Journal of Nuts 5(2):33-37, 2014
ISSN: 2383 – 319x

DNA Extraction of Almond without Phenol and Liquid Nitrogen

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Received: 7 October 2014 Accepted: 10 December 2014

Abstract

Genomic DNA extraction with a high quantity and quality is a basic requirement in molecular biology. The DNA obtained was free of any contamination proteins, polysaccharide, polyphenols and colored pigments. These compounds would interfere with the genomic isolation procedures and downstream reactions such as restriction enzyme analysis and gene amplification. The isolated genomic DNA was found suitable since this method reduce these compounds. Hence, it can be employed for preparations inter sample sequence repeats (ISSR) and cloning. The protocol also excludes the need for liquid nitrogen and toxic phenols. In this method. A260/A280 absorbance ratio of extracted DNA was 1.7 to 1.8. The protocol covers many and divers.

Keywords: DNA, Extraction, Liquid nitrogen, Phenols, Polysaccharide.

Introduction

Almond belongs to the family of Rosaceae. Twenty-one almond species and six natural hybrids grown in different climatic regions have been found (Kadkhodaei *et al*., 2010). The main world producer is Iran with more than 400,000 tons followed by Turkey, USA and Syria (Faostat, 2004).

In many plant species, extracting large amounts of high quality, high molecular weight DNA can be a challenge due to high amounts of phenolic compounds, high levels of DNA and the presence of large amounts of organellar DNA (Lutz *et al*., 2011). With the modified Doyle and Doyle (1987) CTAB method, it is feasible to extract qualified DNA from leaves of almond. It is difficult to obtain from almond because of its high amounts of secondary metabolic substances such as polyphenols, polysaccharides, quinone and tannins, which would interfere with DNA extraction procedures and the activity of DNA enzymes and restriction endonucleases (Michiels *et al*., 2003). During an isolation procedure. polysaccharides are found to form complexes with nucleic acids forming a gelatinous mass, thereby physically inhibiting the DNA from the action of DNA modifying enzymes e.g. restriction enzymes, DNA polymerase, ligase, etc (Porebski, 1997; Amani, 2011). PVP forms complex with latex lactones, actucin and other phenolics. The PVP complexes accumulate at the interface between the organic and the aqueous phases by centrifugation after addition of chloroform. CTAB binds to fructans and other polysaccharides and forms complexes that are removed during subsequent chloroform extraction (Maltas *et al*., 2011; Dani 2006; Zidani *et al*., 2005). The objective of this study was to develop efficient means for extracting DNA of different plants in an open laboratory environment and, a method that eliminates the need to use liquid nitrogen and toxic phenol. This CTAB (Cetyl trimethy lammoniu bromide) protocol was suitable for genetic engineer for example inter-simple sequence repeats (ISSR) (Sahu *et al*., 2012; Brown 2006).

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Materials and Method

Collection of plant material

The progeny samples were collected and labeled in a laboratory of department faculty of agriculture, university of Islamic azad damghan. This research was conducted during years 2011-2012.

DNA Extraction and Isolation

The preheat suspension buffer (PH:8) contained 2% CTAB, 2% PVP, 50mM EDTA, 1M Tris-HCl, 1/4M NaCl, and 0.2% β-mercaptoethanol (to be freshly added just before use) in a water bath at 60◦C. Leaves (10-30 mg) were grinded into a fine powder in an ice cold condition using PR chilled. The solution was transferred into a 2 ml centrifuge tube.

Note: To avoid usage of liquid nitrogen, the following method was successfully employed. 10µl Proteinase K was added to a solution that contained the leaf sample. This was inverted, gently mixed and incubated at 60°C for 45 minutes. The suspension was centrifuged at 13,000 rpm for 10 minutes at room temperature. The supernatant was carefully transferred to a new 2 ml centrifuge.

Tube and an equal volume of chloroform-isoamylalcohol (24:1) were added and mixed gently by inversion 30 times, followed by centrifugation at 13000 rpm for 10 minutes. The aqueous phase solution was carefully transfered into a new tube. Double volume of Chloroform-Isoamyl alcohol was added and inverted gently 15 to 20 times and centrifuge at 13000 rpm for 15 minutes.

The sample was allowed to cool to at room temperature. 300µL of 7M sodium acetate was added and placed on ice for 30 minutes. The suspension was centrifuged at 12,000 rpm for 15 minutes. The upper clear aqueous layer was carefully transferred to another 1.5 ml microfuge tube. The upper aqueous phase was taken and two third volumes of ice-cold isopropanol were added.

It was then kept at 20°C for more than 30 minutes and centrifuged at 13400 rpm for 10 minutes. The supernatant was discarded. The DNA pellet was washed with 75% ethanol twice and air dried.

Finally, the DNA pellet was dissolved in 200µl sterilized double distilled water. 3µL RNase (10mg/mL) was added and kept at 37°C for 30 minutes followed by chloroform: isoamyl alcohol extraction and ethanol precipitation. The DNA pellet was washed with 75% ethanol twice and air dried. 20 to 40µL (depending upon the pellet) of sterilized double distilled water was added to dissolve the precipitate. The quality of extracted DNA was analyzed by means of agarose gel electrophoresis stained with ethidium bromide (Fig. 1). In order to demonstrate the efficiency of the method, ISSR-PCR was performed with UBC872 (GATAGATAGATAGATA) in the following conditions: 94ºC; 5 minutes, 94ºC; 5 seconds, 40.7ºC; 45 secondes, 72ºC; 1 minute, for 35 cycles and finally extension 72ºC; 7 minutes. The PCR products (5µl) were resolved on an agarose gel (2%), which was subsequently stained with ethidium bromide and visualized under UV light (Fig. 2).

Results

Chromosomal DNA Isolation from plant

Plant species were much more resistant to cellular lysis resulting from the extensive concentration of cell wall and componed phenolic. This study aimed to present a suitable protocol to achieve an easy to handle and highly efficient extraction system of chromosomal DNA. By using this simple and rapid protocol, it was possible to isolate DNA and perform PCR for a large number of samples in a single working day. The method was based on a modification of procedure described by Doyle and Doyle, and consisted of

only three steps prior to DNA analysis: suspension, lysis and purification by precipitation of the proteins with high salt concentrations. Fresh and young leaf materials were the first choice to obtain good-quality DNA because mature leaves contained higher quantities of polyphenols and polysaccharides, which made it very difficult to isolate DNA of good quality (Porebski, 1997; Amani, 2011)

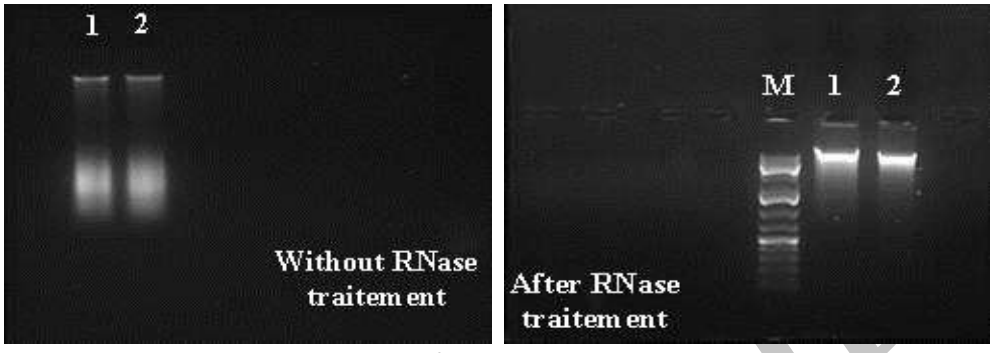

Fig.1. Electrophoresis of genomic DNA. DNA samples, Almond

The extracted DNA quality for almond was assessed by spectrophotometry and PCR amplification, respectively. The A260/A280 absorbance ratio ranged from 1.7 to 1.8 for DNA extracted with the pretreatment of distilled water, indicating the isolated DNA was free from protein contamination. Due to the removal of phenol as an inhibitor of Taq DNA polymerase, there was no protein contamination. The addition of 7M sodium acetate was found useful in the formation of non-gelatinous CTAB/DNA pellet and removal of color contaminants, resulting in the precipitation of white instead of colored pellet (Hu *et al.*, 2009). On the other hand, high concentrations of β-mercaptoethanol, helped reduce the browning in DNA preparations produced by the oxidation of phenolics.

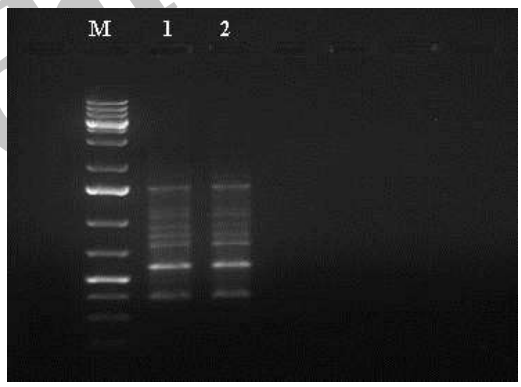

Fig.2. ISSR profile of two DNA sampl of Almond.

Evaluation of Quantity and Purity of Extracted DNA

The extracted DNA quality for almond was assessed by spectrophotometry and PCR amplification, respectively. The A260/A280 absorbance ratio ranged from 1.7 to 1.8 for DNA extracted with the pretreatment of distilled water, indicating the isolated DNA was free from protein contamination. Upon electrophoresis on 1% agarose gel, thick, sharper and distinct bands were obtained. The absence of smears indicated a high purity in the nucleic acids extracted (Fig. 1).

PCR amplification

We evaluated the quality of the extracted DNA through ISSR-PCR. The amplified PCR products

of leaf DNA showed identical band patterns and similar intensity to that of leaf tissue (Fig.2). ISSR-PCR amplification tests were performed on all samples using primer and protocols previously optimized in the agarose gel. This further confirmed the purity of DNA extracted with this method, free from polysaccharide and polyphenol contamination. The results indicated that isolated DNA was amenable to further downstream applications and this method was suitable for other plant such as African violet.

Discussion

The efficiency and the speed of this method together with the use of inexpensive facilities and the absence of toxic chemicals made the current method an attractive alternative for the extraction of plant DNA. These results showed that the DNA produced by this simple, low cost, fast and safe protocol can be used in PCR-based techniques on a wide range of organisms, and in laboratories lacking state-of-the-art equipments and technology. By removing phenol as inhibitor of Taq DNA polymerase, protein contamination was not observed. The addition of 7M sodium acetate was found useful in the formation of non-gelatinous CTAB/DNA pellet and removal of color contaminants, resulting in the precipitation of white instead of colored pellet (Hu *et al*., 2009). On the other hand, high concentrations of β-mercaptoethanol, helped reduce the browning in DNA preparations produced by the oxidation of phenolics (Zidani *et al*., 2005; Cortés, 2010). Ethanol precipitated the DNA and RNA while isopropanol selectively precipitated DNA, leaving RNA and polysaccharides in the solution Ethanol undoubtedly plays vital role in DNA precipitation, but special care has to be made in the removal of ethanol after precipitation (15). Due to these specific kits, chromosomal DNA was free of contamination. It is simple, rapid and universally competent method for isolation of genomic DNA from a variety of samples. Thus, we suggest application of this protocol for efficient and adequate PCR amplification and order library analysis.

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