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Method

Comparison of two DNA extraction protocols from leave samples of *Cotinus coggygria*, *Citrus sinensis* and Genus *juglans*

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Abstract: High quality DNA is essential for molecular research. Secondary metabolites can affect the quantity and quality DNA. In current research two DNA isolation methods including CTAB and Delaporta (protocols 1 & 2 respectively) were applied in three leave samples from *Cotinus coggygria*, *Citrus sinensis* and *Genus juglans* that their leaves are rich of secondary metabolites. We successfully isolated DNA from *C. coggygria, C. sinensis* and Genus *Juglans* using the two protocols described above. Good quality DNA was isolated from *C. coggygria*, *C. sinensis* and Genus *Juglans* using protocol 1, while protocol 2 failed to produce usable DNA from these sources. The highest amount of DNA (1.3-1.6) was obtained from them using protocol 1. As we discovered, procedure 1 may work better for plants with secondary metabolites.

Key words: DNA extraction; secondary metabolites; *Cotinus coggygria*; *Citrus sinensis; Genus juglans.*

Introduction

High quality DNA is essential for molecular research. Secondary metabolites as contaminations are common problem in plant DNA extraction (1-2). These contaminating compounds can affect the quantity and quality DNA caused low efficiency modifying enzymes, such as restriction endonucleases and *Taq* polymerase (3). In many plant species there are high contents of polysaccharides, polyphenols or other secondary metabolites that can affect DNA extraction and purification. Many factors, such as choice of plant tissue, tissue preparation, and modifications of the extraction buffer, can help in DNA extraction for difficult plant species (3). Large amounts of complex polysaccharides can make extraction of usable DNA impossible, rendering the aqueous portion of the extraction too viscous to allow for efficient separation of DNA from the contaminating polysaccharides. These polysaccharides can also tightly adhere to the DNA, preventing access by modifying enzymes (4). Medicinal plants, crop plants, fruit trees, ornamental plants and desert shrubs are known for high contents of secondary metabolites, polysaccharides and polyphenolics (5).

The *Cotinus coggygria*, *Citrus sinensis* and *Genus juglans* plants leaves are rich of secondary metabolites. Herbal and aromatic plants are attracting more attention among contemporary plant researchers because some human diseases resulting from bacterial antibiotic resistances have gained worldwide concern. These plants contain exceptionally high amounts of polysaccharides, polyphenols, and other secondary metabolites that have medicinal properties (6). *Cotinus coggygria* Scop contains many phenolic compounds, phytochemical investigations of it, a medicinal and tinctorial plant used since antiquity (7). Citrus is one of the most important commercial fruit crops grown on all continents of the world. *Citrus* family had a rich source of phytochemicals such as flavanones, polyphenols, anthocyanins and hydroxycinnamic acids, which are beneficial to most pathological conditions which includes, high cholesterol and antiinflammation; complications related to diabetes and cancer prevention (8). Polysaccharides are common in Juglandacea leaves which makes it rather difficult to obtain high quality genomic DNA from their tissues (9).

In current research two DNA isolation methods were applied in *Cotinus coggygria*, *Citrus sinensis* and *Genus juglans* leaves.

Materials and Methods

Three leave samples from *Cotinus coggygria*, *Citrus sinensis* and *Genus juglans* were collected for DNA extraction. Leaves after collection were brought to laboratory in liquid nitrogen and stored at -20˚C. The leaves were subjected to two published genomic DNA extraction protocols, which comprised of Murry and Thompson (1980) (10) (protocol 1) and Dellaporta *et al* (1983) (11) (protocol 2) developed in our laboratory. The procedure for these protocols had been described in

this paper.

DNA Isolation

Protocol 1

100 milligram of fresh leaf tissue was weighed in an electronic balance and ground into a fine powder in liquid nitrogen by a pestle and mortar, then transferred into a 1.5 ml microfuge tube. 500 µl of extraction buffer (2% CTAB, 50 mM Tris-HCl (pH 8), 5% NaCl, 20 mM EDTA, 0.5% PVP, 2% ß-Mercaptoethanol (freshly)) was added to the tube and mixed for 5 s. In order to disrupt plant cells completely, the tube was placed in a water bath at 65 ºC for 60 min. To each tube add 400 µl of Chloroform: Iso Amyl Alcohol (24:1) and mix the solution by inversion. After mixing, the tubes were centrifuged at 8000 rpm for 10 min and transferred the upper aqueous phase to a clean microfuge tube. To each tube added equal cold absolute ethanol and then incubated at -20 °C for 1 hour, centrifuging at 12000 rpm for 10 min and then remove all the supernatant. 1 ml of washing buffer (ammonium acetate 1 M, Ethanol 100%, DDW) was added to the tube and after mixing; centrifuge the tubes at 10000 rpm, 4 ºC for 10 min. All the supernatant removed and repeat this step again. Add equal ice cold 70 % ethanol and centrifuge the tubes at 10000 rpm, 4 ºC for 5 min. The DNA resuspend in 50 µl sterile DNase free water (10).

Protocol 2

100 milligram of fresh leaf tissue was weighed in an electronic balance and ground into a fine powder in liquid nitrogen by a pestle and mortar, then transferred into a 1.5 ml microfuge tube, 500 µl of extraction buffer (100 mM Tris, pH 8.0, 50 mM (EDTA), pH 8.0, 500 mM NaCl, 2.5% SDS) was added to the tube and mixed for 5 s. In order to disrupt plant cells completely, the tube was placed in a water bath at 65ºC for 60 min. Add 500 µl potassium acetate 5 M to each tube and then placed at -20 ° C for 15 minutes. To each tube add 400 µl of Chloroform : Isoamyl alcohol (24:1) and mix the solution by inversion. After mixing, centrifuge the tubes at 8000 rpm for 10 min. Transfer the upper aqueous phase to a clean microfuge tube. To each tube add equal cold absolute ethanol and then placed at -20 ° C for 1 hour. Centrifuge at 12000 rpm for 10 min and then removed all the supernatant. Add equal ice cold 70 % ethanol and centrifuge the tubes at 10000 rpm, 4 ºC for 5 min. The DNA resuspended in 50 µl sterile DNase free water (11).

Gel electrophoresis

Samples run on 1% agarose gel were utilized to have a visible test of quantity and quality of extracted DNA. The gel was run in 1x TAE (Tris-base, glacial acetic acid, 0.5 M EDTA) and Staining with novel juice. It was visualized and photographed under a UV light source.

UV Spectrophotometric Analysis

UV-Visible spectrophotometer was used to measure the absorbance of isolated genomic DNA at *A*260 and *A*280 nm. While the purity of extracted DNA was determined based on the ratio of A260/A280, the yield was measured according to the formula (DNA (µg) = A260 x 50 x Dilution factor). Dilute the genomic DNA with DDW and use Eppendorf UV Biophotometer to measure the ratio A260 / A280 and DNA concentration.

Results

We successfully isolated DNA from *Cotinus coggygria*, *Citrus sinensis* and Genus *Juglans* using the 2 protocols described above. Good quality DNA was isolated from *C. coggygria*, *C. sinensis* and *Genus Juglans* using protocol 1, while protocol 2 failed to produce usable DNA from these sources. The highest amount of DNA was obtained from them using protocol 1 (Fig 1). In protocol 1 the isolated DNA had normal spectra in which the A260/A280 ratios were 1.3-1.6 while protocol 2 it was 1.2-1.4 (Table 1). Using protocol 1, colorless DNA from the 3 plant sources was obtained. With protocol 2, the isolated DNA was dark brown or yellow, and, for the most part, unusable. In protocol 1, the extraction buffer contains high amounts of PVP and β-mercaptoethanol, which prevent oxidation of the secondary metabolites in the disrupted plant material. CTAB is used as a detergent in the extraction buffer to separate polysaccharides from DNA. As we discovered, procedure 1 may work better for plants whit secondary metabolites.

Discussion

Many plant DNA extraction protocols for removing polysaccharides have been reported (4. 12-15). In some woody fruit crops that contain high polysaccharide levels, such as crops of *Citrus* spp., the protocols could only be used on vigorous tissue (13, 16). Crops of *Citrus* spp. and other tropical or subtropical fruits are perennial woody plants. Polysaccharide content, even in young tissue, is higher than in field crops (8). Several modified DNA protocols that have removed polysaccharides have recently been reported (4, 12, 17). All were unsuccessful in removing polysaccharides from crops of *Citrus* spp.

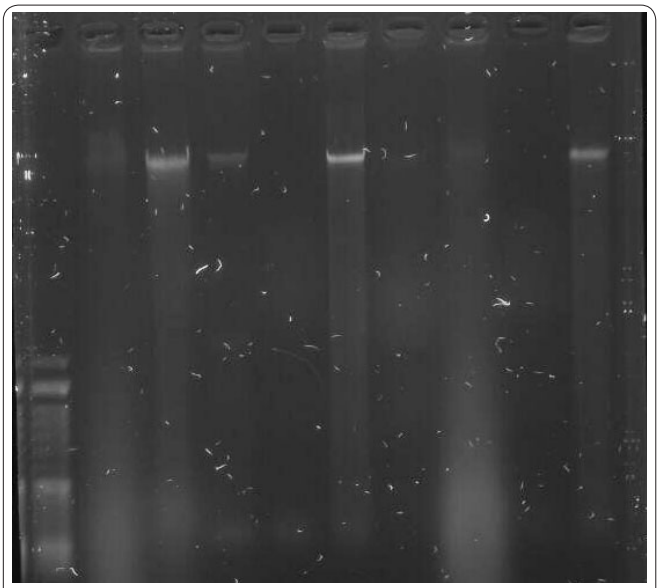

Figure 1. Agarose gel electrophoresis for extracted genomic DNA from three species and two methods. Where 1: size marker, 2: *Citrus sinensis* via CTAB, 3: *C. sinensis* via Delaporta, 4: *Cotinus coggygria* via CTAb, 5: Blank, 6: *C. coggygria* via Delaporta, 7: Blank, 8: *Juglans* via CTAB, 9 Blank and 10: *Juglans* via Delaporta.

and other fruits. Isolating high quality DNA for RFLP analysis of some materials, such as withered and old frosted *Citrus* leaves, was difficult. DNA samples were hyaloplasm gel-like (almost insoluble in TE buffer); A260/280 ratios were always less than 1.5; and a peak of 270 nm, corresponding to the peak of a combination of phenol and polysaccharides, was usually scanned (18).

DNA extraction has been reported in plant (19) and non-plants (20) previously.

In conclusion, the current research indicates that the highest amount of DNA was resulted from studied plants using CTAB protocol. The CTAB may work better for plants with secondary metabolites.

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