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Comparison among different method of DNA extraction from corn and their role in PCR

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Abstract

Besides DNA purity, mild precipitation conditions were found to be beneficial in obtaining less low-molecular mass nucleic acids in the final DNA extract. The homogenization step and the amount of sample extracted were also found to be crucial in keeping the extraction procedure robust. 50 Corn samples were collected from local markets of Baghdad city. DNA was extracted with three extraction methods. chemical method (CTAB), Wizard kit and modified method which is rapid .The result of purity is (1.75-2.0) and DNA concentration is (0.7-44µg) for the modified method respected by CTAB method was 1.7-1.99 and concentration was 0.1-12.5µg and finally Wizard kit was 1.6-1.7 and the concentration was0.02-10µg. Among the 3 methods tried, the modified method was found to be suitable for PCR amplification with high purity and yield of genomic DNA.

Keywords: DNA extraction, CTAB, Zein, PCR.

Introduction

Most of DNA isolation protocols having lengthy steps using different hazardous reagents and required to remove interfering substances that often co–precipitate with the extracted DNA (Kotchoni *et al.,* 2009). CTAB method and its modifications (Huang *et al.,* 1987; Doyle *et al.,* 2000) were extensively used in different laboratories, but these methods are time consuming (Cheng *et al.,* 2003). The method containing CTAB, a cationic surfactant which is a hazardous chemical may cause irritation to skin and respiratory system. Sodium dodecyl sulfate (SDS) has also been used as an alternative to CTAB (Aljanabi and Martinez, 1997). RNase treatment also consumes time and money mostly. Most of the methods required unsafe liquid nitrogen (Sharma *et al.,* 2003), or freeze – drying lyophilization (Sperisen *et al.,* 2000) for proper tissue grinding and these facilities are more expensive to many laboratories. High cost per sample is main problem with commercially available DNA isolation kits (Kang and Yang, 2004: Ahmed *et al.,* 2009) make them an unattractive option otherwise DNA isolation from large number of samples could be a costly affair in concern with money, safety and time.

Various protocols for DNA extraction have been successfully applied to many plant species (Doyle and Doyle 1987: Ziegenhagen and Scholz, 1993), which were further modified to provide DNA suitable for several kinds of analyses (Ziegenhagen and Scholz, 1998). They were tested various DNA isolation protocols i.e., Phenol-chloroform method (Hari Prasad, 1998; Dellaporta *et al.,* 1983), and CTAB method, mong the 3 methods tried, CTAB method (Doyle and Doyle 1987), was found to be suitable. Modifications were made to minimize polysaccharide, and to simplify the procedure for processing large number of samples. Thus the protocol derived for genomic DNA isolation is efficient, inexpensive, simple, rapid, high yield and pure. The isolated DNA would be suitable for further molecular studies.

The procedure described here is modified method in our lab. Containing least chemicals with a rapid procedure to get extremely pure DNA and consequently ideal for a large number of samples.

Materials and Method

Plant Material: Corn samples were collected from various parts of local market and used for DNA extraction without in liquid nitrogen.

DNA Extraction Protocol: To assess the effect of modifying a number of factors, including changing the chemicals added to the extraction buffer, varying incubation time, and fluctuating incubation temperature, we selected one method (5) that produced high DNA yields, demanded comparatively little working time, and allowed for systematic modification. For this assessment, all components and their concentrations need to be

known. We used three methods.

Chemical extraction: DNA extraction from maize foods was performed by using method of (Doyle and Doyle, 1987), 100 mg of ground homogeneous sample was transferred into a sterile 1.5ml reaction tube. 300µl sterile dH2O was added and mixed with a loop. Then 500µl of CTAB-buffer (20g/l CTAB, 1.4M NaCl, 20Mm EDTA, 100Mm Tris HCl) was transferred to the reaction tube. 20µl of Proteinase K (20mg/ml) was added and shaked gently. The mixture was incubated at 65C◦ for 30-90min. in water bath. Before the last 5 minutes of incubation 20µl of DNAse free pancreatic RNAse (10mg/ml) was added and tubes were incubated at 65 C◦ for 5 min. Tubes were centrifuged in microcentrifuge Hettich Micro 12-24 for 10min. at 13.000 ×g. In order to remove significant quantities of protein, supernatant was carefully collected with a micropipet and transferred to a tube containing 500µl chloroform/isoamylalcohol (in the ratio at 24/1 respectively). After a gently mix for 30s, sample was centrifuged for 5min. at 13.000 rpm. The upper layer of separated liquid was collected and transferred to a new sterile tube. 2V of CTAB precipitation buffer (5g/l CTAB, 0.04M NaCl) was added and the sample was mixed by pipetting. Then tube was kept at room temperature for 60 minutes. So as to obtain pellet, tubes were spinned for 5min. at 13.000 ×g. Supernatant was discarded and pellet was collected. After dissociation of precipitation in 350µl NaCl (1.2M), 350µl of chloroform: isoamylalcohol (in the ratio at 24/1 respectively) was added and gently mixed for 30s. Then tubes were recentrifuged for 10min. until phase separation occurs. The upper layer was transferred to a new reaction tube. 0.6 volume of isopropanol was added and gently mixed in order to precipitate nucleic acid. Tubes were centrifuged for 10 minutes at 13.000 ×g. Supernatant was discarded and 500µl of 70% ethanol solution was added. After a gentle mixing, tubes were centrifuged for 10min. at 13.000 ×g. Supernatant was discarded. Pellet was collected and samples were kept at room temperature until the ethanol completely evaporated. Collected pellet was redissolved in 100µl sterile dH2O (Somma, 2002). Nucleic acids were analysed by agarose gel electrophoresis. DNA was kept at –20 ◦C for further use.

Wizard extraction: Corn powder (100mg) in 1.5ml micro centrifuge tube mixed with 600µl of Nuclei Lysis Solution, and vortex 1–3sec. to wet the tissue. Incubate at 65°C for 15min.. 3µl of RNase Solution was added to the cell lysate, and mix the sample by inverting the tube 2–5 times. Incubate the mixture at 37°C for 15 minutes. Allow the sample to cool to room temperature for 5min. before proceeding. 200µl of Protein was added Precipitation Solution and vortex vigorously at high speed for 20sec. Centrifuge for 3min. at 13,000–16,000 × *g*. The precipitated proteins will form a tight pellet. Carefully remove the supernatant containing the DNA (leaving the protein pellet behind) and transfer it to a clean 1.5ml micro centrifuge tube containing 600µl of room temperature isopropanol. Centrifuge at 13,000–16,000 × *g* for 1min. at room temperature. Carefully decant the supernatant. Add 600µl of room temperature 70% ethanol and gently invert the tube several times to wash the DNA. Centrifuge at 13,000–16,000 × *g* for 1min. at room temperature. Carefully aspirate the ethanol using either a drawn Pasteur pipette or a sequencing pipette tip. The DNA pellet is very loose at this point and care must be used to avoid aspirating the pellet into the pipette. Invert the tube onto clean absorbent paper and air-dry the pellet for 15min. Add 100µl of DNA Rehydration Solution and rehydrate the DNA by incubating at 65°C for 1hr. Periodically mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature or at 4C◦and kept the DNA at -20°C.

Modification method: Take 100 mg of grinding corn in eppendorf tube. Add 800 µl of lysis buffer (detergent prepared in the Lab.) and incubate at 65C◦ for 1hr. Spin at13000 rpm for 5min. Transfer the supernatant in to new tube containing 500µl of chloroform. Spin at 13000 rpm for 5min. Take the upper phase and transfer in to new tube containing 500 µl of isoprobanol. Spin at 13000 rpm for 5min and discard the solution carefully. Put 500µl 70% ethanol, spin at13000 rpm for 5min, discard the solution carefully, and invert the tube upside down on filter paper to become dry. Add 100µl of sterile D.W, and kept at -20°C.

Quantification and visualization of DNA: DNA quantified by measuring optical density (O.D.) at A260 and A280 with UV/Vis spectrophotometer major science the quality of DNA was analyzed by agarose gel electrophoresis. Samples were prepared by taking 5µl of DNA and 1µl of 10X bromophenol blue dye (0.25% bromophenol blue and 50% glycerol) on a glass slide. Samples were subjected to electrophoresis in 1X TAE buffer for 1hr. at 80V on 0.8% agarose gels. The gels were photographed under a gel documentation system
(Biorad, 200).

Polymerase Chain Reaction (PCR): PCR amplification was carried out in a PCR mix of 25µl on a Applied biosystem 9700 thermal cycler. The final concentrations of each PCR reaction were as follows: 12.5µl Go Taq Green Mix (Promega); 100ng of genomic DNA; 1µl (1-10µM stock concentration)

of each primers; and complete the final volume with D.W.

Oligonucleotide primers: Alpha DNA synthesized oligonucleotide primers. (Canda) at the final concentration of 100mM. All oligonucleotide primers were diluted to the concentration of 10 pmol/l with sterilized deionized water and stored at -40°C until use. The sequences and amplification conditions are presented in Tables (1) and (2).

Results and Discussion

The present protocols were applied to 50 corn samples. DNA isolation from foodstuffs is affected by their secondary metabolites yielding polysaccharides and protein contaminations. Three different protocols, CTAB based Protocol 1, Wizard based Protocol 2, and modified based Protocol 3 followed for isolation of DNA from corn, were used hazardous chemicals and consume different time span to complete procedure. Obtained quality and quantity of DNA, use of chemicals and time-consuming steps of these three protocols were compared using spectrophotometer at the absorbance of 260 and 280nm (Table 3).

In Protocol 1, chemicals CTAB and EDTA were used and steps were more time consuming than others, making this protocol expensive. Yielding A260/280 ratio 1.75-1.99. This finding was in accordance with the result of Chen and Ronald (1999), they have used the CTAB protocol for extracting total DNA from grains of rice and maize. The CTAB method gave not only high DNA yield but also good DNA quality with the A260/ A280 in the range of 1.75 to 1.99 for raw materials and animal feeds, respectively. This purity of DNA was good enough for PCR amplification and other molecular applications (Yamaguchi *et al.,* 2003). This is in agreement with (Song *et al*., 1995; Wang *et al*. 1998; Chen and Ronald, 1999) who have shown that successfully detected from the genomic of transgenic rice lines extracted by the CTAB protocol (Figure 1).

In protocol 2 (Wizard method), DNA extract according to our protocol shows less presence of low molecular mass nucleic acids. A feasible explanation for this occurrence is in the mild precipitation conditions, which diminish the possibility of shorter nucleic acid molecules to precipitate. On the other hand, the Wizard method produced moderate qualities of DNA with the presence of traces of proteins with A260/A280 ratio of 1.6 and 1.7 respectively. However, according to (Pich and Schubert, 1993), the A260/A280 was 1.6-1.7, indicating the absence of contaminants (Figure 2).

Modified Protocol 3, capitulate good quality and quantity of genomic DNA, giving A260/280 2.0 indicating pure DNA than other followed protocols. Additional precipitation steps, removed large amounts of precipitates (detergents, proteins, etc.) by modified speed and time of centrifugation. We found these modified steps necessary to standardize and increase the quality and quantity of genomic DNA. The degree of purity and quantity varies between applications (Zidani *et al.,* 2005). The present modified method yielded of 0.7-44µg DNA (Figure 3).

Table (1): Sequences of oligonucleotides used in this study

Primer	Targets	Sequences	Amplicon
Ze-03 (R)	Maize zein	AGTGCGACCCATATTCCAG	277 bp
Ze-04 (F)		GACATTGTGGCATCATCATTT	

Table (2): PCR amplification conditions

PCR Program	Cycles	Temperature	Time
Initial denaturation		95°C	3 min
Denaturation	35	96 °C	1 min
Annealing		60 °C	1 min
Extension		72 °C	3 min

Table (3): Comparison of isolated DNA for purity, yield and isolation time using different protocols

Method of extraction	DNA Yield (µg/ml)	Ratio 260/280	No. of samples
CTAB method	0.1-12.5 µg	1.75-1.99	50
Wizard method	0.02-10 µg	1.6-1.7	34
Modified method	0.7-44 µg	1.7-2	22

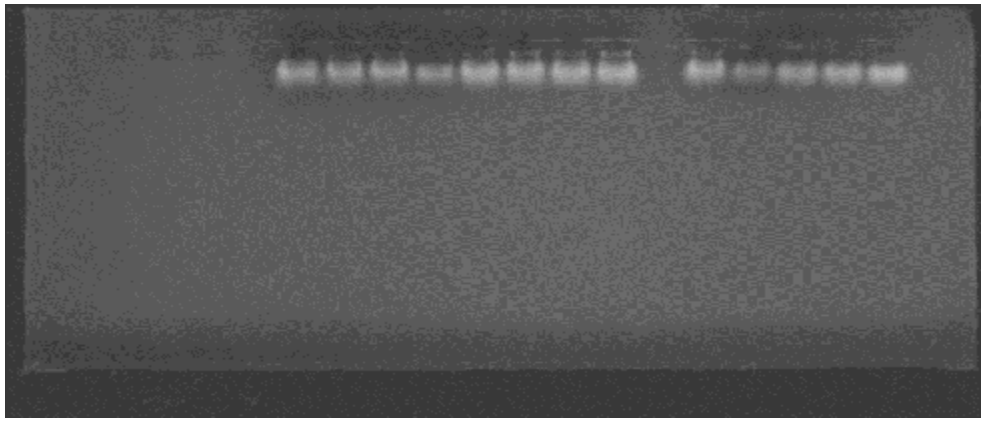

Figure (1): Analysis of purity of genomic DNA Using CTAB Methods.

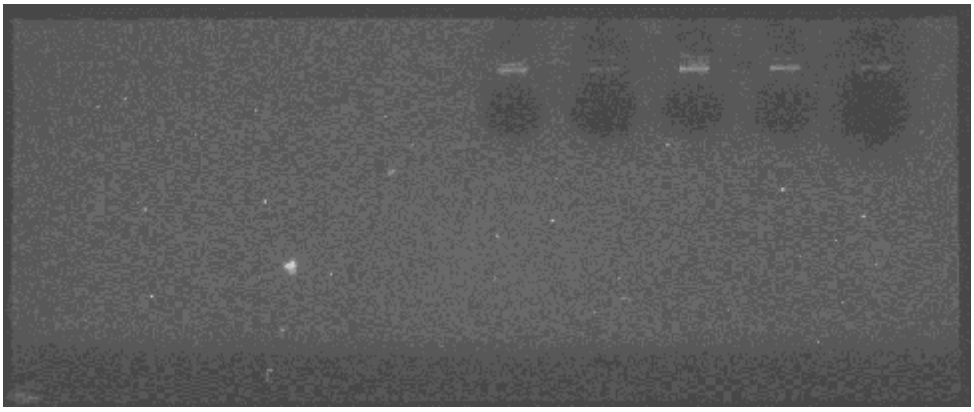

Figure (2): DNA isolation Using Wizard Methods

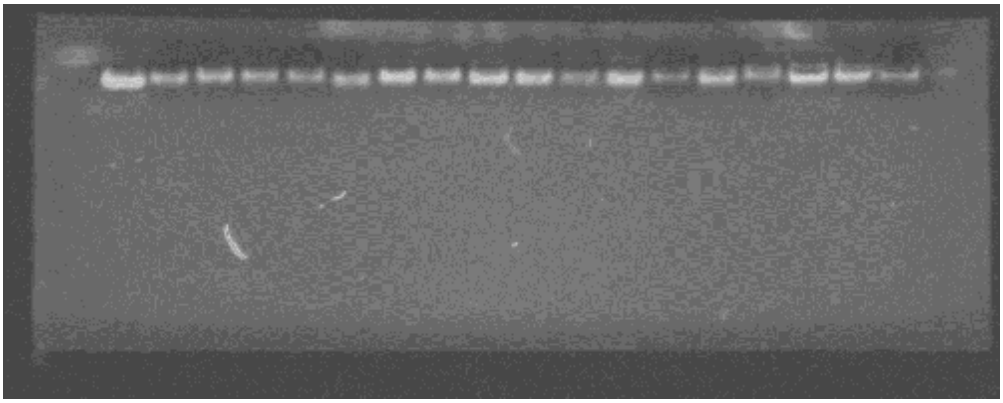

Figure (3): DNA isolation Using Modified Method.

Comparative obtained data of different protocols presented in (Table 3) concerns the remarkable difference between purity data obtained from the different analytical methods. In particular, we observed that the in the extraction step the modified method gives the best purity ratio (1.7-2.0) and high concentration of DNA (0.7-44µg) if it is compared with other two methods (CTAB, Wizard). Above protocol is independent to use of liquid nitrogen, CTAB, HCl and EDTA with more advantages of its simplicity, rapidity and cost effectiveness. Addition of essential steps makes protocol reliable and yielding higher quantity of genomic DNA. An even inexperienced person could isolate pure DNA by following simple and safe steps described in given protocol. Use of general

laboratory equipments and chemicals in present method gives further potential and scope for pure DNA isolation from plants.

DNA extracted using various method is good enough in terms of quantity and quality for PCR amplification. In this study, DNA extracted was subjected to PCR amplification with primer pair Z0-e3/Z0-e4 targeting the zene gene (Pauli *et al.,* 2000) for products derived from maize. 277bp amplicons were amplified from soybean and maize derived DNA sources respectively using DNA templates extracted by the three different protocols. This showed that the quality and quantity of the DNA were good enough for PCR amplification. The modified method was successful to produce the amplifiable DNA stocks by PCR (Figure 4).

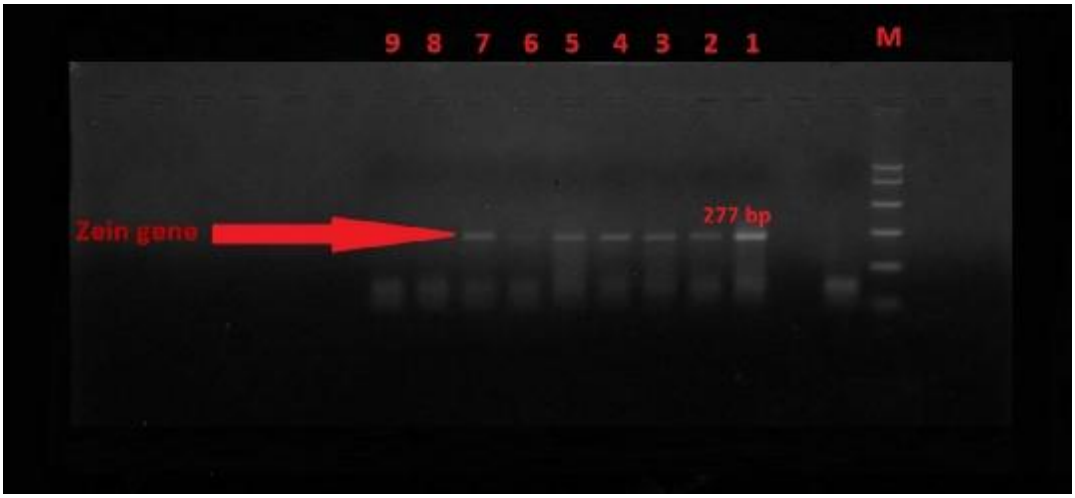

Figure (4): Agarose gel electrophoresis of PCR amplicons using DNA templates extracted by the following methods; Modified method (lanes 1 to 3), the CTAB method (lanes 4 to 6), Wizard method (lanes 7 to 9)Lane M: 100 bp DNA ladder (Promega).

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