European Scientific Journal November 2014 edition vol.10, No.33 ISSN: 1857 – 7881 (Print) e - ISSN 1857- 7431

OPTIMIZATION OF GENOMIC DNA EXTRACTION PROTOCOL FOR MOLECULAR PROFILING OF BANANA / PLANTAIN (*MUSA SPECIES*)

Romana Siddique
BRAC University, Bangladesh

Abstract

Extraction of pure and high molecular weight genomic DNA (deoxyribonucleic acid) is a prerequisite for genetic analysis of plants. However, the presence of polysaccharides and polyphenols in plants represent a great challenge as it interferes the isolation of pure DNA and downstream reactions like PCR amplification. For this species, standard protocols do not produce high quality PCR amplifiable DNA. Here, in this study, an optimized protocol based on the standard CTAB (cetyl trimethyl ammonium bromide) protocol is described. The major changes in the optimized protocol are the addition of antoxidant compounds-namely polyvinyl pyrrolidone (PVP) and 2-mercaptoethanol, in the extracton buffer; the increasing of CTAB (3%,w/v) and sodium chloride (2M) concentration; and an extraction with organic solvents(phenol and chloroform). The yield of extracted DNA ranged from 150-200 µg / µl and the A260/A280 value was between 1.7-1.8 indicating minimal levels of contamination. Thus this protocol proved amenable for PCR and is suitable for further work on diversity analysis of *Musaceae* species.

Keywords: Banana, *Musa* spp., DNA isolation, CTAB, polysaccharides, polyphenols

Introduction

The banana and plantains (*Musa species*) belonging to the family Musaceae are one of the world's most important subsistence crops. In Bangladesh, banana is popular for its year round year availability, abundant production as well as high acceptability to the consumers. It is a rich source of carbohydrate and also rich in some minerals, notably phosphorus, calcium and potassium. Banana is particularly rich in vitamin-C and also contains significant amounts of several other vitamins (INIBAP 1987).

The fruit is easy to digest free from fat and cholesterol. Banana powder is used as the first baby food. It helps in reducing risk of heart disease when used regularly and is recommended for patients suffering from high blood pressure, arthritis, ulcer, gastroenteritis, and kidney disorders.

Beside these it has great medicinal value. Plant genetic resources conservation is an essential component in meeting the demand for future food sustainability and security. Isolation of semi-intact, high quality genomic DNA is a crucial pre-requisite step for molecular biology applications such as polymerase chain reaction (PCR), rapid amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), PCR-RFLP, Southern blotting, and library construction of crop, forestry, desert and medicinal plants.

Plant leaves of crop, tree and medicinal plants are rich in secondary metabolites, polysaccharides and polyphenolics that are problematic during isolation of genomic DNA. . DNA samples are unstable for long term storage too (Lodi *et al*., 1994; Sharma *et al*., 2002). Several plant DNA extraction protocols for removing polysaccharides have been reported (Porebski *et al*., 1997; Schlink and Reski, 2002). Various protocols for DNA extraction have been successfully applied to many plant species. We have tested previously mentioned DNA isolation protocols in some fruit crops that contain high polysaccharide level such as different banana (*Musa species*), lichi (*lichi chinensis* S.) guava (*psidium guajava* L.).But these methods resulted in DNA with lot of impurities and not very suitable for PCR, RAPD, RFLP AND SSR analysis. In this study a simple and modified protocol to isolate high yield and purified DNA is described which is suitable for further downstream molecular research.

Materials and methods

Plant materials

Seven banana (*Musa* species) cultivars Amritsagar, Sabri, Champa, Anupom, BARI kola-1, Mehersagar and Kabri were used in this research. One gram of young and healthy leaves from each sample was harvested for DNA isolation.

Reagents and solutions

An extraction buffer consisting of 3 % CTAB (w/v), NaCl (2 M), 100 mM Tris-HCl pH 8.0 and 20 mM EDTA pH 8.0 was prepared. After being autoclaved for 20 min, 1.5% (w/v) PVP, 2 % (v/v) 2-mercaptoethanol were added to the extraction buffer, immediately before use. In addition, Phenol: chloroform: isoamyl alcohol (25:24:1, v/v/v), chloroform: isoamylalcohol (24:1, v/v), Ribonuclease A (10 mg/ml), proteinase K (20 mg/ml), Ethanol (70 %, 100 %), Sodium acetate (3M) solution (pH 8.0), and TE buffer (Tris

HCl, 10 Mm,pH 8.0 , 1mM EDTA; pH 8.0) and absolute isopropanol were the additional solutions required.

DNA isolation protocol

a. Freshly harvested young and healthy leaves of *Musa species* (1g) were ground to fine powder in presence of liquid Nitrogen using mortar and pestle.

b. The tissue powder was quickly transferred into a clear autoclaved 50 ml centrifuge tube and then 10 ml of pre-warmed (65 °C) CTAB-buffer was added and mixed very well.

c. After that 40 µl of proteinase K (20 mg/ml) was added and mixed again by inversion for 1 min.

d. The mixture was incubated at 70 °C for 30 min with gentle mixing by inverting the tubes.

e. The tube was centrifuged at 13,000 rpm for 5 minutes and the supernatant was transferred to a clean centrifuge tube.

f. An equal volume of phenol: chloroform: isoamyl alcohol (25:24:1, v/v/v) was added, mixed by using gentle inversion and centrifuged at 10,000 rpm for 5 min.

g.The supernatant was transferred to a clean centrifuge tube and an equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed properly by inverting the tubes 20-25 times to form an emulsion and centrifuged at 13000 rpm for 10 minutes.

h. Again the supernatant was carefully decanted and transferred to a new tube and was precipitated by adding one volume of pre chilled (-20oC) iso-propanol, mixed by gentle inversion incubated at -20oC for a minimum of one hour.

i. The samples were centrifuged at 13,000 rpm for 15 minutes. The supernatant was discarded and the obtained pellet was washed twice and thrice with 70 % ethanol. Decanted the supernatant and air dried DNA pellet at room temperature until the whitish pellet turned to transparent. Finally, the pellet was resuspended in 300 µl of TE Buffer or deionized water and stored at -20ºC .In case of DNA contamination with RNA , an additional step needs to be performed, by adding 6µl of RNAase (10 mg/ml) and to incubate it for 1 hour at 37ºC . All the centrifugation steps were carried out at room temperature to avoid precipitation with CTAB, DNA degradation and to obtain good quality of DNA.

Concentration, purity and quantity of the extracted DNA

The quality and quantity of the extracted DNA were measured using a UV spectrophotometer at 260 nm and 280 nm.The purity of DNA was determined by estimating the ratio of absorbance at 260 nm to that of 280

nm. In order to verify DNA integrity, samples of DNA were subjected to gel electrophoresis on 1 %. Agarose gel,stained with ethidium bromide (Sambrook *et al.*1989) and compared with lambda DNA marker (used to determine the concentration). The nucleic acid concentration was estimated following (Sambrook *et al.*1989).

Results

DNA extraction using CTAB method reported by Doyle and Doyle gave very low yield of DNA. This may be due to the presence of high amount of polyphenols, polysaccharides, proteins and other secondary metabolites (Fiorentino *et al.* 2007, Males *et al.* 2006, Zambani *et al.* 2008) which either lead to embedding of DNA into a sticky gelatinous matrix (Do, N *et al. 1991)* or promote DNA degradation (John, M.E., 1992).Accordingly, Doyle and Doyle CTAB protocol was modified (described in methods and material) to improve DNA yield and quality.

The isolated DNA was of high quality as it showed a reading in between 1.7 to 1.8 after calculating the ratio of absorbance 260/280 nm Fig.(1).

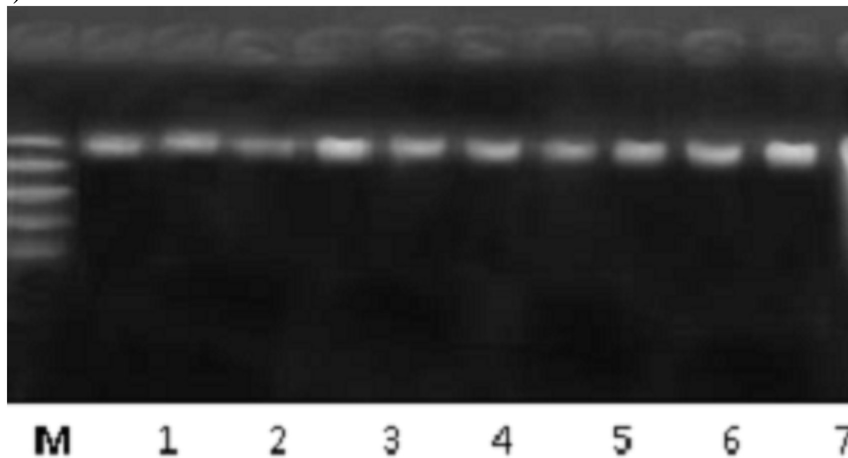

Fig (1): Electrophoretic pattern of DNA extracted by modified CTAB method from *Musa species* leaves. The electrophoresis was performed in 1% (w/v) agarose gel.

Lane M: 1 kb molecular weight marker.
Lane 1-7: Genomic DNA isolated from different banana cultivars.

Discussion & conclusion

Banana plants are economically important due to its nutritional value. Therefore, it is crucial to preserve genetic material of *Musa species* for their sustainable exploitation. At present several banana varieties have many synonyms in different regions which make identification difficult.

Differentiation of cultivars through morphological features is inefficient and inaccurate. Various types of DNA based molecular techniques are used to evaluate the genetic variability of plants. These approaches require both high-quality and quantity of DNA, for which *Musa species* presents a great challenge.

In this study Doyle and Doyle protocol was successfully improvised by using high salt concentration to remove polysaccharides, by adding antioxidants to remove phenolic compounds and proteinase K to get rid of proteins. The modified protocol described here made it possible to extract high quality clean genomic DNA from *Musa species* leaves. The protocol described here is simple, inexpensive, reliable, rapid, and less hazardous and yields appreciable levels of DNA. The optimized DNA isolation will aid in future molecular characterization and further downstream applications like genetic diversity analysis, phylogenetic studies and most importantly in developing conservation strategies of this promising economically important plant.

References:

Aljanabi, S.M.; Martinez, I (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucl. Acids Res. *25*, 4692–4693.

Bryant, J.A (1997). DNA extraction. In Methods in Plant Biochemistry; Dey, P.M., Harborne, J.B., Eds.; Academic Press: San Diego, CA, USA; Volume 10b, pp. 1–12.

A.; Monaco, P (2007).Polyphenols from the hydroalcoholic extract of *Arbutus unedo* living in a monospecific Mediterranean woodland. Biochemical Systematics and Ecology. *35*, 809–811.

Cheng, Y-J.; Guo, W-W.; Yi, H-L.; Pang, X-M.; Deng, X (2003). An efficient protocol for genomic DNA extraction from *Citrus* species. Plant Mol. Biol. Rep., 21, 177a–177g.

Cota-Sánchez, J.H.; Remarchuk, K.; Ubayasena, K (2006). Ready-to-use DNA extracted with a CTAB method adapted for herbarium specimens and mucilaginous plant tissue. Plant Mol. Biol. Rep. 24, 161–167.

Doyle, J.J.; Doyle, J.L (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin. *19*, 11–15.

Do, N.; Adams, R.P (1991). A simple technique for removing plant polysaccharide contaminant from DNA. BioTechniques *10*, 162–166.

De Kloet S.R. (1984). Rapid procedure for the isolation of high molecular weight RNA and DNA from yeast using lyticase and sodium dodecyl sulfate. J. Microbiol. Methods. 2(4):189-96.

Gendimenico G.J., Bouquin P.L. and Tramposch K.M. (1988). Diphenylamine colorimetic method for DNA assay: A shortened procedure by incubating sample at 500C .Analytical Biochemistry. 173:45-48
Fiorentino, A.; Castaldi, S.; D'Abrosca, B.; Natale, A.; Carfora, A.; Messere, John, M.E. (1992).An efficient method for isolation of RNA and DNA from plants containing polyphenolics. Nucleic Acids Research. *20*, 2381–2385.
Lodhi, M.A., YE, G.N., Weeden, N.F. and Reisch, B.I (1994). Simple and Efficient method for DNA extractions from grape vine cultivars and *Vitis* species. Plant Molecular Biology Reporter, 12, 6-13.
Males, Z.; Plazibat, M.; Vundac, V.B.; Zunta, I (2006). Qualitative and quantitative analysis of flavonoids of the strawberry tree—*Arbutus unedo* L. (*Ericaceae*). Acta Pharmaceutica .*56*, 245–250.
Michiels A., Van den Ende W., Tucker M.,Van Riet L. and Van Laere A (2003). Analytical Biochemistry.315 (1), 85-89.
Mandelkern M., Elias J.G., Eden D. and Crothers D.M (1981). The dimension of DNA in solution. J. Mol. Biol. 152(1):153-61.
Moyo M., Amoo S., Bairu M.W. and Finnie J.F (2008). Optimizing DNA isolation for medicinal plant. South Africal Journal of Botany. 74: 771-775
Porebski, S., Bailey, L.G. and Baum, B.R(1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and ployphenol components. Plant Molecular Biology Reporter. 15, 8-15.
Peterson, D.G.; Boehm, K.S.; Stack, S.M (1997).Isolation of milligram quantities of nuclear DNA from tomato (*Lycopersicon esculentum*), a plant containing high levels of polyphenolic compounds. Plant Mol. Biol. Rep. 15, 148–153.
Psifidi A., Dovas C.I. and Banos G. A.; 2010. Comparison of six methods for genomic DNA extraction suitable for PCR-based genotyping applications using ovine milk samples. Mol. Cell. Probes. 24(2):93-8.
Reichardt, M.; Rogers, S (1994). Preparation of plant DNA using CTAB. In Current Protocols in Molecular Biology; Ausubel, F., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., Struhl, K., Eds.; John Wiley and Sons: New York, NY, USA; pp. 2.3.3–2.3.7.
Schlink, K. and Reski, R (2002). Preparing high-quality DNA from Moss (*Physcomitrella patens*). Plant Molecular Biology Reporter. 20, 423a-23f.
Sharma, A. D., Gill, P.K. and Singh, P (2002). DNA isolation from dry and fresh samples of polysaccharide rich plants. Plant Molecular Biology Reporter. 20, 415a-15f.
Sambrook, J.; Fritsh, E.F.; Maniatis, T (1989) .Molecular Cloning, a Laboratory Manual; Cold Spring Harbor Laboratory Press: New York, NY, USA.
Saenger W. (1984). Principles of Nucleic Acid Structure, Springer-Verlag: New York. 90-123.

Syamkumar, S.; Lowarence, B.; Sasikumar, B (2003).Isolation and amplification of DNA from rhizomes of turmeric and ginger. Plant Mol. Biol. Rep21, 171a–171e.

Wang Y. and Taylor D.E (1993). Biotechniques, 14, 748-750.

Watson J.D. and Crick F.H.C.; 1953 Molecular structure of nucleic acid: A structure for deoxyribose nucleic acid. Nature. 171(4356): 737-8.

Zamboni, A.; Pierantoni, L.; de Franceschi, P (2008).Total RNA extraction from strawberry tree (*Arbutus unedo*) and several other woody-plants. IForest-Biogeoscience Forestry *1*, 122–125.