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ORIGINAL ARTICLE

Botanical and genetic characteristics of *Celtis australis* L. and *Celtis occidentalis* L. grown in Egypt

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Abstract *Celtis australis* L. and *Celtis occidentalis* L. are deciduous ornamental trees, grown in Egypt. This study presents a comparative investigation of the botanical features of the stems, stem barks and leaves of both plants. Furthermore, the DNA of both plants was extracted from leaf samples and analyzed using 10 decamer random primers. A total of 159 random amplified polymorphic DNA (RAPD) markers were identified. Seed protein profiling was also performed and revealed low variation in both plants' SDS–PAGE profiles.

1. Introduction

Family Ulmaceae is a large family, containing about 15 genera and 200 species.[1] The largest genus, *Celtis*, includes about 60 species distributed in the temperate and tropical zones.[1,2] Among these species are *Celtis australis* L. and *Celtis occidentalis* L. which are commonly cultivated in the Egyptian gardens for shade purposes.

C. australis L. (Mediterranean hackberry, European nettle tree, lote tree, nettle tree)[3] is a deciduous tree native to the Mediterranean region and south western Asia. The leaves and fruits are astringent and stomachic. Decoctions of the leaves and fruits were used as remedy for amenorrhoea, heavy menstrual bleeding and colic. The leaves and fruits were also used to astringe the mucous membrane in peptic ulcers, diarrhoea and dysentery.[4] The plant is considered an important remedy for bone fracture, pimples, contusions, sprains and joint pains in Indian traditional medicine.[5]

C. occidentalis L. (Hackberry, American Hackberry) is a medium-size deciduous tree native to North America. Native Americans used decoctions prepared from the bark as an aid in menses and to relieve sore throat, and the wood extract in treatment of jaundice.[6,7]

Reports on the botanical characteristics of genus *Celtis* were found fragmentary[8–10]; in addition those on both species under

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investigation were almost lacking. Botanical characterization of closely related plant species is nowadays greatly supported via examination of various decisive genetic criteria such as isoenzymes, DNA or seed proteins.[11]

The concept of DNA fingerprinting has been, in this respect, increasingly applied to establish the ancestry of plants. It is reported as a promising tool for the authentication of medicinal plant species and especially useful in species or varieties that are morphologically and/or phytochemically indistinguishable.[12]

Seed protein profiling, obtained by polyacrylamide gel electrophoresis (PAGE), as well, is frequently used in taxonomy to provide valid evidence for addressing various taxonomic problems and has found wide application in resolving systematic relationships.[13] This is on account of several advantages including the high stability of seed proteins which are unaffected by environmental conditions.[14] Since differences between species is based on gene differences, and direct comparison of genes is both difficult and time consuming; therefore, these differences could be easily measured by comparing the products of gene activity, i.e. by using protein as genotype markers.[15] This technique is also considered rapid and inexpensive.[16]

Hence the objective of this work was targeted towards discrimination between *C. australis* L. and *C. occidentalis* L. through establishment of different botanical and genetic criteria which could be helpful in this respect.

2. Experimental

2.1. Plant material

Samples of *C. australis* L. and *C. occidentalis* L. used in this study were collected during the years 2008–2010 from El-Orman Botanical Garden and the Agricultural Museum, Giza, Egypt. Identity of the plant material was kindly verified by Dr. Mohamed El Gebaly, botanist specialist.

2.2. Botanical profiling

Specimens for morphological studies were dried according to standard herbarium techniques and voucher samples were kept in the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University. Photographs were taken using a Casio Digital camera.

Anatomical investigations were performed on cross-sections of the old and young stems, stem bark and leaves which were preserved in 70% alcohol and on air-dried finely powdered samples. The photographs were taken using a Leica DFC500 digital camera.

2.3. Genetic profiling

2.3.1. DNA fingerprinting

Entire fresh leaves of both plants under investigation were separately freeze-dried and ground to fine powder under liquid nitrogen prior to DNA isolation.

2.3.1.1. DNA extraction. DNA was extracted from 10 g of leaf tissue in 1.5 ml microfuge tubes using the DNA extraction method described by Williams et al.[17]

2.3.1.2. Oligonucleotide primers. A total of 10 random decamer oligonucleotide primers from A, C, D, E, G, M and Z kits (Operon Technologies Inc.) were used to amplify *Celtis* genomic DNA having the following sequences: OPA-18: AGGT GACCGT, OPC-11: AAAGCTGCGG, OPC-20: ACTTCGC CAC, OPD-12: CACCGTATCC, OPE-08: TCACCACGGT, OPG-09: CTGACGTCAC, OPG-18: GGCTCATGTG, OPM-06: CTGGGCAACT, OPM-08: TCTGTTCCCC, OPZ-20: ACTTTGGCGG.

2.3.1.3. Polymerase Chain Reaction (PCR). PCR amplification was conducted with 25 μl of reaction mixture containing 1% Triton 10-× reaction buffer (100 mM Tris–HCl (pH = 8.3), 500 mM KCl, 0.01% (w/v) gelatin), 2.0 μl $MgCl_2$ (25 mM), 2.5 μl of each dNTP (2 mM), 3 μl primer, 0.3 μl of Taq polymerase (Promega), and 2.5 μl of genomic DNA and completed to volume with distilled water. The reaction mixture was overlaid with two drops of mineral oil. The amplification reaction was carried out in a Thermocycler Perkin–Elmer Cetus 480 (Warrington, UK). The thermocycler was programmed for one cycle of 5 min initial strand separation at 94 °C and for 40 cycles each 1 min at 94 °C for denaturation, 1 min primer annealing at 36 °C, a 7 min primer elongation at 72 °C, followed by one cycle of final primer extension at 72 °C for 10 min.

2.3.1.4. Gel electrophoresis and staining. PCR products were separated in 1.4% agarose gel by electrophoresis in TE buffer (10 mM Tris–HC1, 1.0 mM EDTA, pH = 8.0) with a constant power of 100 V for about 3 h. The products were stained with ethidium bromide and then visualized and photographed under UV light using Bio-Rad Gel Doc-2000 (UK).

2.3.2. Seed protein profiling

2.3.2.1. Sample preparation. A sample (0.1 g) of mature seeds of each plant was powdered and mixed with 1 ml sample buffer (Tris borate solution (pH 8.2) (1:3 v/v). The slurry was centrifuged at 6000 rpm for 10 min. The supernatant containing the protein extract was used immediately for electrophoresis. The seed proteins of *C. australis* L. and *C. occidentalis* L. were analyzed using continuous polyacrylamide gel electrophoresis according to the method described by Stegmann.[18]

The prepared protein extracts were diluted with sample buffer, and then 500 μl of 10% SDS (sodium dodecylsulphate) were added followed by 25 μl of mercaptoethanol. They were placed in a boiling water bath for 5 min and 5 μl of bromophenol were added as a tracking dye. Ten microliters of each sample were loaded and 10 μl of marker protein mixture were used as standard.

2.3.2.2. Running conditions. The runs were carried out at a constant voltage of 200 V. Usual runs took approximately 120 min.

2.3.2.3. Gel staining and destaining. The separated protein fractions were stained with excess of coomassie brilliant blue stain R-250 for about 1 h. After gel staining, the gel was transferred to the destaining solution (15 ml ethanol, 50 ml glacial acetic acid and 300 ml distilled water) to remove excess stain.

2.3.2.4. Scoring of seed protein data. The protein binding profile in the gel was photographed. The banding profile was also scanned using SynGene System, version 4.00A (Gene tools