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COMPARATIVE OPTIMIZED DNA ISOLATION PROTOCOLS FOR SIX ACCESSIONS OF SWEET POTATO (*Ipomoea batatas* [L] Lam.) IN NIGERIA USING FTA PLANT SAVER CARD AND DNA ZOL

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Abstract: **DNA Isolation is a necessary protocol in Molecular Biology Research. Comparative Optimized DNA Isolation Protocol for six accessions of Sweet Potato (*Ipomoea batatas* [L] lam.) in Nigeria using FTA Plant saver card and DNA Zol was carried out with the aim of establishing a suitable and effective DNA extraction protocol from the leaves of sweet potato by comparing the bands formed after amplification using 5 Simple Sequence Repeats (SSR) primers. The accessions (Mothers Delight, King J, Butter Milk, DanZaria, NR 8164 and 87/TIS0087) collected from the National Root Crop Research Institute (NRCRI) Abuja, Nigeria were used as plant materials. Young leaves from the tip of the main vine of 20 days old plants were obtained and subjected to FTA Plant saver card and DNA Zol extraction protocols. Polymerase Chain Reaction in a touch down fashion and Agarose gel electrophoris were then performed on the extracted DNA and the bands formed were viewed under U-V light. The result showed that although both the FTA Plant Saver card and DNA Zol extraction method were able to extract a considerable amount of DNA, the bands formed from the DNA extracted using DNA Zol extraction protocol were more intense and clearer than those formed from the DNA extracted through FTA Plant Saver Card. This is due to the fact that DNA Zol utilised a higher amount of total genomic DNA than FTA plant Saver card for amplification. The DNA Zol extraction protocol is therefore recommended as an effective and efficient DNA extraction protocol for the potato plant.**

Keywords: **DNA Isolation, FTA plant saver card, DNA Zol, Sweet potato, Protocol, Accession, Amplification, Bands, Polymerase Chain Reaction and Molecular Biology.**

1. INTRODUCTION

Sweet potato (*Ipomea batatas* [L] Lam), is a dicotyledonous plant that belongs to the family Convolvulaceae. It has large, starchy, sweet-tasting, tuberous roots hence the plant is seen as a root vegetable. Sweet potato is only distantly related to the potato (*Solanum tuberosum* L) and does not belong to the family Solanaceae commonly known as the nightshade family, but both families belong to the same taxonomic order which is the Solanales. The plant is an herbaceous perennial vine, bearing alternate heart-shaped or palmately lobed leaves and medium-sized sympetalous flowers. Sweet potato cultivars with white or pale yellow flesh are less sweet and moist than those with red, pink or orange flesh [5]. Generally,

sweet potatoes are consumed because their nutrients contain beta carotene that prevents vitamin A deficiency in many developing countries [9]. DNA isolation is a process of purification of DNA from samples using a combination of physical and chemical methods. The first isolation of DNA was done in 1869 by Friedrich Miescher [3]. Currently, DNA isolation is a routine procedure in molecular biology analysis. As stated by [1], the first step in Molecular Biology research is nucleic acid isolation and purification followed by amplification and also, Nucleic acids commonly extracted in routine molecular work are: bacteria plasmid DNA, chloroplast DNA, mitochondrial DNA, mRNA extractions and total genomic DNA extractions. For the chemical method of isolation, there are different kits and procedures and selecting the correct procedure will save time on kit optimization and extraction protocol and Polymerase Chain Reaction (PCR) sensitivity detection is considered to show the variation between the commercial kits [10]. As reported by [8], the main objective of various DNA isolation methods is to develop a relatively quick, inexpensive and consistent protocol to extract high quality DNA with better yield. In general, a lot of comparative work on FTA Plant saver Cards and DNA zol has been carried out in the last few years and a lot of recommendations has been made. [1] For instance compared these methods using the Eggplant and recommended the FTA method for timely and quality DNA extraction and amplification from large number of samples. Also, [6] compared both methods using *Plectranthus species* (*Plectranthus esculenthus* and *Plactranthus roduntifolius*) and reported that only the DNA zol was efficient in extracting sufficient DNA for PCR amplification of the plants.

2. METHOD

Potato plant materials

Six potato accessions (Mothers Delight, King J, Butter Milk, DanZaria, NR 8164, 87/TIS0087) collected from the National Root Crop Research Institute (NRCRI) Abuja, were planted in the field at the research farm in the University of Agriculture, Makurdi, Benue State, Nigeria and used as plant material.

DNA Extraction Method 1

Genomic DNA was extracted from young leaves located at the tip of the main vine of the potato plant using FTA Plant Saver card protocol as reported by [1] with slight modifications. The protocol was carried out in the Molecular Biology laboratory of the University of Agriculture, Makurdi, Nigeria as follows:

i. Young leaves for each of the accessions numbered NC 1 – NC 6 were collected from the tip of the main vine of a 20 day old plant and placed carefully on the FTA Plant Saver cards and overlaid with a transparent parafilm
ii. The leaves were pounded gently using a laboratory pestle until greenish sap is transferred on the FTA Plant Saver card
iii. The parafilm was afterward removed and the FTA Card air dried for one hour
iv. Using the Harris punch, 2 discs (2 mm diameter) from the air dried sample on the FTA card was cut into a 1.5 ml tube
v. Two hundred µl of 70% ethanol was added into the tube, allowed to soak for 5 minutes and vortex for 30 seconds before discarding the liquid, leaving the discs in the tube
vi. Step V above was then repeated
vii. Two hundred µl of FTA purification reagent was added to the tube, allowed to soak for 5 minutes and then vortex for 30 seconds before discarding the liquid gently, leaving the discs in the tube
viii. Step VII above was repeated
ix. A micropipette was afterwards used to remove the leftover liquid in the tube leaving the disc behind in the tube
x. The tube was air dried for an hour and stored in a freezer at -20°C for further use

DNA Extraction method 2

Genomic DNA was extracted from young leaves located at the tip of the main vine of the potato plant using DNA zol protocol as reported by [1] with slight modifications. The protocol was carried out in the Molecular Biology laboratory of the University of Agriculture, Makurdi, Nigeria as follows:

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i. One gram of fresh and healthy leave sample from the tip of the main vine of a 20 day old plant was weighed and placed in a mortar in which 5 ml of absolute ethanol was added to submerge the leaf tissue for 30 minutes

ii. Excess ethanol was decanted and the leaves pulverized and transferred into labelled micro-centrifuge tubes (1.5 ml) bearing numbers representing the accession codes

iii. DNA zol reagent (750 µl) was dispensed into the tube and allowed to stand for five minutes

iv. Chloroform (750 µl) was added to the tube and allowed to stand for 5 minutes

v. The tube was centrifuge at 10,00 ×g for 10 minutes and the supernatant transferred into a new labelled tube

vi. Absolute ethanol (750 µl) was added to the tube containing the supernatant and allowed for 5 minutes after which the tube was centrifuge at 5000 × g for 5 minutes

vii. Seventy parent ethanol (750 µl) was added to re-suspend the pelletized DNA and allowed to stand for 5 minutes

viii. The tube was centrifuge at 5000 × g for 5 minutes and the liquid portion was gently decanted leaving the pelletized and pure DNA

ix. The tube was air dried for 1 hour and then stored in a freezer at -20°C for further use.

DNA Amplification

DNA Amplification was done at the Molecular Biology laboratory of the University of Agriculture, Makurdi, Nigeria using the following steps:

Selection of Primers

Five (5) primers listed in table 1 below were used to amplify total genomic DNA by PCR.

Table 1: List of Primers and their Sequence

S/N	Primer Name	Forward Sequence	Reverse Sequence
1	IB02	CTGTGGATCTGTTCTTTGAACC	TTCCATGTGGAGTGTGAAGTAT
2	IBS139	CTATGACACTTCTGAGAGGCAA	AGCCTTCTTGTTAGTTTCAAGC
3	IBS166	TCCGTCTTTCTTCTTCTTCTTC	ATACACTAACTGCATCCAAACG
4	IBS199	TAACTAGGTTGCAGTGGTTTGT	ATAGGTCCATATACAATGCCAG
5	Ibu4	GGCTGGATTCTTCATATTTAGC	GCTTAATGGATCAGTAACACGA

Polymerase chain reaction

PCR Amplification was done using a 2720 Thermal Cycler and covered the following steps in a touch down fashion:

i. Initiation: This was done at a temperature of 95°C for 4 minutes.

ii. Denaturation: This was carried out at a temperature of 95°C for 30 seconds.

iii. Annealing: This was carried out at between 55.0°C for 30 seconds.

iv. Polymerization: This was done at a temperature of 72°C for 50 seconds.

v. Step 2 to 4 was repeated for 40 cycles.

vi. Final extension: This was carried out at a temperature of 72°C for 50 seconds.

Each tube loaded into the P.C.R machine contained G.E Healthcare premix (13µl), 1 µl of primer (forward and revers primers that have been constituted) and 1 µl of genomic DNA.

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Agarose gel electrophoresis:

Preparation of gel

Agarose gel electrophoresis was done in the Molecular Biology laboratory of the University of Agriculture, Makurdi, Nigeria using the following steps:

i. Five grams of agarose powder was weighted into an Erlenmeyer flask containing 500 mL of TAE
ii. The content of the flask was swirled and the top covered with a paper towel
iii. The flask was microwaved till the agarose powder is completed dissolved and the content of the flask crystal clear
iv. This was allowed to cool a little and then 1.5 µl of ethidium bromide was added
v. The content of the flask was emptied into a casting tray with combs already inserted
vi. After cooling, the combs where removed and the casting tray was placed in a gel thank containing adequate water

Loading into wells

i. After Polymerase Chain Reaction amplification, 1 µl of DNA loading dye was added into each of the tubes and then made to spin for 10 seconds
ii. The content of the tubes was carefully loaded into separate wells
iii. DNA ladder (100 kb) was loaded into wells before each set of wells containing a set of DNA for each primer
iv. The gel tank was covered properly and a constant volt of 120 was applied for 60 minutes

U-V Light

After the DNA has separated, the gel was then transferred to a Bench top transilluminator and the gel image was viewed

3. RESULTS

The result for the experiment is shown in Plate 1 below. The result showed that both the FTA plant saver card and the DNA zol extraction protocols were able to trap a considerable amount of DNA from the leaves of the potato plant. However, the bands formed from the DNA extracted using DNA zol were clearer and intense than those formed from the DNA extracted with FTA plant saver card.

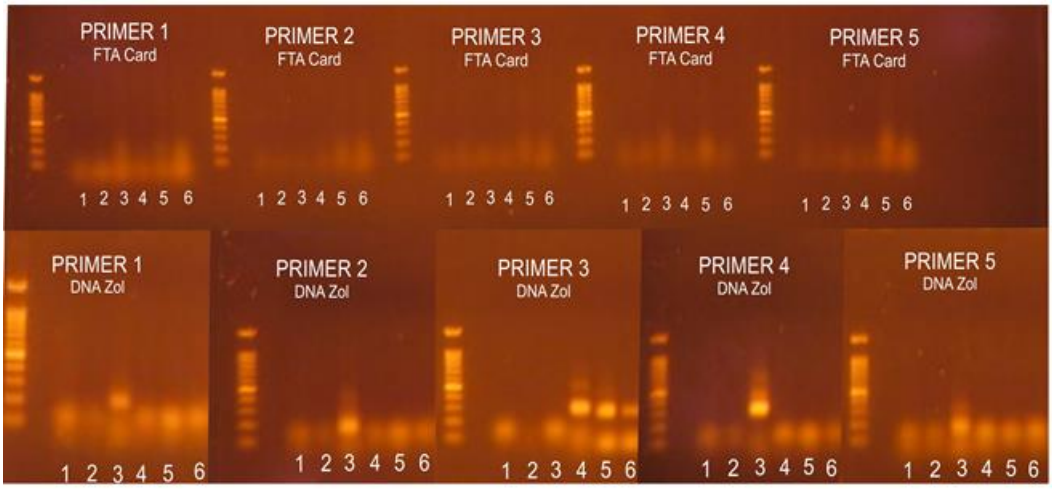

Plate 1: Gel image for the two DNA isolation protocol

4. DISCUSSION

In comparing the FTA Plant saver card and the DNA Zol extraction methods, different authors have taken different stands as to which method is preferable to the other because both protocols have been reported to be effective especially on different plant species and have their peculiar merits and demerits. For an instance, [1], recommended the FTA Plant

saver card over the DNA Zol while [6] took a different stand by recommending the DNA Zol over the FTA Plant saver card. This is simply because they compared both methods on different plant species. Their divergent stand however, does not in any way suggest that any of the protocol is ineffective in DNA extraction because this study has shown that both methods are effective in DNA extraction especially on the sweet potato plant. Apart from FTA Plant saver cards and DNA zol, other DNA extraction methods have been used on the potato plant and reported to be effective. The Cetyl Trimethyl Ammonium Bromide (CTAB) method is the most widely used and reported in literatures. [7] And [2] reported this method according to Doyle and Doyle (1990) with minor modifications while [4] reported the same method but as proposed by Murray and Thompson (1980) with modifications.

5. CONCLUSIONS

FTA plant saver card and DNA zol are both effective DNA isolation protocols depending on the plant species that they are used on. For the potato plant especially when the sample size is large, the DNA zol extraction protocol is preferable and therefore recommended.

Plate 2: Mothers Delight

Plate 3: King J

Plate 4: Butter Milk

Plate 5: DanZaria

Plate 6: NR 8184

Plate 7: 87/TIS0087

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