he was abducted two months later, was the result of a fracture caused by a blow to his skull. The most critical evidence to shed light on the incident was the wooden ladder used to climb to the baby's window. After identifying many suspects, the police found wooden beams during a search in the house of carpenter Bruno Richard Hauptman. Arthur Koehler, a tree identification expert, microscopically examined four trees, both handmade and commercial, for growth rings and knot patterns and compared them with the ladder found in Charles Lindberg's house. Upon examination, the structure of the sixteen-year-old growth rings and nodes matched the wooden beams found in Hauptman's home. Lindberg's kidnapping was admitted into evidence as the 'Crime of The Century,' and Hauptman was arrested [19].

Case Study 2

Molecular marker techniques are based on the amplification of various types of primers using the PCR technique to detect differences in protected regions in the DNA molecule [20]. DNA fingerprinting is regularly done in conjunction with the PCR technique. Disadvantages of this method are the difficulty in collecting DNA samples with a meticulous study and environmental factors. Since trace amounts of DNA samples can be used as evidence, great attention should be paid to the risk of cross-contamination when collecting findings from crime scenes and during preservation. The amount of DNA isolated from samples is insufficient for trace evidence DNA fingerprint studies. PCR allows obtaining enough DNA to create a DNA fingerprint from a minimal amount of DNA. This technique was used for the first time in a murder case in England. The use of DNA gained a different dimension with an unsolved murder that took place in England in 1983. In Narbourough, a 15-year-old girl named Lynda Mann was abducted, raped and she was found dead the next day. Three years after the incident, a 15-year-old girl named Dawn Ashworth was similarly raped and killed. Richard Buckland was arrested in connection with both events, although he only claimed responsibility for the second incident. DNA fingerprinting (typing), developed by Alec John Jeffreys to look for markers of genetic diseases, was used to find the killer(s) based on semen in corpses. Jeffreys examined semen samples from these two murder cases and concluded that both murders were committed by the same person. In the studies conducted, the DNA of Richard Buckland, who was arrested for two murders, did not match either murder. Buckland, who was released as a result, stated that the reason he took responsibility for the second incident was the pressure he was under. Later, 5500 men living in that region were included in the research. Colin Pitchwork persuaded his friend to give the test instead of him. However, one day, when he was at the pub, he told how he deceived the inspectors before. This was heard, and the police were informed. The sperm sample taken from Pitchwork was matched with both murders, and Pitchwork was arrested in 1987 [21].

Case Study 3

In an incident that took place in America in 1992, a woman was strangled and thrown into an area next to an empty factory. What makes this incident interesting is that the DNA of a plant growing at the crime scene was used to solve the case, not the suspect's DNA. The murdered woman was found near a *Palo verde* (syn. *Cercidium floridum*) tree in Phoenix, Arizona. The first-degree suspect denied being at the crime scene (alibi), but during the search of the pickup truck belonging to the man, seeds of this tree were found. Detectives asked Dr. Timothy Helentjaris who was working at Arizona University and discovered the DNA fingerprints of many agricultural plants if he could help to solve this murder case. He was given seeds from 12 different *Palo verde* trees at the crime scene, including one from the tree that was close to the body at the murder site. In addition, 18 different *Palo verde* seeds grown in the Phoenix area were also provided. The seed samples taken from the suspect's pickup truck and the seeds of the tree closest to the body were similar and matched. In this murder case, which was solved by using *Palo verde* seeds as primary evidence, it was revealed that the suspect was the murderer by proving that he was at the crime scene [22].

In recent years, studies on forensic palynology in our country have been carried out by conferences and seminars given within the scope of the 'Crime Scene Investigation and Identification Basic Training Course' organized by the Criminal Investigation and Technical Investigations Training Branch Directorate (KATEM) within the Criminal Department of the General Directorate of Security. After this training, a Crime Scene Investigation expert's palynological evidence collected from the scene is sent to Hacettepe University Palynology Laboratory for spore and pollen analysis [23]. The theft incident that took place in Sakarya province in our country is the first theft case solved by forensic palynology. With the help of palynological evidence and evidence obtained by fingerprint experts, it is proven that the suspect is not guilty and the suspect is acquitted. Palynological evidence was used in court for the first time and was recorded in official records [24].

Case Study 4

A 14-year-old girl went missing in Washington State. The girl was last seen with a man who was 10 years older than her. After an extensive search, no evidence was found regarding the girl's disappearance. Two days after the girl's disappearance, the suspect was arrested. After the suspect's arrest, the girl's body was found wrapped in a carpet in a grassy area near her home. Help was sought from a forensic botany expert to find out how long the body had been wrapped in the carpet. The carpet in which the body was wrapped caused a colour change in the grass. Since the grass cover at the scene was not exposed to sunlight, chlorophyll destruction occurred. The forensic botanist designed an experiment to determine how many days it would take to achieve the same colour change through chlorophyll degradation. In the experiment, 30 garbage bins were placed in the grassy area near the event. The garbage bins are designed in a similar structure to the carpet in which the body was wrapped. Every day, he lifted a trash can and took a photo of the grassy area. The loss of chlorophyll observed on the seventh day matched the appearance of the grassy field when the case was found. Thus, it was determined that the body was found seven days after being thrown onto the carpet [14].

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Case Study 5

In July 2011, during road maintenance work, human skeletal remains were found in an uncultivated area adjacent to a main road. First, a sampling strategy was applied to conduct a comparative analysis between samples obtained from local vegetation and human remains. The presence of a large root was detected in the victim's skull, entering the right acoustic hole. *Rubus* sp., a thorn species, was found under the victim's left shoe has been found. Likewise, a *Phytolacca americana* root was detected growing along the right shoe and the holes of the shoe and tightly surrounding the entire shoe. As a result of the examination of the detected samples, it was found that the roots on the side of the vertebra showed a 1-year development, the sample on the left shoe showed a 3-years growth, and the roots on the right shoe showed a 6-years developmental growth. The results obtained were used to estimate the time after death (post-mortem interval) and it was determined that the body belonged to a man who disappeared in 2003 [14].

Material and Methods

Materials

Nine independent exotic/tropical plant species (*Ananas comosus* (L.) Merr. (Figure 1a, 1b), *Chamaedorea elegans* Mart. (Figure 2a, 2b), *Nolina recurvata* (Lem.) Hemsl. (*Beaucarnea recurvata* Lem.) (Figure 3a, 3b, 3c), *Pandanus veitchii* hort. (4a, 4b), *Phoenix dactylifera* L. (Figure 5a, 5b), *Phormium tenax* J.R.Forst. & G.Forst. (Figure 6a, 6b), *Sansevieria trifasciata* Prain (Figure 7a, 7b), *Theobroma cacao* L. (Figure 8a, 8b), *Washingtonia filifera* (Linden ex André) H.Wendl.) (Figure 9a, 9b)) cultivated in the greenhouses or gardens of AHBG were selected and used their leaves (0.5-1.0 g) for molecular analysis as materials.

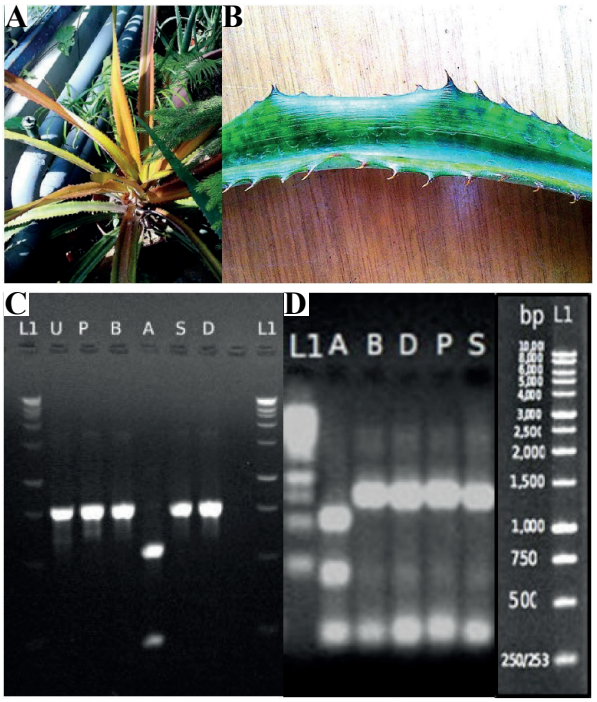

Figure 1. Ananas comosus (L.) Merr. plant (**1A, 1B**) and its DNA bands (**1C, 1D**)

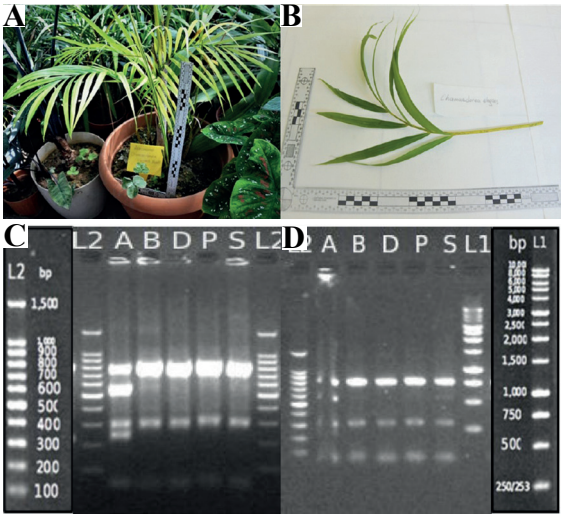

Figure 2. Chamaedorea elegans Mart. plant (**2A, 2B**) and its DNA bands (**2C, 2D**)

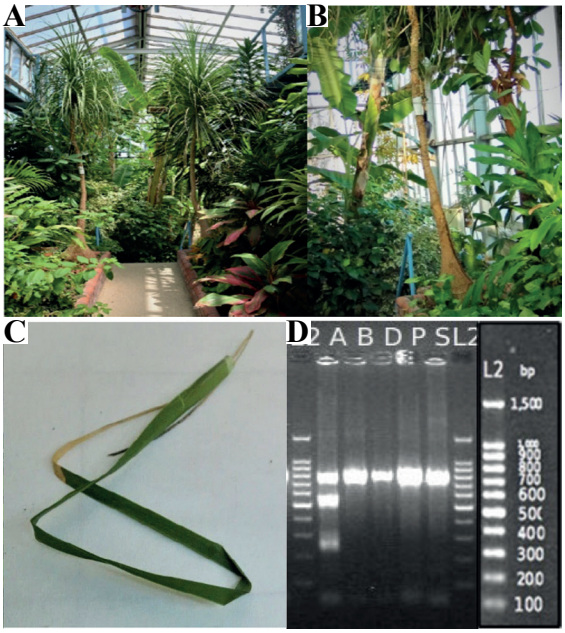

Figure 3. Nolina recurvata (Lem.) Hemsl. (Beaucarnea recurvata Lem.) plant (**3A, 3B, 3C**) and its DNA bands (**3D**)

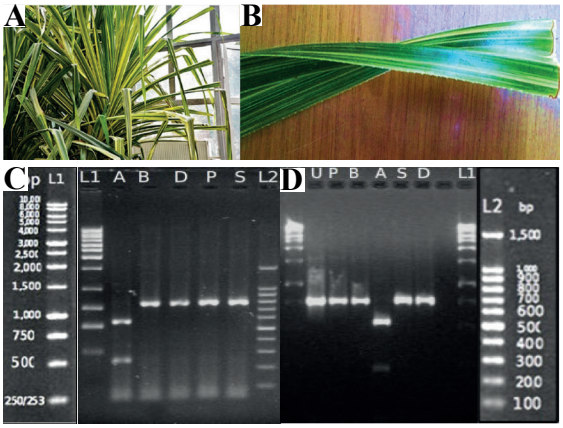

Figure 4. Pandanus veitchii hort. plant (**4A, 4B**) and its DNA bands (**4C, 4D**)

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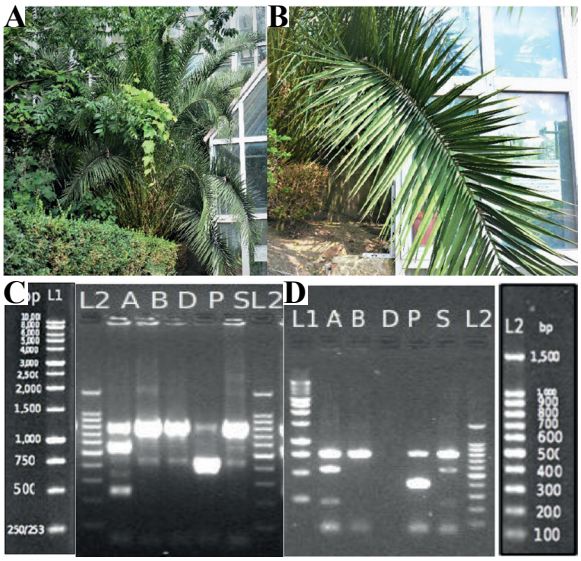

Figure 5. Phoenix dactylifera L. plant (**5A, 5B**) and its DNA bands (**5C, 5D**)

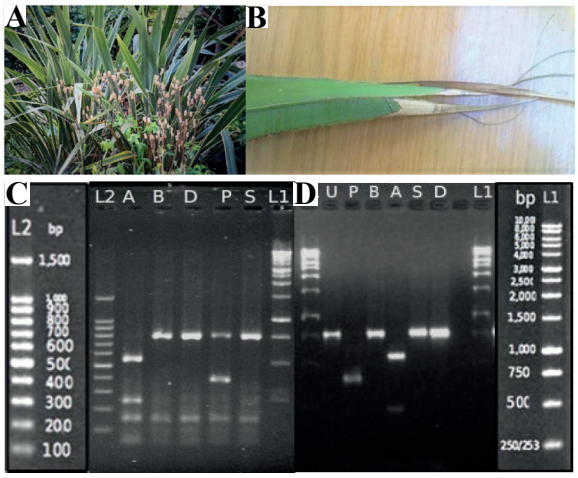

Figure 6. Phormium tenax J.R.Forst. & G.Forst. plant (**6A, 6B**) and its DNA bands (**6C, 6D**)

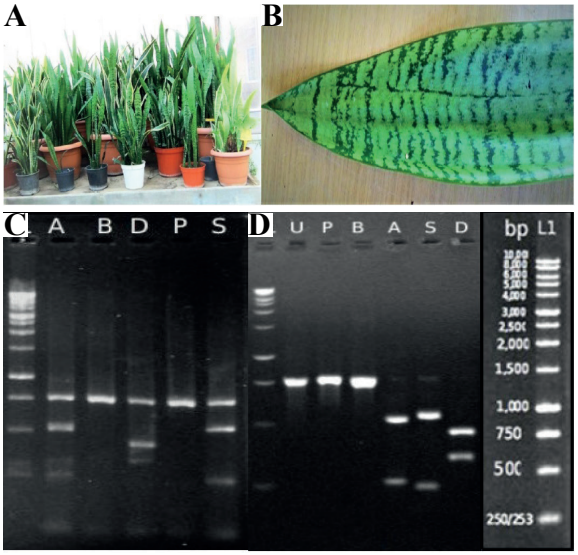

Figure 7. Sansevieria trifasciata Prain plant (**7A, 7B**) and its DNA bands (**7C, 7D**)

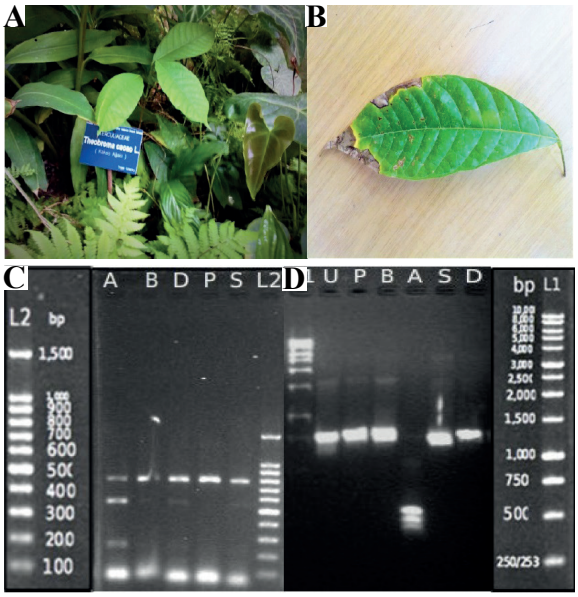

Figure 8. Theobroma cacao L. plant (**8A, 8B**) and its DNA bands (**8C, 8D**)

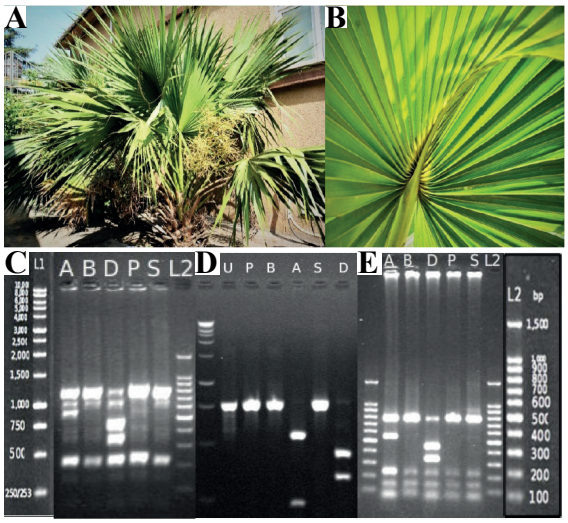

Figure 9. Washingtonia filifera (Linden ex André) H.Wendl.) plant (**9A, 9B**) and DNA bands of it (**9C, 9D, 9E**)

Methodology

The exotic and tropical plant taxa cultivated in AHBG were taken and kept at -80°C until examination, as the research did not require ethics committee approval. The samples were cleaned with distilled water and dried in the laboratory before the DNA isolation. The samples were pulverized with the help of liquid nitrogen (-196°C), then isolated with the DNeasy® Plant Mini Kit and modified the Cetyl Trimethyl Ammonium Bromide (CTAB) DNA Protocol for plants containing high polysaccharide and polyphenol components [25]. DNA amounts of all samples were measured numerically with the Quant-iT™ dsDNA Highly Sensitive (HS) Kit on the Qubit® Fluorometer device. The Agarose Gel Electrophoresis Technique was used to visualize the DNA bands formed after cutting by the restriction enzymes of amplified DNA fragments. The plastid gene, *rbcL*/

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RuBisCO, specific to plants and found in large numbers in every plant cell, was selected so interfering animal and fungal DNAs were eliminated. The *rbc*L gene was amplified by standard PCR using a pair of oligonucleotide primer sets (Forward: 5'-(1) TGTTTACTTCCATTGTGGGTAATG3'; Reverse: 5'CTGGTAGAGAGACCCAACTTGA (749)-3') and digested with various restriction enzymes. Five different restriction enzymes (*AccI (A): GT' MKAC M=A/C K=G/T, BamHI (B): G'GATCC, DraI (D): TTT'AAA, PstI (P): CTGCA'G, SacII (S): CCGC'GG and Uncut (U)*) were used to cut PCR products. Then 2% Agarose Gel Electrophoresis was carried out and stained DNA bands were visualized under ultraviolet (UV) light at 260-360 nm with Ethidium Bromide (EtBr) which shows fluorescence properties. Plants were typed and evaluated according to band length differences by using DNA ladders.

Results

The *rbc*L gene was analyzed with randomly selected exotic/tropical plant species using the CAPS markers [26]. PCR products were digested with cutting enzymes and run on agarose gel. The differences between plants could be determined according to the cutting areas of the enzymes. Due to different restriction enzyme digestions, each plant (*Ananas comosus* (L.) Merr. (Figure 1c, 1d), *Chamaedorea elegans* Mart. (Figure 2c, 2d), *Nolina recurvata* (Lem.) Hemsl. (*Beaucarnea recurvata* Lem.) (Figure 3d), *Pandanus veitchii* hort. (4c, 4d), *Phoenix dactylifera* L. (Figure 5c, 5d), *Phormium tenax* J.R.Forst. & G.Forst. (Figure 6c, 6d), *Sansevieria trifasciata* Prain (Figure 7c, 7d), *Theobroma cacao* L. (Figure 8c, 8d), *Washingtonia filifera* (Linden ex André) H.Wendl.) (Figure 9c, 9d, 9e)) was evaluated according to specific band patterns. It was observed that both two DNA isolation methods [27] worked well in this research for all plant species and we could see both monomorphic (uncut) and polymorphic separated band patterns clearly after cutting the PCR products with the restriction enzymes. However, it may be necessary to additionally determine the size of the particles produced by each restriction enzyme by base sequence analysis. In our study, we tried to determine whether there was a cut and the cut locations based on whether bands were formed or not by looking at the agarose gel images. In a similar experiment, Dunbar and Murphy (2009) observed that when the DNA amplicon was not cut by the enzyme, it resulted in a single band of an uncut particle with a size of 771 bp. Since the enzyme cutting points, which appear as a single band on the agarose gel, are close to each other, a single band is observed. It was concluded that the cutting locations can be determined only after a detailed base pair (index) analysis. Likewise, it is thought that new DNA bands may be formed as a result of contamination of the samples with another plant (grass), and the plant or plants that the different bands belong to can be revealed by detailed base sequence analysis. Therefore, before genetic analysis, it is recommended to wash the leaf sample under running water and, if necessary, with detergent.

Discussion

DNA sequence differences between genotypes, known as base polymorphism, are used in systematics. In recent years, DNA sequence differences have become widely used as systematic characters in taxonomic studies. Various advantages are associated with these genetic traits. For instance, environmental factors do not affect them. Additionally, mitochondrial and chloroplast genomes, which are maternally inherited, can be studied separately from nuclear genomes. It is possible to determine the phylogenetic origin and kinship relationships of plants by detecting different characters from each parent [28]. Genetic variations are responsible for the differentiation of taxa and the differences between populations of a taxon. DNA base polymorphisms are changes in the DNA sequence of the same gene that occur without causing a change in gene function. Natural polymorphisms occurring in the DNA sequence are used in systematic studies to elucidate the phylogenetic and kinship relationships of populations and taxa [29]. Natural polymorphisms occurring in the DNA sequence facilitate the adaptation of species to their environment, allowing them to survive in the evolutionary process. In addition, if these naturally occurring polymorphisms are transferred from individual to individual through sexual reproduction and become permanent in populations, they may also contribute to the speciation mechanism over time [30].

Although several studies have been conducted on the use of plants in forensic science, no cases involving DNA analysis of plant-derived industrial materials have been documented in Türkiye. Plant fibre examinations of crime scene evidence in the 'Criminal Police Laboratory' are conducted at the microscopic level in our country. It is thought that this method, which can be used to identify evidence, will be an alternative to the microscopic analyses currently used in criminal laboratories. Microscope examination depends on the experience and even interpretation of the expert and may not be sufficient to characterize all plant species. In addition, phenotypically close plant species may be genotypically very distant from each other. Molecular markers developed to eliminate this confusion are successfully used to reveal genetic differences in plants and the relationships more accurately between plant species in terms of morphological and genetic classification [30]. Single nucleotide changes such as Single Nucleotide Polymorphisms (SNPs) and insertions-deletions (InDels) occurring on the DNA change the recognition sites of endonucleases, causing the formation of DNA fragments of different lengths. It is based on the digestion of locus-specific PCR products with one or more endonucleases and the execution of the products in agarose or polyacrylamide gel. If there is no polymorphic band in an example, it is necessary to increase the number of restriction enzymes or make a index analysis to find the differences between samples. Thus, providing a species-specific match and identification can be performed. CAPS markers are codominant, locus-specific, and can easily distinguish homozygous-heterozygous alleles. The

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advantages of the CAPS method include the need for a very low amount of DNA, the ability to distinguish codominant alleles, and the fact that it is simple and inexpensive. DNA sequence changes that occur due to mutations can create a limiting effect by affecting the recognition sites of endonucleases and therefore prevent the of high polymorphisms such as AFLP. Despite this, CAPS is widely used in gene mapping studies and molecular identification studies [17].

The natural genetic diversity of plants based on DNA is used in various scientific research areas such as taxonomic studies, determining relationships, identifying cultured and wild forms, evolutionary issues, parentage determination, identifying individual genetic variations, creating genome maps, determining subspecies or races within a species, identifying whether plant taxa are monophyletic or polyphyletic, revealing natural taxa, determining phylogenetic relationships among taxa, preparing phylogenetic trees and cladograms, identifying polymorphisms, population genetics, plant breeding, revision studies, and forensic medicine applications.

The large sequence difference indicates that individuals evolved by diverging from both the ancestral cell and each other a long time ago. Their scarcity indicates that these creatures are close relatives and may even be included in the same species [3]. Since DNA sequences diverge and change during evolution, differences between sequences can be used to calculate the evolutionary distance between them. Genetic comparisons are generally considered the most accurate method to characterize evolutionary relatedness between species [20]. This method eliminates some misleading findings obtained through phenotypic comparisons. Evolutionary distances between species are shown in 'evolutionary tree' or 'phylogenetic tree' diagrams. These diagrams show the evolution of sister taxa from a common ancestor and the divergence of taxa over time.

There are some features that an ideal molecular marker should have. The first of these is that it can distinguish species. Second, there needs to be a standard DNA region that can be used for many different plant taxa. Third, the target DNA region must carry phylogenetic information that can easily distinguish the taxonomic groups (genus, family, etc.) to which the species belong. Fourth, the marker area should be very reliable and protected. It should also be suitable for DNA amplification and sequencing. Finally, the target DNA region must be long enough to allow gradient DNA amplification. Molecular marker techniques are based on the amplification of various types of primers using the PCR technique to detect differences in protected regions in the DNA molecule [20].

To elucidate forensic cases, trace amounts of biological material must often be examined at the DNA level. Morphological examination of the plant is not sufficient to determine the origin of the examined sample [5]. Plant taxa that are phenotypically close to each other may be genotypically very distant from each other. Molecular markers were developed to eliminate this confusion; it is successfully used to reveal genetic differences in plants and to more accurately reveal the relationships between plant taxa in terms of morphological and genetic classification [30]. The most appropriate method to reveal the evolutionary relationship and process between taxa in molecular systematic analyses is to transform the information obtained into family trees through various flow charts and biostatistical analyses [31,32].

Classical identification and determination studies based mostly on morphological characters are not sufficient to define systematic categories of plant groups. This has resulted in a focus on molecular methods in taxonomy studies in recent years. Molecular systematics researchers use molecules instead of phenotypic characters in their studies. The difference between the amino acid or nucleotide sequences of a homologous (same origin) macromolecule belonging to two different species also shows the evolutionary distance between these species. Because after two different species separate from a common ancestor and begin to evolve, many spontaneous mutations occur in their DNA. Non-coding parts of chloroplast DNA (cDNA), such as introns and intergenic segments, are more prone to change than the coding parts. Therefore, it has been revealed that the information character potential of these non-coding regions is higher than the coded regions. For this reason, they are more popular in inter-taxon phylogenetic studies [20].

Mapping of restriction sites of the chloroplast genome is widely used. However, its applicability is limited to taxonomic levels. Comparison of DNA sequences is the area within molecular systematics where the greatest progress has been made. Theoretically, any degree of difference can respond to comparative sequencing studies. In practice, plant systematists have focused on two slowly evolving sequences (*rbc*L and ribosomal RNA (rRNA) genes). DNA sequences sought for comparison, including rapidly changing chloroplast genes, chloroplast introns, intergenic intervals, and non-coding portions of the nuclear ribosomal DNA (rDNA) repeat, are evolving much more rapidly. The two primary sources of molecular variation used for phylogenetic purposes have been the chloroplast genome and the nuclear rDNA repeat region. The mitochondrial genome in plants has been less used in phylogenetic studies, in contrast to animal systematics, where the mitochondrial genome plays a central role. Providing cDNA data for phylogenetic reconstruction in angiosperms is mostly achieved by two approaches. These; restriction site mapping of the entire chloroplast genome and sequencing of the *rbc*L gene.

Restriction mapping has several advantages and is recommended for phylogenetic studies. 1) It is technologically simpler than many methods in molecular biology and allows researchers to compare many taxa simultaneously. 2) The chloroplast genome is large enough that many sites can be sampled per enzyme. Enzymes truncated 100 times per genome can be easily mapped. 3) In the case of hybridization or crossover when cpDNA passes from one lineage to another, the difference as a result of cutting

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with restriction enzymes of the chloroplast genome inherited by a small lineage group may not reflect a difference at the species level. 4) Most phylogenetically informative variation occurs at cutoff sites of non-coding regions. 5) Homoplasy is a similarity in features that is not due to a common ancestor. Most often, homoplasy is seen as a similarity in morphological features. However, homoplasy can also occur in other types of traits, such as similarity in genetic sequence, life cycle types, and even behavioural traits. Information obtained from restriction cut mapping shows a remarkably low degree of homoplasy between the same or related species. Recent studies have shown that the inverted region of cpDNA, which accounts for approximately 20% of the total genome complexity of most angiosperm cpDNAs, can be reliably mapped even for subclades of angiosperms. Many studies using restriction enzymes have shown that closely related species give the same results. There are 20 genes in the chloroplast genome (16S rRNA, 23S rRNA, psbA, psbD, psaB, psbB, psbC, psaA, rbcL, atpB, ndhA, atpA, ndhD, rpoB, rpoClh (h: contains a single large intron), ndhAh, rpoA, ndhF, rpoC2, matK (orfK)). Within cpDNA, comparative gene studies have focused on the large subunit of ribulose-1,5-bisphosphatecarboxylase (*rbc*L) [33].

Conclusion

Although whole plant parts are collected for later evaluation in forensic investigations, leaves are generally used for genetic analysis. Although DNA isolation can be done from living and dried flowers, decaying wood tissue, seeds, fruits and roots, since these structures contain polysaccharides that cause secondary thickening such as wood tissue, lignin and suberin, it becomes difficult to obtain DNA and the amount of DNA obtained is lower than the leaf. For this reason, although all plant tissues can be used for DNA isolation, using a fresh and healthy leaf sample (≥ 0.1 g) without insect traces will be more convenient for genetic studies. However, for successful isolation, appropriate tissue selection must be made, considering the age, condition and contribution of the tissue to the original DNA content. Drying plant samples in silica gel for genetic analysis is very important for preserving the samples. Since dried samples are sensitive to fungal infection, the leaf sample taken as a sample can be stored in a ziplock plastic bag by adding a few grams of silica gel. The material dried in silica gel can be stored at room temperature. DNA isolation from dried samples has also been performed on very old herbarium samples (150 years old), from seeds obtained in archaeological sites, and even from fossil samples [14]. On the other hand, freezing vegetal samples in liquid nitrogen and storing them in -80°C cabinets are also among the methods used for DNA isolation.

As a result, we successfully identified different types of exotic/tropical plant species and compared them with each other by looking at their band gaps. This method can be used to identify and compare plant fibres and plant-based industrial materials that may be found at crime scenes. Analysis of data obtained in comparative restriction site mapping and DNA sequencing methods requires sufficient taxon and character sampling to obtain the best possible estimate of phylogenetic relationships. It is hoped that by increasing the number of fibrous plants and increasing the restriction enzymes studied, the limited number of samples included in the study will serve as a resource. In comparative restriction site mapping and DNA sequencing methods, analysis of the resulting data requires sufficient taxon and character sampling to obtain the best possible estimate of phylogenetic relationships. DNA analyses can be performed as an alternative to microscopic studies using the *rbc*L/RuBisCO gene, which is specific among plants and obtained from the chloroplast.

Conflict of Interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical Approval

The exotic and tropical plants used in the study were legally cultivated at Istanbul University's Alfred Heilbronn Botanical Garden. The study did not involve experiments on humans, animals, or any narcotic or poisonous plants. The plants were chosen for their fibrous cell structure. Since the study does not have any harmful side effects on living things or nature, it does not require permission from an ethics committee.

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