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Full Length Research Paper

Application of fast technology for analysis (FTA)$^{\rightarrow}$ for sampling and recovery of deoxyribonucleic acid (DNA) for molecular characterization of cowpea breeding lines for *Striga* resistance

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***Striga gesnerioides* (Willd) Vatke is a significant constraint to cowpea production in the dry savannas of sub-Saharan Africa. Yield losses caused by *S. gesnerioides* in these regions are estimated in millions of tons annually and the prevalence of *Striga* soil infestation is steadily increasing. Conventional breeding efforts have developed some cowpea lines with *Striga* resistance as well as other important agronomic traits but it is time-consuming and difficult to pyramid favorable traits. The use of marker-assisted selection (MAS) makes it easier to select plant traits and reduce the time needed to develop new varieties. The potential of Fast Technology for Analysis (FTA) as an effective technology for sampling and retrieval of DNA from plant tissue and their subsequent molecular analysis was assessed in the laboratory. DNA was successfully recovered from the leaf tissues of cowpea pressed into the FTA® Classic card and the DNA obtained from the FTA papers was found to be suitable for molecular analysis by PCR-based techniques. The marker efficiency of Sequence Characterized Amplified Region (SCAR) marker MahSe2 and C42B was 93% in detecting SG3 resistance. This study demonstrated that the application of MAS using FTA technology has the potential to put the breeding process on a fast track and increase the efficiency of breeding activities.**

Key word: Marker assisted selection, *Striga gesnerioides*, race, FTA→□PlantSaver Card, PCR, tissue prints, DNA.

INTRODUCTION

Striga gesnerioides presents a major constraint to cowpea production and productivity in sub-Saharan Africa. The parasitic weed is difficult to control and, once established few means are available to counter the impact on yield. Yield losses raging from 83 to 100% have been reported (Aggarwal, 1985; Emechebe et al., 1991). As a result, development and deployment of resistant crop varieties remain the most effective ways to combat the menace presented by *S. gesnerioides*. Classical tools have been used in identifying resistant and susceptible genotypes. Often this classification is affectted by the environment and a long time is needed to develop resistant varieties. Therefore, an effective method is required to facilitate the molecular characterization of cowpea lines for resistance to *Striga,* and speed-up the process of crop delivery to farmers. Access to simple, low cost tools for the molecular study is central to generating the knowledge required. Intact high molecular weight plant deoxyribonucleic acid (DNA) is

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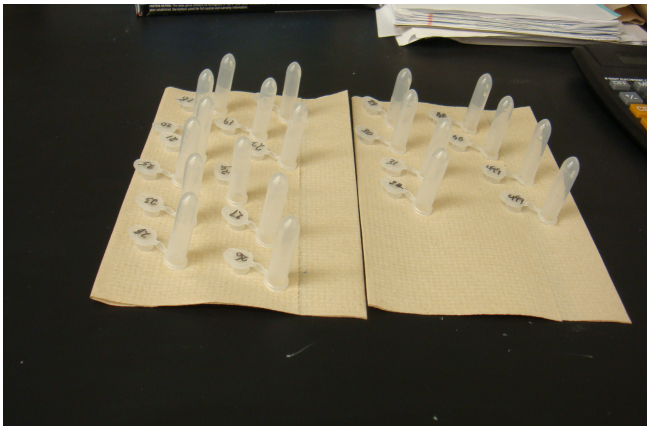
a



b

Figure 1. a. FTA sampling method; b. Processed 2 mm FTA disc.

essential for molecular studies and genomic DNA library construction. A frequent limitation for studying DNA at molecular level is the ability to obtain reliably high quality DNA. Plant tissues to be analyzed must be collected and preserved to maintain integrity of the DNA until they can be processed. This poses challen-ges when sample numbers are large and when working in the field, especially in the tropical and subtropical regions where laboratory facilities are limited. Field and greenhouse studies are thus constrained by the resources required. Sample collection and transportation place restrictions on the number and size of samples that can be collected in a given time. Timely processing or storage of the samples before they deteriorate can also be difficult in locations where access to well equipped laboratory facilities is limited. Previously, traditional isolation methods were used, in which plant tissue is grind in liquid nitrogen and transferred to a preheated extrac-tion buffer (Dellaporta et al., 1983; Mohapatra et al., 1992).

Liquid nitrogen can be difficult to procure in remote locations thus, a method not requiring its use would be helpful. FTA$^{\rightarrow}$ cards (Whatman$^{\rightarrow}$) provide an alternative method. A simple tool is the use of FTA$^{\rightarrow}$ technology for collection, storage and retrieval of genomic DNA for molecular study. FTA$^{\rightarrow}$ technology has great potential for the collection and retrieval of DNA especially when operating in developing countries and regions remote from laboratory facilities. FTA$^{\rightarrow}$ matrix cards developed by Whatman is a novel method for the rapid collection, purification, and analysis of genomic DNA from a wide range of biological sources, whole blood, tissues, plasmid, plant materials, and microorganisms (Hide et al., 2003, Lampel et al., 2004, Whatman Inc. FTA Protocols, 2002). FTA-treated matrix cards are impregnated with a proprietary mix of chemicals containing strong buffers, a free radical trap, and protein denaturants that lyses cell membranes on contact, physically entrap DNA, and stabilize and protect DNA from nuclease, oxidation, ultraviolet-visible (UV) damage and microbial and fungal degradation (Kubo and Fujita, 2006; Smith and Burgoyne, 2004). FTA$^{\rightarrow}$ cards have been successfully used for the collection and long-term storage of bacterial cells for molecular analysis (Rajadram et al., 2006), leaf tissue of a variety of plants, including soybean (Lin et al., 2000), tomato, tobacco, and grapes (Ndunguru et al., 2005). They have been used for the transport and molecular genetic analysis of scleractinian corals in remote environmental locations (Crabbe, 2003), and for sampling, recovery and molecular characterization of viral pathogens and virus-derived transgenes from plant tissue (Ndunguru et al., 2005). They have also proven to be useful for large- scale plant DNA isolation for use in MAS (Mbogori et al., 2006). To-date, there are few record of its potential use in the collection of cowpea leaf material for long-time storage and retrieval of total genomic DNA. Therefore, we assessed the potential of the FTA$^{\rightarrow}$ matrix card system as an effective technology for the sampling and retrieval of genomic DNA from cowpea tissue and its subsequent molecular analysis.

MATERIALS AND METHODS

Plant material

Cowpea (*Vigna unguiculata* (L.) Walp.) parents, F_1 and the derived F_2 populations were grown under standard greenhouse conditions in IITA Kano station, Nigeria, in a pot culture inoculated with *Striga* seeds. Genomic DNA was extracted from leaf tissue for one month-old plants using the FTA$^{\rightarrow}$ Plantsaver cards. The second young leaf was excised from the plant and placed in a square of the FTA card. The leaf sample was covered with a parafilm paper and a pestle was used to press the leaf sample onto the FTA$^{\rightarrow}$ paper until both sides of the FTA were soaked with the extract (Figure 1a and b). In circumstances where the pestle was stained by the leaf sap as a result of parafilm paper damage, a paper towel soaked in 70%

Figure 2. A 2 mm diameter disc being removed from FTA plant saver card for DNA processing and molecular analysis.

ethanol was used to clean the pestle in between samples to prevent cross- contamination.

Preparation of FTA tissue print

The excised young leaf was placed on the FTA® Plantsaver card covered with parafilm paper, and brief pressure was gently applied with a pestle until plant material was sufficiently transferred to the card. The cards were allowed to dry for one hour; plant material was brushed off with tissue paper. After air drying, FTA® cards were placed in a paper pouch and stored at ambient temperature in a dry location.

Preparation of samples for polymerase chain reaction (PCR) analysis

A disc from the dried FTA tissue print was removed using a clean Haris$^{\rightarrow}$ micro-pouch (Figure 2) and the disc was placed directly into a 1.5 ml eppendorf tube. In between samples, the Haris$^{\rightarrow}$ micro pouch was cleaned with a tissue dampened with 70% ethanol and then a disc was taken from a blank sample less FTA$^{\rightarrow}$ card to prevent cross-contamination. The disc was washed twice with 200 µl of 70% ethanol, and incubated for 5 min between each wash. A repeat wash with 200 µl of FTA reagent, was incubated for 3 min at room temperature. The liquid was discarded. The tubes were inverted and drained on a paper towel for air-drying for approximately 1 h. After drying, the disc was transferred to a PCR tube for PCR analysis.

PCR analysis

PCR analysis was done with 2 primers MahSe2 and C42B. The PCR mixture (25 µl final volume) contained, in addition to the purified 2 mm FTA DNA disc containing the DNA sample, a final concentration of 18 µl of sterilized water, 2.5 µl each of balanced dNTPs mix and 10X PCR buffer, 0.05 µl of Taq polymerase (5U/µl), and 1 µl of each of the forward and reverse primers (0.1-0.6 µl) (synthesized by IDT Corlville, IA). PCR reactions were performed on a heated lid thermal cycle using the thermal cycle (Biometra) operated as follows: 35 cycles of denaturation at 94 $^{\circ}$C for 30 sec, followed by annealing at 57.5 $^{\circ}$C for 30 sec and extension at 72 $^{\circ}$C for 2 min. A final extension cycle of 10 min at 72 $^{\circ}$C was added to ensure completion of the final amplification products. For the C42B marker, a similar procedure was followed, but the annealing temperature used was 67.5 $^{\circ}$C.

Analysis of PCR product

A 25 µl of the final PCR product was electrophoresed on a 3% agarose gel with ethidium bromide staining. The gels were run for approximately 1 h 30 min at 120 voltage in 1 X Tris acetic acid (TAE) buffer (45 mmol L-1 glacial acetic acid, 0.5 mmol l-1 ethylenediaminetetra acetic acid (EDTA), (pH, 8.4). A 1 kb DNA molecular marker ladder loaded in the first well for band size determination of PCR products. The ethidium bromide-stained gel was visualized on an UV transilluminator and photographed using a Polaroid camera.

RESULTS AND DISCUSSION

DNA was successfully recovered from leaf tissue of cowpea pressed into FTA$^{\rightarrow}$ classic cards. Plant DNA eluted from FTA$^{\rightarrow}$ cards stored for over nine months at room temperature was found suitable for molecular analysis by the PCR-based techniques in a manner equivalent to that offered by traditional isolation methods. Two primers, MahSe2 and C42B, linked to *Striga* resistant gene in LG1, were used to discriminate between resistant and susceptible lines in the F_2 populations. DNA of the resistant lines using markers linked to the specific *Striga* race 3 (SG3) was detected from the population collected on FTA cards. Unique bands were produced by the two markers. The primer MahSe2 identified resistant lines carrying double bands while the susceptible lines carried single bands. On the other hand, C42B identified resistant lines with a single band; a susceptible line had no band (Figures 3 and 4). PCR amplification of deoxyribonucleic acid (DNA) generated from cowpea DNA eluted from FTA cards was compared with that

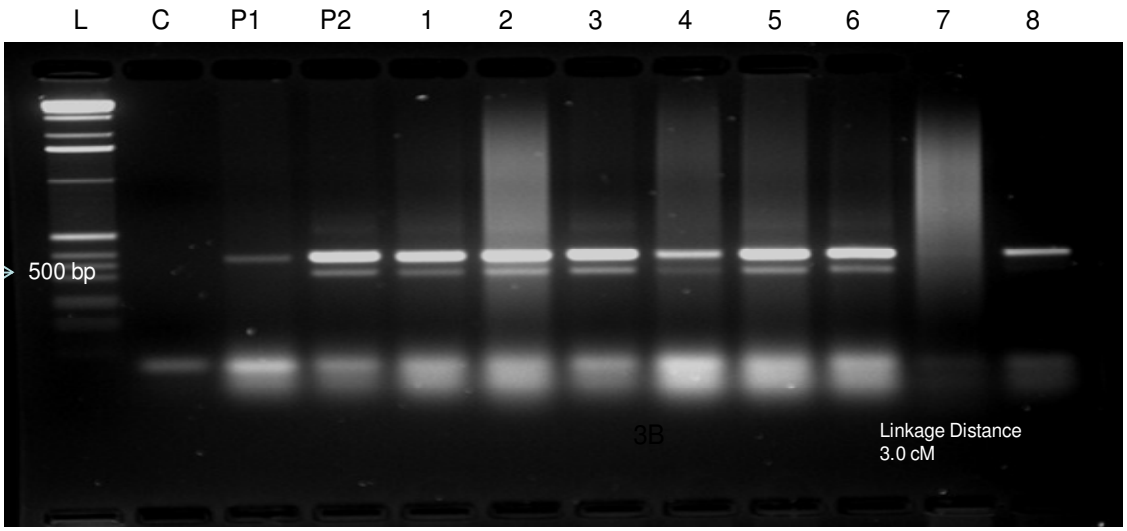


Figure 3. the results of the PCRamplification of the DNA based on the use of the SCAR marker mahSe in 3% agarose gel with ethidium bromide staining. It has a topmost band and lower band. L represent 1Kb ladder to confirm fragment size. C-blank reaction, susceptible parent P1-Borno Brown, resistant parent P2IT97K-499-35, and 1-8 are F2 progencies

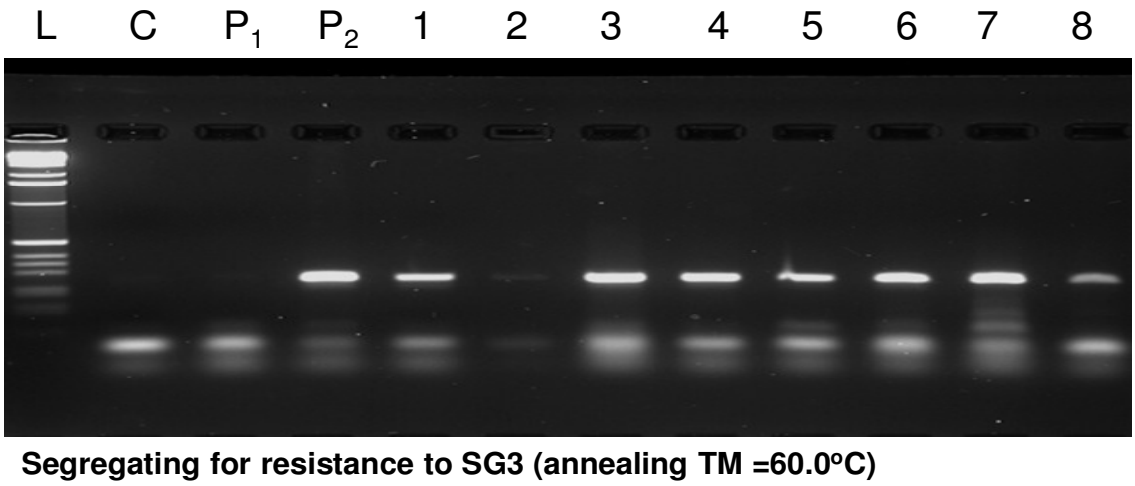


Figure 4. The results of PCR amplification of DNA based on the use of the SCAR maker Mahse2 in 3% agarose gel with ethidium bromide staining. It has a single band L represents 1 Kb ladder, control fragment size, susceptible parent P1-Borno Brown, resistant parent P2IT97K-499-35, and 1-8 are F2 progencies

of conventional breeding method. FTA was found to be suitable for the recovery of genomic DNA and for use in molecular characterization of the segregating population for resistance and susceptibility to *S. gesnerioides*. The marker score corresponded with the phenotypic score implying that the marker was reliable in molecular characterization of cowpea genotypes for resistance or susceptibility to *Striga*. Results obtained from FTA$^{\rightarrow}$ sampled materials were effective and reproducible in all the three segregating populations used. The studies described here demonstrate that FTA offers a simple, sensitive, and specific tool appropriate for molecular characterization of plant genomic DNA isolated from cowpea leaf tissues. We believed that the application of this technology has the potential to significantly enhance the cowpea breeding program and the efficiency of breeders to speed-up the process of developing and deploying cowpea *Striga* resistant varieties to farmers.

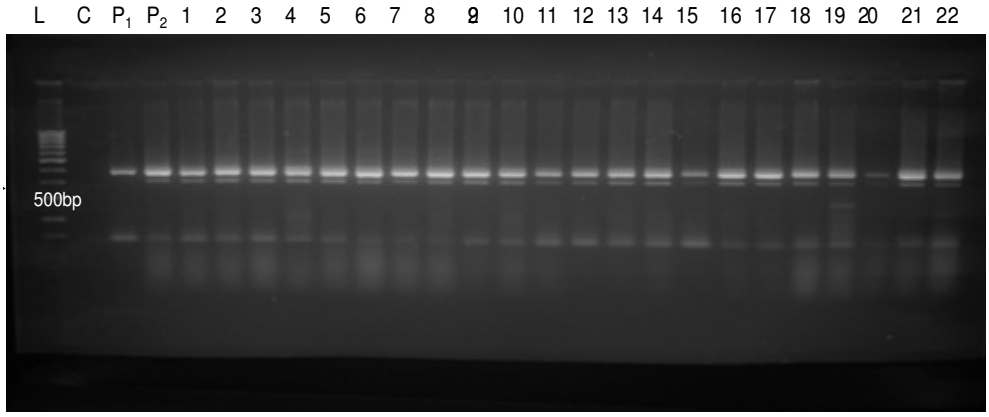


Figure 5. PCR amplification of DNA based on the use of BIONEER Accupower→ PCR premix with the SCAR maker Mahse2 in 3% agarose gel with ethidlim bromide staining. L represents 1 Kb ladder, to confirm fragment size. C-blank reaction, susceptible parent P1-Borno Brown, resistant parent P2IT97K-499-35, and 1-8 are F2 progencıes. Segregating for resistance to SG3 (Annealing Tm= 57.5°C). Resistant genotypes carry double bands while susceptible genotype had single band.

There was a positive significant correlation (0.864) between the two makers and a correlation of 0.91 to that of the phenotypic score.

This indicates that these markers are efficient in discriminating between resistance and susceptibility in segregating population and that the marker techniques are valuable. A correlation values above 0.8 has been suggested to be good association between markers (Rohlf, 1993). FTA→ technology was also used to sample F_3 cowpea plants for molecular characterization using BIONEER AccuPower→ PCR PreMix (Accupower→ PCR PreMix is a new, and ready-to use PCR reagent optimized PCR amplifications). The PCR amplification also produced uniform banding patterns and a good resolution on 3% agarose gel, indicating reliability of the markers and confirming the result obtained in the F_2 segregation, regardless of the method used. The AccuPower→ PCR PreMix yielded the expected result in the F_3 populations tested (Figure 5) with primer pair MahSe2. The results obtained here indicate that it is possible to use this procedure for marker genotyping in cowpea for resistance to *S. gesnerioides*. Similar results have been reported in other crop using this procedure for marker genotyping (Lange et al., 1998; Drescher and Graner, 2002). The ability to obtain and store the prints at ambient temperatures means that these tests could be employed for wide-scale studies in the field to enhance cowpea breeding programme for researching cowpea resistance to *S. gesnerioides*. The benefits of this technology have important implications for improving the efficiency of the molecular characterization of cowpea genotypes for resistance to *Striga* in the laboratory, especially when working in remote areas and in developing countries where access to laboratory facilities, chemicals, and equipment is limiting. Results obtained from FTA→ sampled materials were effective and reproducible in our hands from the three populations used. The studies described here that the demonstrated FTA offers a simple, sensitive and specific tool appropriate for molecular characterization of plant genomic DNA isolated from plant tissues. We conclude that the application of this technology has the potential to significantly enhance the cowpea breeding program, and the efficiency of breeders to speed-up the process of developing and deploying cowpea *Striga* resistant varieties to farmers.

This study demonstrates that FTA is an effective, economical and sensitive method for sampling, storage and retrieval of genomic DNA from cowpea leaf tissues, growing under greenhouse condition. This study was able to provide information that plant tissue samples can be stored on FTA→ cards for up to one year at ambient temperature without compromising the integrity of the sample.

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