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Comparative optimized protocols for DNA extraction and purification using FTA plant saver card and DNA zol methods for *Plectranthus species (Plectranthus esculenthus and Plactranthus roduntifolius)* in Nigeria (Livingstone and Hausa potato)

Inalegwu Bawa[1], Danjuma Yakubu[1*], Catherine Danmaigona[2], Amos Stephen[2]

[1]Department of Biochemistry, University of Agriculture, Makurdi, Nigeria

[2]Department of Plant Breeding and Seed Science, University of Agriculture, Makurdi, Nigeria

*Corresponding Author: *bioyaks@gmail.com*

Abstract: **The experiment compared two methods of DNA extraction in Livingstone and Hausa potato. DNA zol and plant saver card were optimized, simplified and presented. The aim of the experiment was to establish a suitable protocol for the extraction of these plants in Nigeria, in order to save time, cost and energy which may be spent during DNA extraction process. 20 day old fresh leaves of Livingstone and Hausa potato were extracted for the experiment. DNA extracted from four different accessions were amplified using 10 SSR primers. The results indicated that only the DNA zol technique was effective in extracting sufficient amount of DNA from the plants. Even though DNA zol requires more leaf sample, it proved to be the best technique for the extraction of DNA from the leaf samples. FTA plant saver card was however unable to trap enough amount of DNA from the leaves. Therefore, DNA zol technique is recommended for the timely and quality extractions and amplification PCR using the outlined protocols in this article.**

Keywords: **Opimization, DNA extraction, FTA plant saver card, DNA zol, Protocols, Livingstone potato, Hausa potato, *Plectranthus esculenthus, Plectranthus roduntifolius, Plectranthus species.***

1. INTRODUCTION

The first step in Molecular Biology research is 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]. The main objective of various DNA isolation methods is development of relatively quick, inexpensive and consistent protocol to extract high quality DNA with better yield [9]. Generally leaf samples contain large quantities of polyphenols, tannins and polysaccharides. The basic principle of DNA isolation is disruption of the cell wall, cell membrane and nuclear membrane to release the highly intact DNA into solution followed by precipitation of DNA and removal of the contaminating biomolecules such as the proteins, polysaccharides, lipids,

phenols and other secondary metabolites by enzymatic or chemical method. The present research, describe two modified methods for plant genomic DNA isolation.

2. MATERIALS AND METHODS

The experiment was carried out in Nigeria, Federal University of Agriculture Makurdi Molecular Biology Laboratory. The DNA was extracted from fresh leaves (20 days) using FTA plant saver card and DNA zol methods. Complex methods were modified using simpler procedures which produced simpler sufficient quality of DNA. DNA was viewed on 1% Agarose gel electrophoresis. The gel images were captured on the UV transilluminator.

Other extractions were followed by PCR amplification of 10 DNAs labeled SSR1 - SSR10 using 10 SSR primers (SSR1, SSR2, SSR3, SSR4, SSR5, SSR6, SSR7, SSR8, SSR9, SSR10). Amplification was done using thermal cycler (Appled biosystem version) where 1µl of DNA extraction from DNA zol and purified FTA disc served as 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. To improve the adaptability of DNAzol reagent to various plant species, several modifications were made to the manufacturer's protocol, which included an additional elution step with chloroform, a higher centrifugation speed for DNA precipitation, an additional wash step with 750 µl of 70% DNAzol reagent and 30% ethanol instead of 70% ethanol, and a 10 min incubation time of DNA pellet to remove the ethanol residue. The chloroform elution step could markedly improve the DNA purity in all *Plectranthus* species tested. It was found that the centrifugation at 5000g, recommended in the manufacturer's protocol, precipitated less DNA than that at 20000 g (Method 1). The protocols optimized are given in (Method 1 and 2).

Method 1: DNA extraction using DNAzol 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.

Label your microcentrifuge tube (1.5 ml - 2 ml capacity) with a number representing the accession code.

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.

Dispense 750 µl DNAzol reagent into the tube Pulverize the leaf tissue in the mortar.

Transfer the homogenize tissue to the tube containing DNAzol. Allow the mixture stand for 5 minutes.

Add 750 µl chloroform to the mixture above. Allow the mixture stand for 5 minutes.

Centrifuge the tube at 10,000 g for 10 minutes.

Transfer the supernatant (portion containing the DNA) into a new and labeled tube.

Add 750µl absolute ethanol to the transferred supernatant to precipitate the DNA for 5 minutes.

Centrifuge at 5000 g for 5 minutes to produce pelletized DNA.

Add 750 ^l of 70% ethanol to resuspend the pelletized DNA. Allow the mixture to stand for 5 minutes.

Further centrifuge at 10, 000g for 5 minutes.

Gently decant the liquid portion leaving the pelletized and pure DNA extracted.

Air dry the tube for 1 hour. Reconstitute the DNA in 100 µl of 1* TE for further use.

Method 2: FTA PlantSaver card method.

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.

Place a leaf sample in a labeled square of the FTA card.

Overlay the sample with a transparent parafilm.

Gently pound the leaf until greenish sap is transferred beneath the paper.

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Remove the parafilm and air dry the card for 1 hour.

Cut 2 discs (2 mm diameter) from the sample into a 1.5 ml tube using the Harris punch.

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.

Repeat the last step.

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.

Repeat the last step.

Transfer the two discs containing purified DNA into a fresh tube using micropipette tips.

Air the discs in the tube for 1 hour.

Store in a freezer at -20°C for further use. Each disc can serve as a DNA template for PCR.

Comment: The FTA card failed to trap enough DNA for PCR reaction.

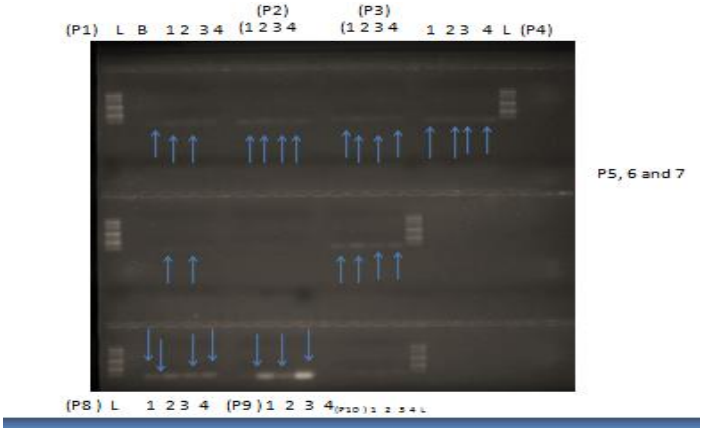

Figure 1. Amplification of nine extracted from DNA zol DNA (P1-P10)

Using nine SSR primers respectively. Legend: M = 50 bp ladder, B = Blank. P1 = DNA bands amplified by SSR1 on sample 1-4, P2 = DNA bands amplified by SSR2 on sample 1-4, P3 = DNA bands amplified by SSR3 on sample 1-4, P4 = DNA bands amplified by SSR4 on sample 1-4, P 5 = DNA bands amplified by SSR5 on sample 1-4, P6 = DNA bands amplified by SSR6 on sample 1-4, P7 = DNA bands amplified by SSR7 on sample 1-4, P8 = DNA bands amplified by SSR8 on sample 1-4, P9 = DNA bands amplified by SSR9 on sample 1-4 and P10= DNA bands amplified by SSR10 on sample 1-4.

Figure 2. *Plectranthus esculenthus* leaves as DNA source (4.8 cm in height).

3. DISCUSSION

DNA extraction is regarded as the first step in the study of plant DNA as it is the most important molecular activity [2]. Of the two methods compared in this experiment, only the DNA zol extracted sufficient quantity of DNA from the leaf samples. Although the use of FTA plant saver card requires only a single leaf sample, it needs serious washing of

discs in order to purify DNA. However, it minimizes the being space use in the laboratory. It has been a recommended method for Extracting DNA from plant accessions [1]. The separation of DNA from the disc to a verified gel becomes another major challenge of the FTA card method, the DNA is amplified directly on the discs to reduce rigor. DNA separation is often done on the discs. Hence the DNA cannot be checked.

4. CONCLUSION

The modified procedure of DNAzol reagent described is rapid, simple and allows yielding genomic DNA of satisfactory yield and purity from a broader range of wild plant species. The procedure may be employed to isolate DNA from different species and these DNA extracts have can be further used for various DNA analyses such as isolation of DNA markers, detection of primer specificity and analysis of fungal biodiversity in our laboratory. This procedure may also be advisable for other *Plectranthus* species.

From the two protocols optimized, only the DNA zol was efficient in extracting sufficient DNA for PCR amplification of the plants. The can help researchers in achieving quality DNA extraction while saving resources. The protocol presented in this experiment may be used in extracting other wild plant species.

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