

Full Length Research Paper

Protocol for isolation of genomic DNA from dry and fresh roots of medicinal plants suitable for RAPD and restriction digestion

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A simple and efficient protocol for isolating genomic DNA from fresh and dry roots of medicinal plants was developed. It involves a modified CTAB procedure using 3% CTAB, 4% β -mercaptoethanol, 2 M NaCl and 5% PVP. The extraction was carried out at 70°C. A slight increase in the concentrations of these chemical components and temperature helped in the removal of secondary metabolites and polysaccharides from the DNA preparation. The quantity and purity of isolated DNA was higher when compared with DNA extracted by the methods of Dellaporta et al. (1983) and Doyle and Doyle (1990). The DNA yield ranged from 33 to 68 μ g per g of root samples and it was 1.47 times greater in dried than fresh samples. The DNA samples were found suitable for analysis with restriction enzyme digestion and random amplification of polymorphic DNA (RAPD). The total duration for DNA extraction from roots of medicinal plants using this protocol was 135 min as compared to 225 min with existing protocols.

Key words: DNA isolation, roots, medicinal plants, secondary metabolite, PCR amplification, restriction digestion.

INTRODUCTION

The traditional systems of medicine such as Unani, Ayurveda and Homeopathy are based on the use of whole plants or their parts, including roots, stems, leaves, flowers and seeds. Even the modern allopathy depends on plants for several lead molecules and preparations. With the development of drug resistance in pathogens and adverse side effects of Allopathic medicines, the interest in herbal drugs for health care is increasing globally, hence maintaining the purity and quality of herbal drugs are necessary. One of the major problems faced by pharmaceutical industry is to maintain the quality and efficacy of herbal drugs is the lack of purity for herbal raw materials. Furthermore, using traditional identification methods to identify the correct plant part needed for the preparation of herbal medicine can be

difficult. Creating a novel system to assess the quality of a medicinal herb and to discriminate adulterants from authentic raw material is essential. Our major objective therefore, is to develop molecular tools for accurate identification of root samples of some important medicinal plant species obtained from market or collected from wild. The isolation of DNA is the first step in developing this technology.

With the increasing use of DNA fingerprinting in plant and its potential use in herbal drug industry, the preparation of good quality and quantity DNA has become a major concern. The extraction from tissue needs to be simple, rapid, efficient and inexpensive when many samples are used, such as in population studies, molecular breeding and screening of raw herbal drug materials. Several methods for extracting plant DNA from different plant parts including roots were developed (Dellaporta et al., 1983; Keim et al., 1988; Doyle and Doyle et al., 1990; Khanuja et al., 1999; Kumar et al., 2003). The methods employed for extracting DNA from

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fresh and dried root samples of medicinal plants however are time consuming and yield DNA in lesser quantity and low purity due to the presence of high levels of polysaccharides, phenolics and other secondary metabolites in these samples.

In this communication, we have described an easy and rapid protocol to extract genomic DNA from the fresh and dry roots of some important medicinal plants. The method involves a modified CTAB procedure of Doyle and Doyle (1990). The DNA obtained through this method was highly pure and proved to be good in restriction digestion with endonucleases and also for polymerase chain reaction conducted with random primers. The time taken for DNA extraction using this protocol was also less as compared to the existing protocols.

MATERIALS AND METHODS

Plant material

Samples of fresh mature roots were collected from the herbal garden of Jamia Hamdard University. These were washed with sterilize distilled water followed by 80% alcohol. Samples from fresh roots were kept at 4°C before use. Another representative of samples of fresh roots was dried at 50°C till their weight became constant. These fresh and dried samples were subsequently processed for DNA extraction.

Solutions

The extraction buffer consisting of 3% (w/v) CTAB, 100 mM Tris-HCl (pH 8), 2 M NaCl, 25 mM EDTA (pH 8), 4% β -mercaptoethanol (v/v), and 5% PVP (w/v) was prepared. In addition, chloroform : isoamylalcohol (24:1, v/v) solution, TE buffer (10 mM Tris-HCl, pH 8, 1 mM EDTA) and 80% ethanol were also prepared and stored.

DNA isolation and purifications

Fresh and dried roots of *Asparagus adscendens*, *Withania somnifera*, *Asparagus racemosus* and *Chlorophytum borivillianum* were surface sterilized with sterile distilled water followed by 80% ethanol. These were cut into pieces of approximately 1 mm size with the sterile blade. The pre-chilled mortar and pestle was used to ground fresh (2 g) and dried (1 g) root samples in the presence of liquid nitrogen. The frozen powder was transferred in 8 ml of the extraction buffer (pH 8) into a 30 ml centrifuge tube. The extraction buffer and frozen powder were mixed well and incubated at temperature 70°C for 30 min. After incubation the mixture was cooled at room temperature and thereafter, equal volume of the mixture of chloroform : isoamylalcohol (24 : 1) was added.

The mixture was centrifuged at 12000 rpm for 10 min at 25°C. The aqueous phase was transferred to a fresh tube and the DNA was precipitated by adding 0.6 volumes of ice-cold isopropanol and stored at -20°C for 30 min. Precipitated DNA was centrifuged at 9000 rpm for 10 min at 4°C. Supernatant was decanted carefully and pellet washed with 80% ethanol. The pellet was dried at 37°C for 12 min and dissolved in 400 μ l of 1x TE. The crude DNA sample was treated with 1 μ l RNase (10 mg/ml stock) for 10 min at 37°C and equal volume of the mixture of chloroform: isoamylalcohol (24:1) was added. It was thereafter centrifuged at 8000 rpm for 10

min at 4°C. The aqueous phase was taken and 0.6 volumes of isopropanol were added. It was then kept at -20°C for 10 min. The mixture was centrifuged at 8000 rpm for 5 min at 4°C and supernatant decanted carefully. The pellet was washed with 80% ethanol twice and dried at 37°C for 10 min. Finally DNA pellet was dissolved in 50 μ l of 1x TE buffer.

Quantification and restriction of DNA

The DNA yields per g fresh and per g dry root tissues were determined using a UV-VIS spectrophotometer (Specgene, Techne, England) at 260 and 280 nm. The purity of DNA was determined by calculating the ratio of absorbance at 260 nm and at 280 nm. The purity of extracted DNA was checked with restriction digestion of the purified DNA samples with EcoRI (4 unit per μ g) for 5 h incubation periods and restricted DNA run on ethidium bromide stained agarose gel (1.2%).

PCR amplification

To check the quality of isolated DNA from the root samples of selected medicinal plants, PCR reaction was performed. The PCR programme had 36 cycles in which first denaturation was carried out at 94°C for 3 min, segment denaturation at 94°C for 1 min, annealing at 35.5°C for 1 min, extension at 72°C for 2 min and final extension for 3 min at 72°C. Amplification reaction was carried out with random primers: 5'GTCGCCGTCA3' (P1) and 5GTTTCGGTCC3' (P2) (Imperial) in 25 μ l volume. PCR products loaded onto 1.2% agarose gel stained with ethidium bromide.

RESULTS AND DISCUSSION

Medicinal plants have large amounts of secondary metabolites. These secondary metabolites make hindrance in DNA isolation and isolated DNA is not suitable for PCR amplification and restriction digestion. We followed the protocol described by Dellaporta et al. (1983) and Doyle and Doyle (1990) for the isolation of genomic DNA from the fresh and dried roots of *A. adscendens*, *W. somnifera*, *A. racemosus* and *C. borivillianum*. DNA extracted, however was very viscous and could not be resolved on the agarose gel. The other problems encountered were low DNA yields and poor PCR amplification as well as restriction digestion. We increased the concentration of PVP from 1 to 5%, β -mercaptoethanol (v/v) 1 to 4%, and NaCl 1 to 2 M and incubation temperature from 65°C to 70°C temperature. These modifications helped in the extraction of DNA of high purity and high quantity. We also eliminated the use of phenol for the purification of extracted DNA, which makes our method less hazardous. We also used 1 μ l RNase A (10 μ g per μ l) per 400 μ l DNA samples instead of 10 μ l RNase A per 400 μ l DNA samples for removal of RNA in other protocols. The DNA yields and resolution on agarose gel however, were much higher and better from both fresh and dried root samples of medicinal plants with the use of our protocol (Table 1 and Figure 1a).

The DNA yield ranged from 48 to 68 μ g per g of dried root tissues and from 33 to 42 μ g per g of fresh root tiss-

Table 1. DNA yields from fresh and dry roots of different medicinal plants.

Plant Species	DNA ($\mu\text{g/g}$ of root)					
	Present method		Dellaporta et al. (1983)*		Doyle and Doyle (1990)*	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
<i>Asparagus adscendens</i>	33 ± 1.0	48.67 ± 2.0	15.0 ± 0.6	20 ± 1.3	14.0 ± 0.5	18 ± 1.0
<i>Withania somnifera</i>	40 ± 2.6	50.67 ± 2.0	11.0 ± 0.4	11 ± 0.3	12.0 ± 0.4	17 ± 0.9
<i>Asparagus racemosus</i>	38 ± 2.6	58.0 ± 2.6	12.0 ± 0.4	12 ± 0.5	12.0 ± 0.4	16 ± 0.9
<i>Chlorophytum borivillianum</i>	42 ± 2.6	68.0 ± 2.6	13.7 ± 0.6	11 ± 0.4	16.33 ± 0.6	20 ± 1.0

Values are mean \pm SE (standard error).

*The DNA was extracted from the fresh and dried roots of medicinal plants strictly in accordance with methods mentioned.

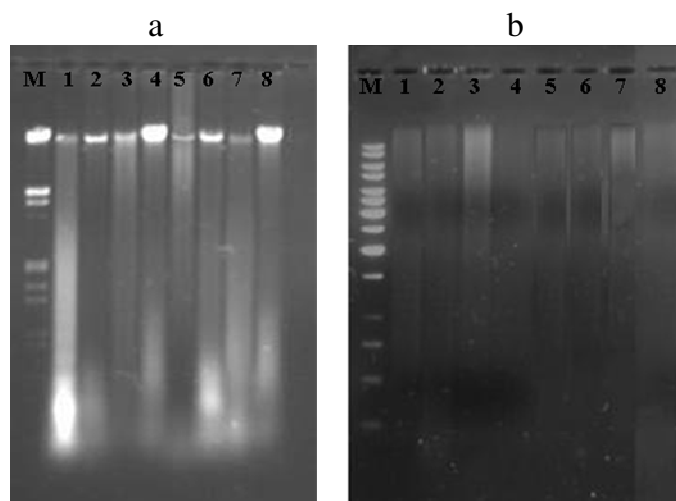


Figure 1. **a.** Ethidium bromide-stained agarose gel of electrophoretically separated crude genomic DNA. Lane M: Markers; Lanes 1, 3, 5 and 7 DNA isolated from fresh root; Lanes 2, 4, 6, and 8: DNA isolated from dry root of *A. adscendense*, *W. somnifera* and *A. racemosus* and *C. borivillianum*, respectively. **b.** Ethidium bromide-stained agarose gel of electrophoretically separated restricted (EcoRI) genomic DNA. Lane M: markers, Lanes 1, 2, 3 and 4: restriction of DNA from fresh root; Lanes 5, 6, 7 and 8: restriction of DNA from dry root of *A. adscendense*, *W. somnifera* and *A. racemosus*, respectively.

ues. The quantities of DNA in dry samples were 1.47 times greater than fresh samples. The quality of DNA samples was checked by determining the ratio of absorbance at A260/280. The ratio obtained varied from 1.8 to 2.0, which suggests that our DNA samples were fairly of good quality. PCR reaction was performed according to the protocol by Williams et al. (1990), using the DNA samples isolated from dry and fresh samples of roots of these medicinal plants with random primer 5'GTGCGCGTCA3' (P1) and 5'GTTTCGGTCC3' (P2) (Imperial) using a Techne Thermalcycler. The DNA isolated using previous methods (Dellaporta, 1983; Doyle and Doyle, 1990) did not give PCR amplification and amenable to restriction digestion. This might be due to the fact that DNA samples isolated using the above prot-

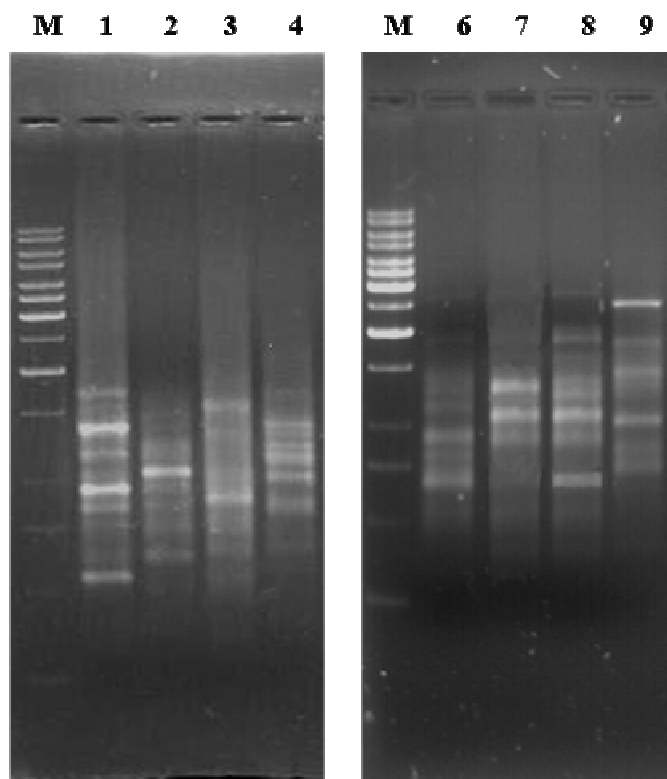


Figure 2. Ethidium bromide-stained agarose gel of electrophoretically separated amplicons of DNA extracted from dry root with two different primers viz. P1 (5'GTGCGCGTCA3') and P2 (5GTTTCGGTCC3'). Lane M: markers; Lanes 1, 2, 3, 4: amplicon obtained with P1; Lanes 5, 6, 7 and 8: amplicons obtained with P2 from dry root of *A. adscendense*, *W. somnifera*, *A. racemosus* and *C. borivillianum*, respectively.

ocols were contaminated with large amounts of secondary metabolites and polysaccharides. The DNA samples isolated using our protocol however were found suitable for the restriction digestion by EcoRI and PCR amplification as evident from the results obtained from this study (Figures 1b and Figure 2).

Based on these findings, it can be concluded that the DNA samples extracted and purified from fresh and dry

roots of *A. adscendens*, *W. somnifera*, *A. racemosus* and *C. borivilianum* using our protocols of high purity and amenable to further processing in cloning experiments as well as DNA fingerprinting. Moreover our method is rapid, as the duration for complete extraction of DNA from the roots of medicinal plants is much less compared to the other published protocol.

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