



COMPARISON BETWEEN THREE DIFFERENT METHODS FOR ISOLATION GENOMIC DNA FROM DRY DATE PALM LEAVES WITHOUT LIQUID NITROGEN

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Abstract

Three different methods for isolation genomic DNA from Date palm leaves is described. The leaves was dried and ground before DNA extraction by CTAB development method [100mM Tris- HCl (pH 8.0), 20 mM EDTA, 4M NaCl, 6% CTAB, 2% Polyvinylpyrrolidone, 0.2% B-mercaptoethanol and 15 ul of proteinase K] and compared with two other methods, the results shows the developing method gives highly quality of DNA ($A_{260}/A_{280} = 1.6$ to 1.9) in all three Date palm cultivars under tests. This study found the development method is very suitable to DNA extraction from dried leaves without liquid nitrogen.

Key words : DNA extraction, leaves, date palm, CTAB.

Introduction

Date palm (*Phoenix dactylifera* L.) is a very important fruit in middle east and it take a special place in Iraqis and Arab people because, it is mentions in holly Quran and some conversations to messenger Mohamed peace up on him, in addition the date palm is oldest known fruit crops in Iraq and some Arab contrary (Chou and Krueger, 2007). In the last years, there is revolution in genetic sciences which depend on genomic studies, some of this studies teak care date palm such as fingerprint and adversary between date palm cultivar by use mutable technique such as RFLP, RAPD, ISSR, SSR and STR and other technique (Khanam *et al.*, 2012 and Khierallah, *et al.*, 2014), DNA sequencing studies (Al-Mssallem *et al.*, 2013) or find the gene action (Al-Qurainy, 2011). The first stapes of all these studies, it is an extraction of whole genomic DNA from leaves or any part of date palm trees but there are many problems on this stapes spatially on leaves. The rigid and fibrous date palm leaves is notoriously difficult in addition high content of Polyphenolic compounds and Polysaccharides in the tissue which intersect with DNA extraction. The another problem in DNA extraction, plant materials are among the most difficult for high-quality DNA extractions. The

key is to properly prepare the tissues for extraction. In most cases, this involves the use of liquid nitrogen flash freezing followed by grinding the frozen tissue with a mortar and pestle. Liquid nitrogen is difficult to handle and it is dangerous in an open laboratory environment such as a classroom, in addition, sometimes we need to extraction DNA from dry leaves tissue, therefore we need a good protocol to extraction high purity DNA from dry leave without liquid nitrogen.

Materials and Methods

Plant material

The young leaves which chosen for DNA extraction must be near to white color or very few chlorophyll, this leaves may be found near to apical shoot, leaves were cleaned it from dust or microbial infection by cotton wetness by 80% alcohol, the leaves were dried aerial for a week until stable weight, then grind to a fine powder using mechanical grinder then a powder was keeping in refrigerator tall used.

Reagents for DNA extraction

The DNA extracted from 0.2 g leaves powder by three procurers. The first procurer was a manual used the CTAB buffer [1.5mL ependorf tub content 100mM

Tris- HCl (pH 8.0), 20 mM EDTA ,4M NaCl, 6%CTAB, 2% Polyvinylpyrrolidone and 0.2% B-mercaptoethanol added directly when used], were added and vortexed for 1 min, 15 ul of proteinase K was added then incubated at 60°C for 30 min,proteinase K allow to react on the sample by breaking down the peptide bonds. After the extracted was cooling, it centrifuged at 14000rpm for 10 min, the 600uL of chloroform: Isomylalcohol (24:1) was added to supernatant phase after transferred into new tube then vortexes to for 12000 rpm for 10 min, The DNA pellet was washed with 600 µL of cold 76% Ethanol followed by centrifugation at 13000 rpm for 5 minutes.

The other procurer for DNA extract from ground leaves date palm 0.5 ml of digestion buffer in Eppendorf tub content 100 mM Tris-HCl (pH 8.5), 0.5% SDS, 2 mM EDTA, 200 ug/ml proteinase K and put in water bath for 2 hr at 65°C. After heat inactivation of the proteinase K for 10 min at 95°C, the tubes were cooled to 4°C and centrifuged for 10 min at 12,000g. The Third procure using Plant DNA Mini Kit, peqGOLD, it used as the as kit instructions.

DNA quantification and agarose gel electrophoresis

Nanodrop was used to determined the quantity and purity genomic DNA isolated from date palm leaves,depend up on the absorbance at a ratio of A260/A280 nm. Then the extracted DNA was electrophoresed on 1% agrose gels at 90 volts, run for 1 h, then visualized using gel electrophoreses system.

Results and Discussion

Extraction genomic DNA from Date palm leaves is very difficult to compare with another plant leaves, because tissue of date palm leaves contents high concentration of fiber, which takes a long time and allots of liquid nitrogen for extract with hard work, which usually uses it in DNA extraction (Tan *et al.*, 2013 and Wong *et al.*, 2014). This is when the leaves are young as well as the old leaves content high chlorophyll which may be effected on DNA extraction, in addition, the liquid nitrogen is dangers in open area such as student's laboratory (Ferdous *et al.*, 2012). Another cause, Date palm leaves a content high level of phenol and polysaccharide (Rafi *et al.*, 2015), which Interferes with the extraction of DNA from tissue leaves and gives errors when estimating. In this working paper, the method of extracting DNA from date palm leaves using the CTAB method was developed to be suitable for DNA extraction from dried date palm leaves or after air drying for young and old leaves, to avoid difficult obtain on leaves powder when the leaves are fresh.

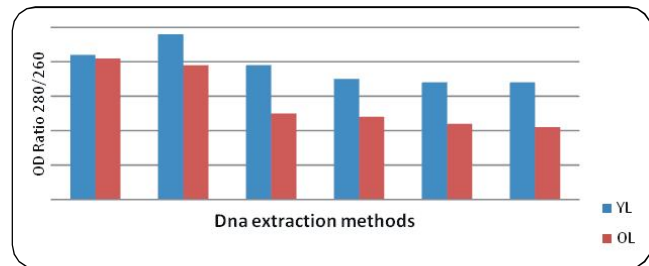


Fig. 1 : OD Ratio 280/260 for three methods of DNA extraction from two date palm leaves cultivars. YL : young leaves, OL : old leaves.

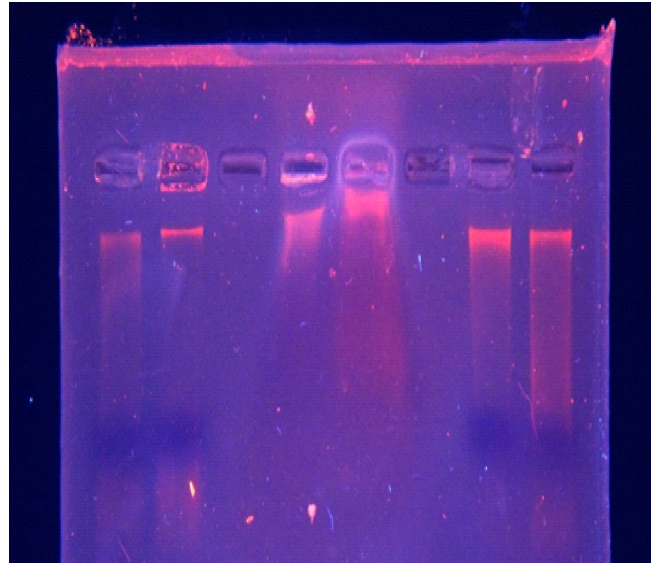


Fig. 2 : Three methods of DNA extraction from two Date palm leaves cultivars by Electrophoreses on 1% agaros from left to right YL: young leaves OL: old leaves Hilawi, Birhi respectively and CTAB development method, SDS and Kit.

This method is cheap and effective and extracts large amounts of DNA. This method was compared with other methods, included whether CTAB could be substituted in the laboratory for other substances, phenol including the method of extraction without CTAB and comparing them with SDS and peqGOLD kit, which was used periodically in the University's Genetic Engineering Laboratories.

The results show, when extraction the genomic DNA from three date palm cultivar, our CTAB development method gave more purity and higher yield of DNA extraction compared with the other two methods (fig. 1), it gives DNA purity between 1.69-1.78. The A260/A280 absorbance ratio was greater than 1.6 in all date palm samples which is recorded good quality (Ahmad *et al.*, 2004) and it succeeded to extract DNA from old and young date palm leaves, in addition, it was extracted from dry leaves powder although the yield of DNA extracted

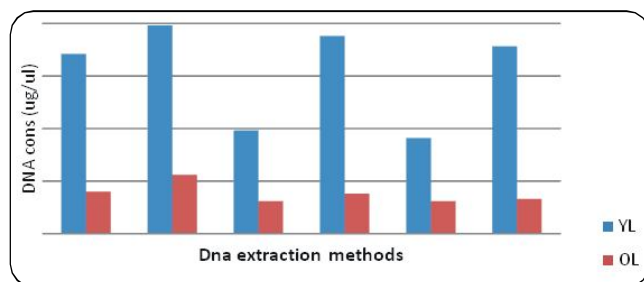


Fig. 3 : DNA concentration (ug/ul) for three methods of DNA extraction from two date palm leaves cultivars. YL : young leaves, OL : old leaves.

from old leaves was less than young leaves, the Barhi cultivar recorded the high purity DNA extraction and Helawi cultivar was recorded high-yield DNA extraction. Whereas the commercial kit method gave a lowest purity and yield of DNA. Agarose gel electrophoresis confirmed the presence of high molecular weight genomic DNA in the gel with a clearer banding pattern and no evidence of substantial band shearing or RNA contamination, Low level of DNA yield in commercial kit method, maybe it is not specific DNA extraction from date palm leaves which contains high level of phenol and polysaccharide, whereas the CTAB development method was work on such as chemical material in date palm leaves and try to decrease effect it (fig. 2).

The NanoDrop spectrophotometer value at A260/A280 was acceptable range (1.6 -1.9), which is a sign to DNA purity and indicate the protein, polyphenol and carbohydrate contaminations. The absorbances at 260 and 280 nm were within the recommended ranges (Doyle and Doyle, 1990). In our study, decreased the contamination of sample with protein, polyphenol and polysaccharide by used the PVP and Bmercaptoethanol in addition proteinase K, the last material work on protein contamination, Proteinase K is an enzyme that cleaves the peptide bond in proteins next to the carboxyl group of hydrophobic amino acid residues (aliphatic and aromatic). Proteinase K itself is a protein, but it is resistant to denaturation by heat, detergents and chaotropic salts and will continue to function happily in them as long as the temperature/concentration is not too high. It is stable (and functional) up to 65°C and will function in temperatures down to 25°C (room temperature). It is often used at higher temperatures (50-65°C) because most nucleases that would chew up your DNA are denatured/inactivated at these temperatures (Cabral *et al.*, 2000 and Shahriar, 2011). We are add PVP and mercaptoethanol for CTAB buffer because the Date palm leaves are rich in phenolics compounds and when we are need to get a high good quality DNA, should be removed this compound or decreased it. Therefore, PVP (polyvinylpyrrolidone) was

a suitable compound to help in removing phenolics from leaves by forming hydrogen bonds with them (Choudhary *et al.*, 2008), while beta-mercaptoethanol was added in the extraction buffers because it is a strong reducing agent which can remove tannins and other polyphenols often present in the crude plant (Souza *et al.*, 2012), a good content of DAN Shown in fig. 3, we noted the high DNA in Birhi cultivars. The young leaves were gives high concentration for DNA compare with old leaves.

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