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Rapid and Efficient Method of Genomic DNA Extraction from Pistachio Trees (*Pistacia vera* L.)

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Abstract: This study was conducted to develop a rapid and efficient protocol for extracting high quality DNA from Pistachio trees suitable for PCR and molecular studies. Genomic DNA was extracted from 12 Pistachio trees using modified QIAGEN DNeasy Plant Mini Kit. The results showed that the modified protocol successfully produced a sufficient amount of DNA with high quality, which was highly confirmed by the purity index values of DNA samples (1.45 to 2.01). In conclusion, the modified protocol can produce high quality DNA from Pistachio trees suitable for PCR studies such as RAPD and AFLP and it can be easily adjusted for other *Pistacia* species.

Key words: Pistachio, *Pistacia vera*, genomic DNA, extraction

INTRODUCTION

Pistachio (*Pistacia vera* L.) is one of the most economically important tree crop in the world. It belongs to the Ancardiaceae family (Cashew family). Among the nut tree crops, pistachio tree ranks sixth in the world production after almond, walnut, cashew, hazelnut and chestnut (FAO, 2005). Total pistachio world production is 501, 000 metric tons (FAO, 2005) and the main producer was Iran followed by USA, Turkey and Syria (FAO, 2005).

Pistacia vera L. (cultivated pistachio) is by far the most economically important species in the genus. It has edible seeds and considerable commercial importance. The other species grow in the wild and their seeds are used as a rootstock seed source and sometimes are used for fruit consumption, oil extraction, or soap production (Ercisli, 2004; Al-Saghir, 2009).

The pistachio is native to the arid zones of Central Asia; it has been cultivated for 3000-4000 years in Iran and was introduced into Mediterranean Europe by Romans at the beginning of the Christian era (Crane, 1978). Pistachio cultivation extended westward from its center of origin to Italy, Spain and other Mediterranean regions of Southern Europe, North Africa and the Middle East, as well as to China and more recently to the United States and Australia (Maggs, 1973; Hormaza *et al*., 1994, 1998). Pistachios are utilized for the most part in the shell for fresh consumption; processed uses include candy, baked goods and ice cream (Ercisli, 2004; Al-Saghir, 2009).

Protocols for extracting genomic DNA from woody tree crops are hampered by phenol substances and polysaccharides produced by these crops including Pistachio (Al-Saghir and Porter, 2006; Oboh *et al*., 2009). Phenolic substances and polysaccharides are found interfere and react with nucleic acids and proteins and inhibit PCR and molecular reactions (Pandey *et al*., 1996; Oboh *et al*., 2009). Therefore, many molecular and breeding studies are hindered by the poor quality of the extracted DNA. No specific efficient protocol for extracting genomic DNA from Pistachio has been published or reported or a protocol that

eliminates polysaccharides associated with the extracted genomic DNA. Rapid, easy and affordable protocol is highly needed particularly in developing countries.

The objective of this study is to develop an efficient, rapid and easy protocol from extracting high quality genomic DNA from Pistachio leaves suitable for PCR and molecular studies.

MATERIALS AND METHODS

Plant Material

Twelve accessions of *Pistacia vera* L. were collected in the field in Jordan in May 2004. The plant materials used for DNA extraction were dried leaves. The study was conducted at Ohio University Zanesville in June 2009.

DNA Extraction

The extraction protocol is a modified DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA) as the following: for each sample, 50 mg of dried leave was placed in bead tube (MO BIO Laboratories Inc., Carlsbad, CA, USA) and 500 µL of AP1 (QIAGEN Inc.) was added. The bead tubes were secured horizontally using the MO BIO Vortex Adapter (MOBIO Catalog No. 13000-V1 or 13000-LV2) or tubes were horizontally secured on a flat-bed vortex pad with tape. The tubes were vortexed at maximum speed for 15 min. Six microliter RNase A stock solution (100 mg mL^{-1}) was added to the mixture and vigorously mixed. The mixture was incubated for 15 min at 65°C and mixed 3 or 4 times during incubation by inverting tube. One hundred and fifty microliter Buffer AP2 was added to the lysate, mixed and incubated for 8 min at 4°C.

The lysate was pipetted into the QIA shredder Mini spin column (lilac) placed in a 2 mL collection tube and centrifuged for 2 min at 20,000x g (14,000 rpm). Then the flow-through fraction was transferred into a new tube without disturbing the cell-debris pellet. Typically 600 µL of lysate was recovered. For some plant species less lysate might be recovered. In this case, the volume should be determined for the next step. 1.5 volumes of buffer AP3/E to the cleared lysate, was added and mixed by pipetting. Then 650-700 µL of the mixture from previous step, including any precipitate that may have formed, was transferred into the Dneasy Mini spin column placed in a 2 mL collection tube then centrifuged for 1 min at 6000x g (corresponds to 8000 rpm for most microcentrifuges) and the flow-through was discarded. The same previous step was repeated with remaining sample, flow-through and collection tube were discarded. The DNeasy Mini spin column was placed into a new 2 mL collection tube and 700 µL Buffer AW was added and centrifuged for 1 min at 6000x g (8000 rpm). The flow-through was discarded and the collection tube was reused in the next step. The previous step was repeated and the column was centrifuged for 2 min at 20,000x g (14,000 rpm). The DNeasy Mini spin column was transferred to a 1.5 or 2 mL microcentrifuge tube and 60 µL buffer AE was added directly onto the DNeasy membrane then incubated for 5 min at room temperature (15-25°C) and centrifuged for 1 min at 6000x g (8000 rpm) to elute. The DNA samples can be stored at 4°C or -20°C.

DNA Analysis

The quality of the extracted DNA samples were analyzed by gel electrophoresis in 1% ultrapure agarose in 1X TBE buffer stained with ethidium bromide (0.5 µg mL^{-1}) at 100 volts using horizontal gel electrophoresis apparatus (Sigma Chemical Co. Louis, MO, USA). The amplified products were visualized under UV light and photographed with digital Olympus

C-7070 camera (Olympus imaging America Inc., Melville, NY, USA). One kilo base pair ladder was used as a DNA molecular marker to estimate the molecular weights of the amplified products.

The DNA concentration and purity was determined using Nano Drop 1000 spectrophotometer (Thermo Scientific Inc., Wilmington, DE, USA).

RESULTS

Gel electrophoresis showed high molecular weight bands for all samples (Fig .1). All the 12 samples showed distinct bands. The gel results clearly showed that the current modified protocol produced a sufficient amount of DNA. The DNA concentration was measured using Nano Drop 1000 spectrophotometer, the DNA concentration values ranged from 7.51 ng to 64.34 ng μL^{-1} (Table 1). These values confirmed the gel electrophoresis results. The purity index of DNA, measured by the ratio of absorbance at 260 and 280 nm using the same instrument, the purity index ranged from 1.45 to 2.01, which clearly illustrates a high quality DNA for all samples (Table 1) produced by the current modified protocol.

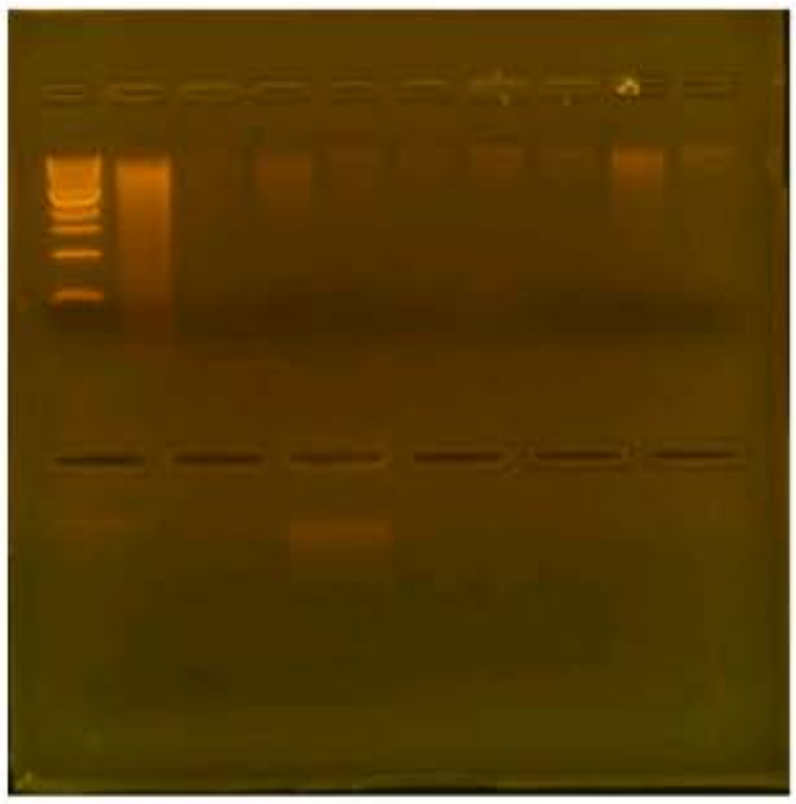

Fig. 1: Ethidium bromide stained gel of Twelve DNA samples extracted from pistachio leaves. First well is DNA molecular weight marker; upper line from left to right S1 to S9, respectively; lower line from left to right S9 to S12, respectively

Table 1: DNA concentration and purity index for all extracted DNA samples from 12 Pistachio trees as measured by Nano Drop 1000 spectrophotometer

Sample ID	User ID	Date	Time (PM)	ng μL^{-1}	A260	A280	260/280	260/230	Constant
	Default	8/15/2009	8:46	64.34	1.287	0.717	1.80	1.70	50.00
S1	Default	8/15/2009	8:48	33.54	0.671	0.359	1.87	1.25	50.00
S2	Default	8/15/2009	8:49	22.61	0.452	0.287	1.57	0.45	50.00
S3	Default	8/15/2009	8:50	10.09	0.202	0.131	1.54	0.43	50.00
S4	Default	8/15/2009	8:51	6.50	0.130	0.067	1.95	0.34	50.00
S5	Default	8/15/2009	8:52	9.75	0.195	0.119	1.63	0.54	50.00
S6	Default	8/15/2009	8:53	9.74	0.195	0.120	1.63	0.45	50.00
S7	Default	8/15/2009	8:54	16.40	0.328	0.180	1.82	0.69	50.00
S8	Default	8/15/2009	8:55	12.77	0.255	0.156	1.64	0.60	50.00
S9	Default	8/15/2009	8:56	12.12	0.242	0.146	1.66	0.56	50.00
S10	Default	8/15/2009	8:57	11.93	0.239	0.165	1.45	0.74	50.00
S11	Default	8/15/2009	8:58	18.53	0.371	0.247	1.50	0.92	50.00
S12	Default	8/15/2009	8:59	7.51	0.150	0.075	2.01	0.45	50.00

DISCUSSION

The original QIAGEN DNeasy Plant Mini Kit (QIAGNE Inc., DNeasy Plant Handbook 2006, http://www1.qiagen.com/literature), which was modified in this study, is not suitable for DNA extraction from hard tissues of woody tree crops (dry or green). In addition, it doesn't yield a high quality genomic DNA from woody tree crops such as pistachio (Al-Saghir and Porter, 2006). Moreover, the conventional DNA extraction methods like Dellaporta *et al*. (1983) protocol are laborious, time consuming, non-reproducible, yield poor quality DNA and produce many bio hazardous wastes (Oboh *et al*., 2009).

This study is the first to report a specific suitable protocol for extracting genomic DNA from Pistachio trees. Unlike the original QIAGEN Dneasy Plant Mini Kit and Dellpatora *et al*. (1983), the modified protocol is easy, rapid, efficient for extracting high quality genomic DNA. The protocol is reliable in terms of time taken and the amount of plant tissue used. The protocol is affordable and practical for use in developing countries. The protocol doesn't require many or expensive equipments. In this protocol, liquid nitrogen was excluded. It doesn't utilize bio hazardous chemicals. Moreover, ultra centrifuge is used instead of bench centrifuge.

In conclusion, this protocol extracts highly quality DNA with very good quantity from Pistachio leaves (green or dry) suitable for molecular studies and PCR applications such as RAPD and AFLP. It can be easily adjusted for other *Pistacia* species and tree crops.

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