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Comparative Optimized Protocols of DNA Extraction and Purification Using FTA PlantSaver Card and DNAzol Methods for Eggplant (*Solanum* Species) Studies in North Central Nigeria

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Abstract

Two methods of DNA extraction and purification in eggplant molecular research were investigated. FTA PlantSaver card and DNAzol were optimized, simplified and presented. The aim was to discard the rigorous protocols and help save energy, resources and time wasted in DNA extraction during molecular research in eggplant. DNA was extracted from fresh leaf of 18-day-old eggplant seedlings. Quality of genomic DNA extracts was verified on 1% agarose gel electrophoresis and viewed on a UV-transilluminator. DNA extracted from nine (9) different accessions was PCR amplified using nine (9) RAPD primers. Results indicated that both methods were effective in extracting and purifying sufficient quantity of DNA under different requirements. DNAzol method required the use of more leaf samples to extract sufficient quantity of DNA which in turn required that more seeds should be sown per accession. However, sufficient quantity of DNA could be extracted from even a single leaf sample using the FTA card method which may be preferred in a situation of low seed viability. Therefore, FTA card method is recommended for timely and quality DNA extractions and amplification using simple protocols outlined in this article.

Keywords

Optimization, DNA Extraction, FTA PlantSaver Card, DNAzol, Protocols, Eggplant, *Solanum* Species

Subject Areas: **Biotechnology, Plant Science**

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1. Introduction

Research in plant molecular biology starts with nucleic acid isolation and purification followed by amplification [1]. Nucleic acids commonly extracted in routine molecular work are: bacteria plasmid DNA, chloroplast DNA, mitochondrial DNA, mRNA extractions and total genomic DNA extractions [1]. According to [2], the principles of DNA isolation are the same. They all involve tissue pulverization and digestion of other components except DNA followed by purification. Total genomic DNA extraction is very common in molecular research. Different methods of plant DNA extraction exist each with its unique protocol [2]. The cetyltrimethylammonium bromide (CTAB) method and DNA extraction kits are often reported in literatures [3]-[5]. QIAquick purification kit and other DNA extraction kits are also widely available [6]. In most cases, authors do not disclose the extraction methods used in literature most especially when generalized procedures previously used by others are reported. Though there are many methods of DNA extraction available on the internet, they are often not available in literatures. According to [1], the factors that influence the choice of extraction method are: quality of extraction, cost of reagent, familiarity of procedures and simplicity of protocol. Many protocols on DNA extraction and purification are rather complicated to use. The simple and timely procedures of extracting DNA cannot be over emphasized in molecular research. The aim of this piece was to present the efficacy of FTA PlantSaver card and DNAzol method of plant DNA extraction using optimized simple protocols to be recommended in the molecular research on eggplant.

2. Materials and Method

Research activities were carried out in the Molecular Biology Laboratory of the University of Agriculture, Makurdi Nigeria. Eggplant seeds of nine accessions were planted in small transparent containers labeled D1-D9 respectively. DNA was extracted from fresh and healthy leaf samples (18-day-old) using FTA PlantSaver card and DNAzol methods. Complex protocols were continuously subjected to modification until optimized and simpler procedures were achieved in extracting sufficient quantity of DNA. Quality of genomic DNA extracts was verified on 1% agarose gel electrophoresis. Images were captured on the UV-transilluminator. Other extractions were immediately followed by PCR amplification of nine DNAs labeled D1-D9 using nine (9) RAPD primers: OPP-11, OPV-04, OPQ-07, OPU-19, OPQ-03, V-19, B-18, OPU-13 and OPU-15 respectively. Amplification was achieved on a thermal cycler (Applied Biosystem version) where 1 µl of DNA extracted from DNAzol or 1 purified FTA disc served as the DNA template in the reactions. Amplified products were resolved on 3% agarose gel electrophoresis and viewed on the UV-transilluminator. All results were compared accordingly.

3. Result and Discussion

DNA extraction is the first step in the study of plant DNA as it precedes all other molecular activities [7] [8]. The two methods compared in this report extracted sufficient quantity of DNA from leaf samples. Based on this outcome, the recommended optimized protocols are given in **Table 1**.

Analysis of the DNAzol method shows that more leaf samples are needed to achieve 1 g pulverized leaf tissue required. This suggests that more viable seeds should be planted (**Figure 1**). This becomes a major challenge as it cannot be employed in a situation where few seeds are viable. The use of mortar in pulverizing leaf tissue consumes much space in the laboratory most especially when extracting DNA from many plant sources. Furthermore, there should be a balance between the pulverized tissue and DNAzol reagent in relation to the chloroform in the tube. Adequate knowledge of the use of centrifuge machine is also required for this method. Despite the above challenges, the DNAzol method is a potent means of extracting quality genomic DNA which can be verified instantly on the gel as shown in **Figure 2**. Moreover, the extracted DNA properly stored can be PCR amplified many times when needed [7].

The use of FTA card method, on the other hand, is recommended when at least one leaf sample is required. It is often employed in a situation of low seed viability. At least, one seedling is sufficient enough for DNA extraction. It is a simpler method than DNAzol but requires repeated DNA washing. It does not consume space as one card can be used for a maximum of eight accessions when the squares are properly demarcated [7]-[9]. It is the preferred method when extracting DNA from many accessions (**Figures 3(a)-(b)**). The use of hazardous chloroform is avoided and no centrifugation is required [10]. However, the separation of DNA from the disc to be verified directly on the gel becomes a major challenge. Instead, the DNA in the disc is PCR amplified directly to avert the rigor of DNA separation. This means that the quality of the DNA extracted is often not checked

because of this challenge, but sufficient quantity of DNA in the disc can be amplified to achieve good band profile (**Figure 4**). Based on the comparative analysis of the two methods, the FTA card method is therefore

Table 1. Optimized protocols of DNA extraction using DNAzol and FTA PlantSaver card methods.

DNAzol method	FTA PlantSaver card method
Materials: Fresh and healthy leaf sample (15 - 21 day old); Mortar and pestle; DNAZOL reagent; Absolute ethanol; 70% ethanol; DNAzol-ethanol wash (optional); Chloroform timer; Micropipettes; 1.5 ml microcentrifuge tubes; Vortex machine; Centrifuge machine; Freezer, plastic rack, hand gloves and lab coat.	**Materials**: Fresh and healthy leaf sample (15 - 21 day old); FTA PlantSaver card; PestleParafilm paper; Harris punch; Harris cutting mat; Cotton wool; Desiccator; Absolute ethanol; FTA purification reagent; 70% ethanol; Timer; Micropipettes; 1.5 ml microcentrifuge tubes; Vortex machine; Plastic rack; Freezer; Hand gloves and lab coat.
Label your microcentrifuge tube (1.5 ml - 2 ml capacity) with a number representing the accession code.	Place a leaf sample in a labeled square of the FTA card.
Weigh 1 g of leaf sample and place in a mortar. Add 5 ml of absolute ethanol to submerge the leaf tissue for 30 minutes. Decant excess ethanol.	Overlay the sample with a transparent parafilm.
Dispense 750 µl DNAzol reagent into the tube	Gently pound the leaf until greenish sap is transferred beneath the paper.
Pulverize the leaf tissue in the mortar.	Remove the parafilm and air dry the card for 1 hour.
Transfer the homogenize tissue to the tube containing DNAzol. Allow the mixture stand for 5 minutes.	Cut 2 discs (2 mm diameter) from the sample into a 1.5 ml tube using the Harris punch.
Add 750 µl chloroform to the mixture above. Allow the mixture stand for 5 minutes.	Add 200 µl of 70% ethanol to the tube and allow soaking for 5 minutes. Vortex for 30 minutes before discarding the liquid, leaving the discs in the tube.
Centrifuge the tube at 10,000× g for 10 minutes.	Repeat the last step.
Transfer the supernatant (portion containing the DNA) into a new and labeled tube.	Dispense 200 µl of FTA purification reagent to the tube; allow to soak for 5 minutes. Vortex for 30 minutes before discarding the liquid gently, leaving the discs in the tube.
Add 750 µl absolute ethanol to the transferred supernatant to precipitate the DNA for 5 minutes.	Repeat the last step.
Centrifuge at 5000× g for 5 minutes to produce pelletized DNA.	Transfer the two discs containing purified DNA into a fresh tube using micropipette tips.
Add 750 µl of 70% ethanol to resuspend the pelletized DNA. Allow the mixture to stand for 5 minutes.	Air the discs in the tube for 1 hour.
Further centrifuge at 5000× g for 5 minutes.	Store in a freezer at −20˚C for further use.
Gently decant the liquid portion leaving the pelletized and pure DNA extracted.	Each disc can serve as a DNA template for PCR.
Air dry the tube for 1 hour. Reconstitute the DNA in 100 µl 1× TE for further use.	**Comment**: FTA method is recommended for timely and quality DNA extraction and amplification from large number of samples.

Figure 1. Eggplant seeds and 18-day-old seedling as DNA source (4.8 cm in height).

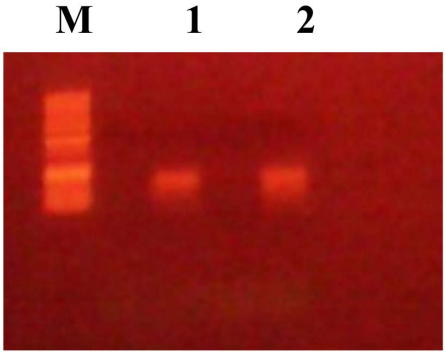

Figure 2. Verified genomic DNA extracted by DNAZOL method. Legend: M represents 50 bp ladder. 1 and 2 represents two DNAs extracted from leaf samples.

(a) (b)

Figure 3. DNA extraction steps using FTA PlansSaver card method. (a) Leaf overlay with parafim; (b) Cutting with Harris punch.

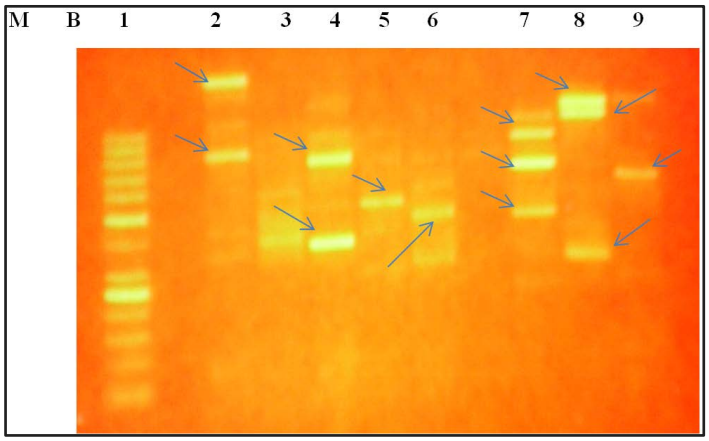

Figure 4. Amplification of nine extracted FTA based DNA (D1-D9) using nine RAPD primers respectively. Legend: M = 50 bp ladder, B = Blank. 1 = DNA bands amplified by OPP-11, 2 = DNA bands amplified by OPV-04, 3 = DNA bands amplified by OPQ-07, 4 = DNA bands amplified by OPU-19, 5 = DNA bands amplified by OPQ-03, 6 = DNA bands amplified by V-19, 7 = DNA bands amplified by B-18, 8 = DNA bands amplified by OPU-13, 9 = DNA bands amplified by OPU-15.

strongly recommended for timely and quality DNA extraction and amplification from a large number of samples. Generally, the two optimized protocols are efficient in DNA extraction which may replace the use of complicated protocols. This would help researchers in achieving quality DNA extraction and also help save resources, energy and time. The protocols presented in this report may therefore be used in extracting DNA from other crops.

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