



An improved method of extracting genomic DNA from preserved tissues of *Capsicum annuum* for PCR amplification

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Abstract

In this study we present a method for the extraction of genomic DNA from the different tissues of the Pepper (*Capsicum annuum*). A standard protocol of Dellaporta was reviewed and modified for DNA extraction from the preserved tissues of the Capsicum sample which was believed to contain high level of polysaccharides. The modified protocol employed yielded a high quality DNA and was found to be suitable for PCR and RAPD analyses. The procedure was also found to be reliable and suitable where some materials are not available and does not require phenol-chloroform extraction. The method also allowed for the preservation of plant tissues for some days from a locality where storage facilities are not accessible. We also discovered that irrespective of the sources of tissues, a good quality DNA was obtained. The quantity of DNA produced from the fleshy mesocarp tissue was more than the quantity obtained from the seeds of the same weight, this is probably due to the hard nature of the seeds and there may have been no complete breakdown of the cell wall to release the cellular contents.

Keywords: *Capsicum*, genomic, mesocarp, pepper, polysaccharides.

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INTRODUCTION

Molecular techniques require isolation of genomic DNA of suitable purity. The growing number of DNA extraction protocols for specific plant species are not always simple and cannot be reproduced for all species (Porebski et al. 1997).

Polysaccharide contamination is a common problem in higher plant DNA extraction. DNA samples are often contaminated with Melicera colloidal Lyalosome, which cannot be dissolved in water or TE buffer (Yun-Jiang et al. 2003).

Plant contaminants like polysaccharides and phenolic compounds are difficult to separate from DNA and are readily identified as they impart a sticky gelatinous brown color to the DNA isolated and interfere with polymerases, ligases and restriction enzymes (Fang et al. 1992, Michard et al. 1995,

Porebski et al. 1997, Csaikl et al. 1998, Tribouch et al. 1998, Belletti et al. 1998, Schlink and Reski, 2002). These contaminants are in abundance in the foliage of perennials, and they co-extract with the DNA (Scott and Playford 1996, Shepherd et al. 2002, Bhattacharjee et al. 2004).

DNA extraction becomes more difficult when working with perennials or tree plant species. Several factors are known to limit the isolation of pure DNA from such species that are rich in impurities; such as terpenes, polyphenols, and polysaccharides. Such factors include the amount of tissue available, the condition of the plant material, the numbers of steps involved in the extraction procedure and the required purity level (Helen

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1995, Bhattacharjee et al. 2004).

According to Bhattacharjee et al. (2004), most tree crops are grown in a location where storage and refrigeration facilities are not immediately available and so collection of leaf or tissue samples becomes almost impossible.

The pepper (*Capsicum annum* L.) is a perennial shrub in tropical areas and can also be grown as a perennial in climate-controlled greenhouses. Some are grown at locations far away from the laboratory facilities and it is impossible to transport samples immediately. For this work therefore, the approach of Bhattacharjee et al. (2004) was employed with modifications

MATERIAL AND METHODS

Plant material

Healthy matured fruits with red/green color were harvested in a 50 mL Falcon tube containing a Dellaporta buffer with just enough quantity to cover the fruits completely.

Tubes were transported under tropical climate condition for more than 24 h before they were stored at 4°C in the laboratory. The samples were predominantly grown in the Northern part of Nigeria and they are locally called Sombo Hausa. For this work the approach of Bhattacharjee et al. (2004) was employed but with a different protocol. In the case of NaCl-CTAB azide solution used by Bhattacharjee et al. (2004) to preserve cocoa samples, a Dellaporta buffer was used to preserve tissues from the Pepper samples before they were transported to the laboratory and refrigerated at 4°C.

DNA extraction

The protocol reported by Dellaporta et al. (1983) was employed with the following modifications: Fruit tissues were brought out of the storage buffer and cleaned with 70% ethanol. Instead of grinding the samples with liquid nitrogen as stated by the Dellaporta protocol, cleaned sample (400 mg) were ground in a mortar with a preheated (65°C) Dellaporta buffer (1600 µL) mixed with 200 µL of 20% SDS and homogenized. Thereafter, 4 mL of RNase, was added, mixed and incubated at 65°C for 30 min instead of adding 1 µL of RNase at the final stage of extraction and then incubated at 37°C for 1 h as stated in the Dellaporta protocol.

In precipitating DNA, 1000 mL of ice-cold isopropanol was added to each tube, gently covered and incubated at -20°C overnight without mixing which is against the Dellaporta protocol of adding 1000 mL (1 mL) of ice cold Isopropanol and mix by inverting gently 4-5 times for the appearance of DNA strands. Instead of washing at the end of the extraction as stated by the protocol, we washed after the second precipitation with ice cold isopropanol by discarding two-thirds of the supernatant carefully without dislodging the DNA. This was done twice by adding 100 mL of 70% ethanol and gently inverting 2-3 times to clean the DNA. This was later spun at 9000 rpm for 10 min to pellet the DNA.

DNA quantification

Extracted DNA was first checked on an 0.8% agarose gel. After electrophoresis it was stained with Ethidium Bromide and viewed under an ultraviolet transilluminator for quality and yield assessments. The quantity of DNA was estimated from the band intensity as compared with the aliquots of standard DNA (Fig. 1).

The quality was further estimated by measuring the OD 260 and OD 280 or absorbance ratio, which varied between 1.7 and 1.9 for both tissues.

RNase treatment

Aliquots of 4 µL of RNase was added to the samples in the extraction buffer after grinding into a paste and homogenizing well before incubation. A complete removal of RNA from the total genomic DNA was recorded after checking on the 0.8% agarose gel (Fig. 1).

PCR amplification using RAPD primers

Two DNA samples, one each from seed and mesocarp were subjected to PCR amplification with a RAPD primer. The total volume of the PCR reaction was 12.5 µL, which contained 2.5 µL of template DNA (1.25 µL of 10 x buffer (10 mM), 1.00 µL MgCl₂ (25 mM), 1.25 µL of 5% Tween20, 0.5 µL of 2.5 mM dNTPs, 0.2 µL of Taq polymerase (Bioline), and 0.5 µL of RAPD primer. The PCR cycle was carried out with a mini-Techne gradient cycler (25 wells type) with the initial denaturation at 94°C for 3 min followed by 45 cycles of 94°C for 20 s, 37°C annealing for 40 s and 72°C for 1 min. It was followed with 72°C for a 7 min extension and stored at 4°C. Amplification products were

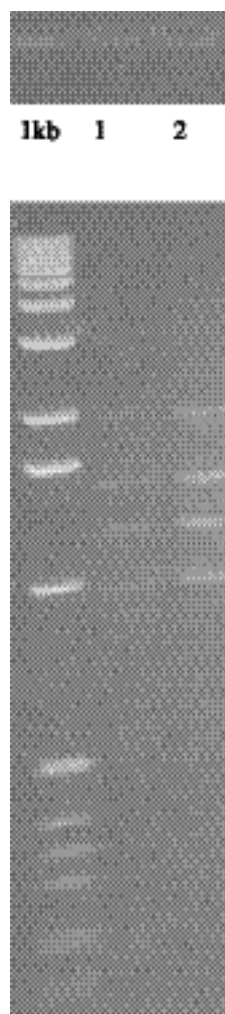


Fig 2. Analysis of the DNA extracted from *Capsicum annuum* using a RAPD Operon primers (AC 13). Lane 1, DNA from mesocarp and Lane 2, DNA from seeds of pepper

viewed in 1.5% agarose gels stained with ethidium bromide (Fig. 2). Results of the agarose test, PCR, and RAPD analysis indicated that the DNA quality had been enhanced despite the low concentration of 2.5 ng. This is possible, because RAPD analysis does not require high DNA concentration; the working dilution for the PCR was optimized at ratio 1 μ L: 1000 μ L 1 of DNA solution to ultra pure water respectively.

RESULTS

DNA extracted from preserved matured pepper fruits using a modified Dellaporta protocol yielded good quality DNA. Results from the agarose gel electrophoresis showed

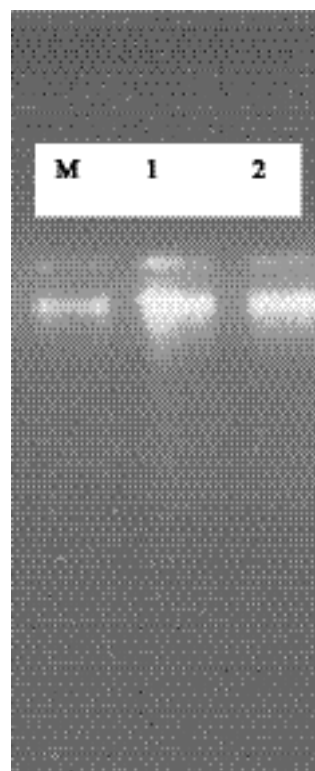


Fig 1. Quality test of 2 DNA samples from *Capsicum annuum* on 0.8% agarose gel. Lane M; λ DNA marker, lane 1; fleshy mesocarp, lane 2; seeds.

a clear and clean DNA. The quantity of DNA was estimated from the band intensity as compared with aliquots of standard λ DNA of 25 ng (Fig. 1). The quality was further estimated by measuring the OD 260 and OD 280 and the absorbance ratio, which varied between 1.7 and 1.9 for both tissues. The amplification of DNA from PCR analysis with RAPD primers was clear as shown in Fig. 2.

DISCUSSION

This method has been standardized to isolate DNA from preserved Capsicum fruit. Preservation of these tissues in a Dellaporta buffer solution yielded a good quality DNA.

Result of the agarose gel and spectrophotometer showed that the polysaccharides and RNA had been removed and DNA quality had been enhanced. It shows that DNA isolated from the various tissues of Pepper had absorbance values that indicate an insignificant amount of contaminating protein and polysaccharides and even the DNA from a starch rich crop appeared undegraded on

agarose. The spectrophotometer result showed the OD260/OD280 absorbance ratio of the DNA solutions ranged from 1.7 to 1.9 indicating a good quality DNA irrespective of the source of tissues.

It was also revealed that the DNA yield from the fleshy mesocarp of the fruit was higher than the yield from the seeds of the same weight (400 mg). This is probably due to the hard nature of the seeds and there may be an incomplete breakdown of the cell wall to release the cellular constituents compared to the fleshy mesocarp, which is softer. This procedure was found to be reliable, and does not require phenol-chloroform extraction. The preservation of tissues from the Pepper confirmed that they could be stored in a Dellaporta buffer for some periods of time in a locality where resources and materials are not accessible Bhattarchajee et al. (2004). The absence of RNA, polysaccharides and the amplification of molecular weight bands are evident of a good quality genomic DNA. It is evident that treatment of the samples with the RNase enzyme at the initial stage of grinding has completely removed the RNA from the samples and this suggested that the enzyme could be added conveniently at this

stage not necessarily at the final stage of extraction as contained in the Dellaporta protocol. The RNA could have been precipitated with the debris and discarded with it when spun at 12000 rpm. The storage in the Dellaporta buffer especially the seeds may have further softens the tissues for easy breakdown of cell wall, making grinding with a pestle and mortar easier and may also have dissolved some polyphenolic compounds and polysaccharides present in the tissues. This has actually replaced grinding with liquid nitrogen to release cell contents. It follows then that where liquid nitrogen is not available the process of extracting DNA will proceed and yield good quality DNA suitable for molecular marker analysis.

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REFERENCES

- Belletti P, Marzachi C, Lanteri S (1998) Flow cytometric measurement of nuclear DNA content in *Capsicum* (Solanaceae). *Plant Systematic and Evolution* 209, 85-91.
- Bhattarchajee R, Maria KA, Peter A, Sunday T, Ivan I (2004) An Improved Semi automated Rapid Method of Extracting Genomic DNA for Molecular Marker Analysis in Cocoa, *Theobroma cacao* L. *Plant Molecular Biology Reporter*. 22, 435a-435b
- Csaikl UM, Bastian H, Brettschneider S, Gauch A, Meir M, Schauerte F, Scholz C, Sperisen B, Vornam ZB (1998) Comparative Analysis of Different DNA Extraction Protocols: A fast, Universal Maxi-Preparation of High Quality Plant DNA for Genetic Evaluation and Phylogenetic Studies. *Plant Molecular Biology Reporter* 16, 69-86
- Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA miniprep version II. *Plant Molecular Biology Reporter* 1, 19-21.
- Fang G, Hammar S, Grumet R (1992) A quick and inexpensive method for removing polysaccharides from plant genome DNA. *Biofeedback* 1, 52-54
- Helen N. Asemota G (1995) A fast, Simple, and efficient miniscale method for the preparation of DNA from tissues of Yam (*Dioscorea* spp.) *Plant Molecular Biology Reporter* 17, 9-14.
- Michaud H, Lumaret JP, Ripoll LT (1995) A Procedure for the Extraction of Chloroplast DNA from Broad-Leaved Tree Species. *Plant Molecular Biology Reporter* 2, 131-137.

- Porebski S, Bailey LG, Baum BR (1997) Modification of a CTAB DNA extraction protocol for plants containing high polysaccharides and polyphenol component. *Plant Molecular Biology Reporter* 15, 8-15.
- Schlink K, Reski R (2002) Preparing high quality DNA from moss (*Physcomitrella patens*). *Plant Molecular Biology Reporter* 20, 423a-423f.
- Scott KD, Playford J (1996) DNA Extraction technique for PCR in rainforest species. *Biotechniques* 20, 974-978.
- Shepherd M, Cross M, Stokoe RL, Scott LJ, Jones ME (2002) High throughput DNA extraction from forest trees. *Plant Molecular Biology Reporter* 20, 425a-425j.
- Tribouch SO, Danilenko NG, Davydenko OG (1998) A Method for Isolation of Chloroplast DNA and Mitochondrial DNA from Sunflower. *Plant Molecular Biology Reporter* 16, 183-189.
- Yun-Jiang C, Wen-Wu G, Hwa-Lin Y, Xlao-Min P, Xivxin D (2003) An Efficient Protocol for Genomic DNA Extraction From Citrus Species. *Plant Molecular Biology Reporter* 21, 177a-177g.

PCR Amplifikasyonu Amaciyla *Capsicum annum*'un Korunmus Dokularindan Genomik DNA Edilmesinde Gelismis Bir Metot

Ozet

Bu calismada, farkli biber (*Capsicum annum*) dokularindan genomik DNA ekstrasyonu icin bir metot sunulmustur. Yuksek miktarda polisakkarit icerdigine inanilan *Capsicum* orneginin korunmus dokularindan DNA ekstrasyonu icin, standart Dellaporta protokolu gozden gecirildi ve degistirildi. Kullanilan degistirilmis protokolle yuksek kalitede DNA elde edildi ve PCR ve RAPD analizleri icin uygun oldugu goruldu. Ayrica yontemin guvenilir oldugu, bazi maddelerin mevcut olmadigi durumlarda kullanilabilecegi ve fenolkloroform ekstraksiyonu gerektirmedigi anlasildi. Metot, saklama imkanlarinin bulunmadigi yerlerde bitki dokularinin birkac gun boyunca korunmasına imkan tanidi. Ayrica, doku kaynagi ne olursa olsun, iyi kalitede DNA elde edildigini tespit edilmistir. Etili mezokarp dokusundan elde edilen DNA miktarı, aynı ağırlıktaki tohumlardan elde edilenden daha fazlaydı. Bu durum, tohumların sert yapısından ve hücre içeriğinin salınması için hücre ceperinin tamamen parçalanamamasından kaynaklanıyor olabilir.

Anahtar Kelimeler: *Capsicum*, genomik, mezokarp, polisakkaritler, RAPD.