# "ABBAS" DNA Extraction Method from plants

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## ABSTRACT

DNA extraction is still problematic in a variety of plants because of the presence of secondary metabolites that interfere with DNA isolation procedures and downstream applications such as DNA restriction, amplification, and cloning. Here we describe a modified procedure based on the polyethylene glycol (PEG-6000) method to isolate DNA from tissues containing high levels of polysaccharides and phenolics. The procedure is applicable for sugarcane, olive, wheat, sorghum, barley, gram, sea buckthorn, amla, autumn olive and related species form leaves and fruits. This modified method contains PEG (1%), 4M NaCl, 0.5M EDTA, 1MTris HCl and SDS (10%) and washed, also reduced the centrifugation times during the separation and precipitation of the DNA. The method has solved the problems of DNA degradation, contamination, and low yield due to binding and/or co- precipitation with starch and polysaccharides. The isolated DNA proved amenable to PCR amplification and restriction digestion. The technique is fast, reproducible and can be applied for PCR based marker's investigations, DNA cloning and modification studies in plants.

**Key Words:** DNA extraction, Polyethylene glycol, PCR amplification and RNA extraction

## 1. INTRODUCTION

The application of DNA technology in agricultural research has progressed rapidly over the last twenty-six years, especially in the area of cultivar identification [1]. Isolation of plant nucleic acids for use in Southern blot analysis, polymerase chain reaction (PCR) amplifications, restriction fragment length polymorphisms (RFLPs), cDNA synthesize, arbitrary primed DNA amplifications (RAPD, SSR-PCR), and genomic library construction are the most important and time-consuming steps. The degree of purity and quantity varies between applications. A good extraction procedure for the isolation of DNA should yield adequate and intact DNA of reasonable purity. The procedure should also be quick, simple and cheap. The extraction process involves, first, breaking or digesting away cell walls in order to release the cellular constituents. This is followed by disruption of the cell membranes to release the DNA in to the extraction buffer. This is normally achieved by using detergents such as sodium dodecyl sulphate (SDS) and Polyethylene glycol 6000 (PEG) in a buffer form to be protected from endogenous nuclease. EDTA is often included in the extraction buffer to chelate magnesium ions, a necessary co-factor for nucleases. The initial DNA extracts sometimes contain a large amount of RNA, proteins, polysaccharides, tannins and pigments, which may interfere with the extracted DNA and a redifficult to separate [2]. Most proteins are removed by denaturation and precipitation from the extract using chloroform and iso-amyl-propanol. RNA, on the other hand, is normally removed by treatment of the extract with heat-treated RNAase. Polysaccharide-like contaminant sare, however, more difficult to removed. Polysaccharide can inhibit the activity of certain DNA-modifying enzymes and may also interfere in the quantification of nucleic acids by spectrophotometric methods [3], NaCl at concentrations of more than 0.5M, together with CTAB is known to remove polysaccharides [4,5]. The concentration ranges mentioned in the literature varies from 0.7M [6] and 6M [7] and is dependent on the plant species under investigation. Some protocols replace NaCl by KCl [8].

The problem of DNA extraction is still an important issue in the field of plant molecular biology. Various plants contain high levels of polysaccharides and many types of secondary metabolites affecting DNA purification. Antioxidants are commonly used to deal with problems related to phenolics. Examples include mercapto-ethanol, Bovine serum albumin, sodium azide and PVP amongst others [6, 9]. Phenol extractions coupled with SDS are also helpful. However, with plants having a high content of poly phenolics, SDS-phenol tends to produce low yields of DNA [10]. Sever all laboratories performed side by side the comparison of all four DNA isolation procedures. Two methods are based on classical principles of lyses and purification. The first one is the commonly used protocol of Doyle and Doyle [11], which has been used successfully in many plant species. The second one, from Guillemaut and Maréchal-Drouard [12] originated from Dellaporta [13] and was later modified [14].

Since the mid-1980s, genome identification and selection has progressed rapidly with the help of PCR technology. A large number of marker protocols that are rapid and require only small quantities of DNA have been developed. Three widely-used PCR-based markers are RAPDs [15], SSRs or microsatellites [16] and AFLPs [17]. Each marker technique has its own advantages and disadvantages. The choice of a molecular marker technique depends on its reproducibility and simplicity. The best markers for genome mapping, marker assisted selection, phylogenic studies, and crop conservation must have low cost, less labor requirements and high reliability. Since 1994, a new molecular marker technique called inter simple sequence repeat (ISSR) has been developed [18]. ISSRs are semi-arbitrary markers amplified by PCR in the presence of one primer complementary to a target microsatellite. In order to encompass the difficulties of DNA extraction in certain plant species some modifications in the basic DNA isolation method were tried. The aim of the investigation was to derive a simple DNA extraction protocol applicable to large varieties of plant material for quick and cheap enzymatic modification and markers based investigations.

## 2. MATERIALS AND METHOD

Several experiments were carried out, however, only the optimized protocol is described here.

### 2.1. PLANT MATERIAL

Plants which were experimented include sugarcane (Saccharum officinarum L.), olive (Olea Europaea L.), wheat (Triticum aestivum), sorghum (Sorghum bicolor), barley (Hordeum vulgare), gram pea (Cicer arietinum), sea buckthorn (Hippophae rhamnoides ssp turkestanica), amla (Phyllanthus emblica), autumn olive (Elaeagnus umbellata) and common beans (Phaseolus vulgaris). Leaves and Fruits were collected from the plants aseptically germinated in the green house at controlled temperature and maintained at Faculty of Agriculture Rawalakot Azad Kashmir Pakistan.

### 2.2 REAGENTS

- An extraction buffer consisting of 1% PEG (6000), 1MTris (pH 8.0), 0.5M EDTA (pH 8.0), 10% SDS and 4M NaCl were prepared.
- 2. Chloroform: ISO-amyl-alcohol (24:1) and 100% and 75% ethanol
- 3. TBE buffer consisting of 5.4% Trizma, 2.72% Boric acid and 2 ml 0.5M EDTA.

### 2.3. PROTOCOL

- 1. Plant material (1g) weighed and ground in a buffer to make slurry
- 2. The slurry was poured in eppendorff tube and kept at 65oC for 30 minutes in an oven.
- 3. Chloroform and isoamyl alcohol in the ratio of 24:1 was mixed with the extract at room temperature in a shaker for 3 minutes and the samples kept undisturbed for 5 minutes
- 4. The contents were centrifuged at 13,000 rpm for 10 minutes.
- 5. Supernatant was collected in a new eppendorff tube and was precipitated in 95% ethanol with gentle mixing and kept for 15 minutes at room temperature.
- 7. It was centrifuged at 13,000 rpm for 5 minutes, and the supernatant was discarded.
- 8. The pallet was Wash with 50%, 70% and absolute ethanol.
- 9. The DNA pellet was dissolved in 200µl TBE buffer and was stored at -800C.



### 2.4. AMOUNT AND PURITY OF DNA

DNA samples from the leaf tissues and fruits were electrophoresed in 1.5% agarose gel, according to the known procedure [19] photographs taken for comparisons and reference.

## 3. RESULTS AND DISCUSSION

A good DNA extraction procedure should yield adequate, intact and pure DNAs in a short time period with low cost and shall be suitable for a large number of plant species [11]. The method used in present experiment i.e. PEG (1%), 4M NaCl, 0.5M EDTA, 1MTris HCl and SDS (10%) "ABBAS DNA extraction method" was completed in approximately 1 hour and 6 minutes and tried in small scale (mini) using 100 mg fresh tissue or in midi scale using 1g tissue. The method was found to be suitable for genomic DNA extraction from various fresh tissues, mature and immature seeds in comparison to earlier described methods [11].
The quality of the extracted DNA was evaluated by different procedures using agarose gel electrophoresis. Figure-1 shows the result of the extracted genomic DNA electrophoresed in 1.5% agarose gel, stained with ethidium bromide and visualized under UV light. In order to check the efficiency and reliability of the method, enzyme digestion and PCR were tested on SSR primers of sugarcane using different genotypes. Polymorphic results were shown in Figure-2. Figure-3 shows the genomic DNA treated with RNase for further purification from unwanted debris (RNA). Figure-4 shows the results of RNase and DNase treatment respectively which may be suitable for the protocol of gene isolation or reverse transcriptase process.

To test the effect of various modifications to our DNA extraction protocol, we used sugar cane, olive, wheat, sorghum, barley, chickpea, sea buckthorn, amla, autumn olive and common beans. The effect of detergents in the DNA extraction buffer was tested through various combinations. Detergents, SDS and PEG, were added to the solution containing 1M Tris-HCl (pH 8.0), 4M NaCl and 0.5 M EDTA. It was observed that the addition of preheated 1% PEG was critical in getting good quality of DNA. Evaluated DNA samples were less time consuming, cheap, easy and best for PCR based marker analysis. Only 1 hour and 6 minutes required for DNA extraction, which was applicable for multiple plant species both from leaves and fruit samples.
Conical tube saves precious time in bringing the tissue from -80°C to 60°C as rapidly as possible resulting in DNA of higher quality [2]. DNA in good quality and amount was extracted with the solution containing 1% PEG. The purity of genomic DNA was dependent on the number of washes. A three-time wash combined with a short-run centrifugation was sufficient for DNA purification and removal of endogenous nucleases or other proteins. As PEG is soluble in ethanol, residual amounts were removed in the subsequent wash. During ethanol precipitation of nucleic acids from 4 M NaCl, polysaccharides remain dissolved in the ethanol [20].The freer the nucleic acids from contaminants, the easier are to re-suspend the pellet. If the pellet obtained from the first ethanol precipitation from 4M NaCl was found to be hard to resuspend, two such precipitations were done and the pellet obtained from the second precipitation usually goes into solution very easily. It was found that washing in 70% ethanol gave better DNA as a result of the removal of any residual NaCl and/or PEG.

### 3.1. PCR AMPLIFICATION AND GEL ELECTROPHORESIS

PCR was carried out in a 20µL reaction mixture, which contained d3H2O 5.8 µl, l0X PCR buffer+ (NH4) 2SO4 2.0 ul, MgCl2(25mM) 3.0 µl, dNTP's (2.5mM) 3.0 µl, Taq (5U/ µL) 0.2 µl, Both forward and reverse Primers (30ng/ µL) 3.0 µl, DNA (30ng/ µL) 3.0 µl. DNA amplification was carried out under the following conditions: 95°C for 5 min, followed by 35 cycles of 94°C for 45 sec, annealing 50°C for 45 sec, and elongation 72°C for 1.0 min, with a final extension at 72°C for 7 min. The PCR products were fractionated on a 1.0% agarose gel using 1X TBE buffer containing 10 mg/ml ethidium bromide and were visualized under UV light, and the gels were photographed using a UV gel documentation system Fig- 2. In general, compared to intact purified DNA, fragmented or partially degraded DNA contains a minor amount of proteins and polysaccharides and they were efficiently amplified in PCRs. However, degraded DNAs were not suitable for long time in PCR, endo-nuclease digestion, Southern blot analysis, genomic DNA library construction studies [21]. In the present study, there were complete digestions with restriction endo-nucleases. The use of several PCR based techniques indicated that polysaccharides and proteins as well as the other compounds preventing PCR were successfully removed during the extraction.

## 4. CONCLUSION

The ABBAS DNA extraction method demonstrated the best yield and reproducibility and remains the method of choice where large amounts of high quality DNA are required. Our protocol provides a fully automated and cost effective solution for large number of plant species.

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**Figure 1.** The evaluated DNA samples (Left to right, sugarcane, olive, wheat, sorghum, barley, chickpea, sea buckthorn, amla, autumn olive and common beans) were found amenable for DNA modifying and PCR based analysis.

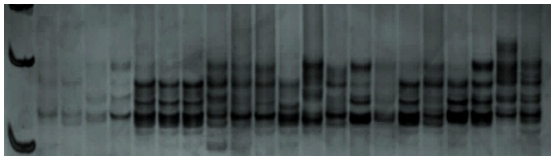

**Figure 2.** Results of Polymerase Chain reaction.


**Figure 3.** RNase treatment.


**Figure 4.** DNase treatment.