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Molecular Characterization of Cowpea Breeding Lines for *Striga* Resistance Using SCAR Markers

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Abstract: *S. gesnerioides* (Willd) Vatke is a major biological constraint to cowpea production in the dry savanna of sub-Saharan Africa. Yield losses caused by *S. gesnerioides* in these regions are estimated in millions of tons annually, and prevalence of *Striga* soil infestation is steadily increasing. The availability of molecular markers tightly linked to *S. gesnerioides* resistance genes opens up the possibility of applying Marker-Assisted Selection (MAS) to cowpea and would fast track the process of developing resistance varieties to the parasite. In the present study, we report the use of Fast Technology for Analysis (FTA) also known as PlantSaver Cards (Whatman® FTA), developed by Flinder Technology associate to retrieve DNA from plant tissue for molecular analysis. A total of 100 F_2 individual plants derived from two crosses were validated for SG3 resistance using two different SCAR markers (MahSe2 and C42B) linked to *Striga* race 3 (SG3) and 5 (SG5) resistance in other segregating populations. Genomic DNA was successfully recovered from leaf tissues of cowpea pressed onto 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 SCAR MahSe2 and C42B in detecting SG3 resistance was 98.5% and 93% respectively. This result revealed the utility of SCAR markers in cowpea breeding programme. Therefore, the application of MAS using FTA technology has the potential to increase efficiency of selection and for molecular characterization of cowpea lines for Striga resistance..

Key words: Marker assisted selection, *S. gesnerioides*, race, FAT technology.

1. Introduction

Striga gesnerioides is a major biological constraint to cowpea production in sub-Saharan Africa. *S. gesnerioides* is difficult to control, and once established few means are available to counter its impact on yield. Yield losses raging from 83%-100% have been reported [1, 2]. As a result, development and deployment of resistance crop varieties remain the most effective manner to combat the menace presented by *S. gesnerioides*. Classical tools have been used in identifying resistant and susceptible genotypes. Often time this classification is affected by the environment and requires a long time to develop resistance variety. Therefore, effective method such as molecular markers is required to facilitate characterization of cowpea lines for resistance to *Striga*, and this would speed-up the process of crop delivery to farmers. Access to simple, low cost tools for the molecular study is central to generate the knowledge required.

A frequent limitation for studying DNA at molecular level is the ability to obtain high quality DNA from plant tissues. Plant tissues to be analyzed must be collected and preserved in order to maintain integrity of the DNA until they can be processed. This poses

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challenges when sample numbers are large and when working in the field, most especially in the tropical and sub-tropical regions where laboratory facilities are limited. Field and greenhouse studies are thus constrained by the resources required for sample collection and transportation, placing restrictions on the number of samples that can be collected in a given time and size. Timely processing or storage of the samples before they spoil can also be difficult in locations where access to well equipped laboratory facilities is limited. Intact high molecular weight plant DNA is essential for molecular studies and genomic DNA library construction. Previous traditional isolation methods required grinding plant tissue in liquid nitrogen and transferring it to a preheated extraction buffer [3, 4]. Liquid nitrogen can be difficult to procure in remote locations; thus, a method not requiring its use would be helpful.

The use of FTA® cards (Whatman®) provide a simple alternative method for collection, storage and retrieval of genomic DNA for molecular study especially when operating in developing countries and regions remote from laboratories facilities. The use of FTA® cards have been reported from a wide range of biological sources like whole blood, tissue, plasmid, plant materials, and microorganisms, etc [5, 6]. FTA treated matrix cards are impregnated with a proprietary mix of chemicals containing strong buffers, free radical trap and protein denaturants that lyses cell membranes on contact, physically entrap DNA, and stabilizes and protects DNA from nuclease, oxidation, UV damage and from microbial and fungal degradation [7, 8]. FTA® cards have also been used in the collection of DNA from bacterial cells for molecular analysis [9]. They have also been used in the collection of leaf tissue of a variety of plants, including soybean [10], tomato, tobacco and grapes [11], molecular genetic analysis of scleractinian corals in remote environmental locations [12], sampling, recovery and molecular characterization of viral pathogens and virus-derived transgenes from plant tissue [11]. It has also proven to be useful for large-scale plant DNA isolation for use in marker-assisted selection [13]

To-date limited successes have been recorded to its potential use in collection of cowpea leaf material for long time storage and retrieval of total genomic DNA. Therefore, we assessed the potential of the FTA® matrix card system as an effective technology for sampling and retrieval of genomic DNA from cowpea tissue and their subsequent molecular analysis using SCAR markers.

2. Materials and Methods

2.1 Plant Material

Cowpea (*Vigna unguiculata* (L.) Walp.) parental lines and the derived F_2 populations were grown under standard greenhouse condition in a pot culture inoculated with *Striga* seeds at IITA, Kano Station, Nigeria in 2008. Genomic DNA was extracted from leaf tissue of one-month old plants using the FTA® Plantsaver cards. The second young leaf was excised from the plant and placed in square of the FTA card. Leaf sample was covered with a parafilm paper and a pestle was used to press the leaf sample extracted onto the FTA® paper until both sides of the FTA were soaked. Paper towel soaked in 70% ethanol was used to clean pestle in between samples to prevent cross contamination.

2.2 Preparation of FTA Tissue Print

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

2.3 Preparation of Samples for PCR Analysis

A disc from the dried FTA tissue print was removed using a clean Haris® micro punch and placed the disc directly into a 1.5 mL Eppendorf tube. In between samples, the Haris® micro punch was cleaned with a tissue dampened with 70% ethanol and taking a disc from a blank, unused FTA® card to prevent cross contamination. The disc was washed twice with 200 µL of 70% ethanol, incubating for five minutes for each wash, followed by a repeated wash with 200 µL of FTA reagent incubating for 3 min at room temperature and the liquid was discarded. The tubes were inverted and drained on a paper towel and air dried for approximately 1 h. After drying the disc was transferred to PCR tube for PCR analysis.

2.4 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 mM each of DNTPs mix and 10 × PCR buffer, 0.05 µL of Taq polymerase, and 1 µL of each of the forward and reverse primers (synthesized by IDT, Coraville, Iowa). PCR reactions were performed on a heated lid thermal cycle (Biometra) using the thermal cycle (Biometra) operated as following conditions: 35 cycles of denaturation at 94 °C for 30 s, followed by annealing at 57.5 °C for 30 s and extension at 72 °C for 2 min. A final extension cycle of 10 min at 72 °C was added to ensure completion of the final amplification products. For C42B marker, a similar procedure was followed but the annealing temperature was adjusted to used was 67.5 °C.

2.5 Analysis of PCR Product

A 25 µL of the final PCR product was electrophoresed on a 3% agarose gel stained with ethidium bromide. The gels were run for approximately 1 hour 30 minutes at 120 volt in 1 X TAE buffer (45 mmol L^{-1} glacial acetic acid, 0.5 mmol L^{-1} EDTA, pH, 8.4). A 1 kb DNA ladder was loaded in the first well for band size determination of PCR products. The ethidium bromide-stained gel was visualized on an UV transilluminator and images photographed using a polaroid camera.

3. Results and Discussion

Genomic DNA was successfully isolated from leaf tissue of cowpea pressed onto FTA® classic cards. Plant DNA eluted from FTA® cards stored for over nine months at room temperature was found suitable for molecular analysis by PCR-based techniques in a manner equivalent to that offered by traditional isolation methods (liquid nitrogen). Two primers MahSe2 and C42B linked to *Striga* resistance 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 race-specific *Striga* (SG3) was detected from the population collected on FTA cards. Unique bands were produced by the two markers. MahSe2 amplified two fragments of similar size to the AFLP fragment observed in B301, one band was present in both the resistant and susceptible genotypes and a lower fragment that was polymorphic, being present in resistant genotypes but absent in susceptible genotypes. On the other hand, the primer C42B identifies resistant lines with a single band while susceptible line had no band (Figs. 1-4).

PCR amplification of DNA generated from cowpea DNA eluted from FTA cards was compared to that of conventional breeding method. FTA was found to be suitable for recovery of genomic DNA and for molecular characterization of the segregating population for resistance and susceptible to *S. gesnerioides*. The marker indicator was quite similar with the phenotypic classification. Results obtained from FTA® 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

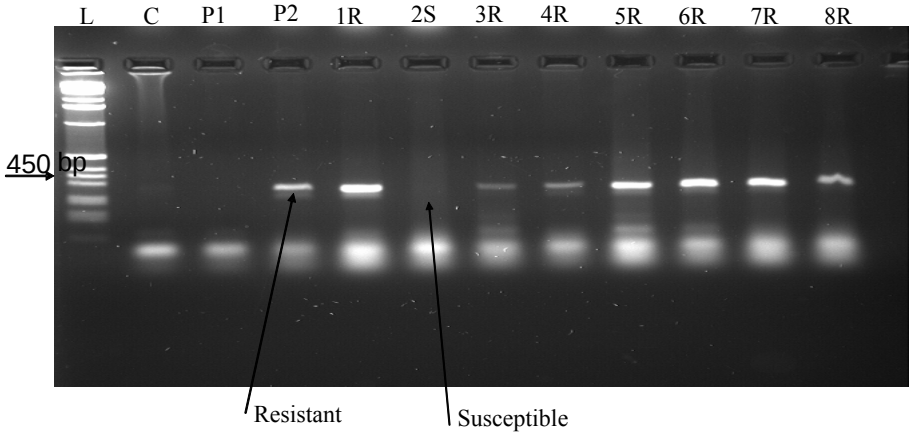

Fig. 1 Results of PCR amplification of genomic DNA by C42B marker for the F_2 progenies derived from Borno brown × IT03K-338-1. L = 1 kb ladder, C = control without Genomic DNA template. P1 = Borno brown, P2 = IT03K-338-1. R and S indicate resistant and susceptible, respectively.

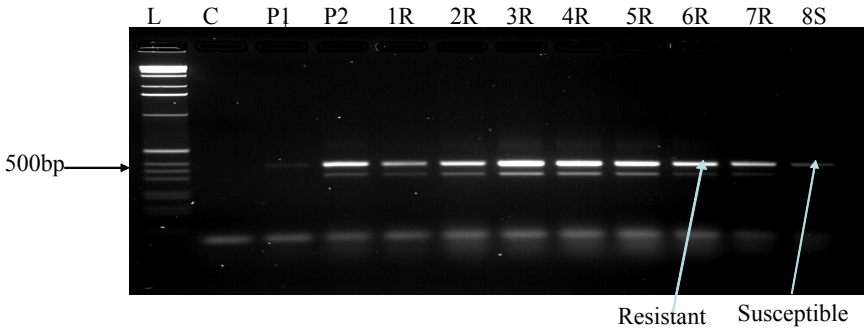

Fig. 2 Results of PCR amplification of genomic DNA by MahSe2 for the F_2 progenies derived from Borno brown × IT03K-338-1. L = 1 kb ladder, C = control without genomic DNA template. P1 = Borno brown, P2 = IT03K-338-1. R and S indicate resistant and susceptible, respectively.

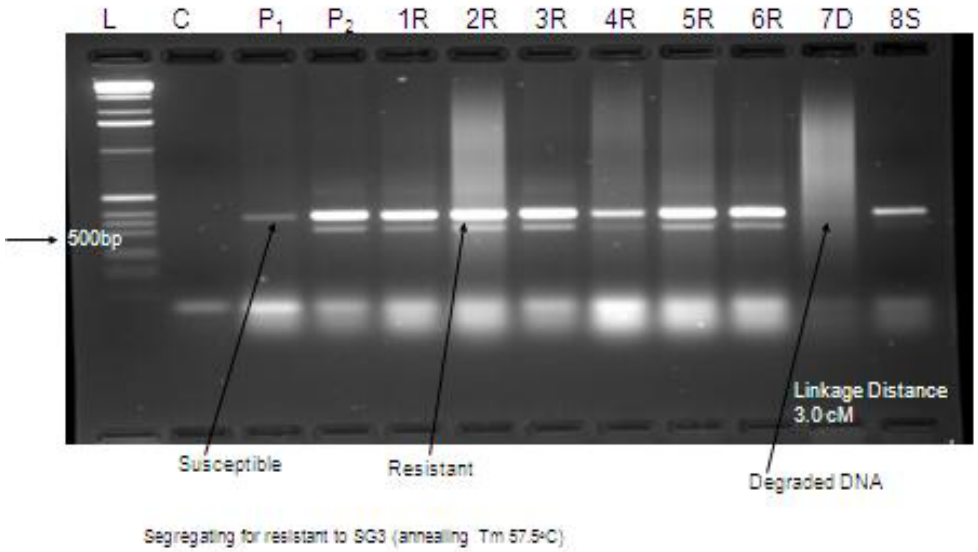

Fig. 3 Agarose gel electrophoretic analysis of PCR amplified product using MahSe2 for the F_2 progenies derived from Borno brown x IT97K-499-35. L = 1 kb ladder, C = control without genomic DNA template. P1 = Borno brown, P2 = IT97K-499-35, line 1-6 are resistant with double bands while line 8 with single band is susceptible. R and S indicate resistant and susceptible.

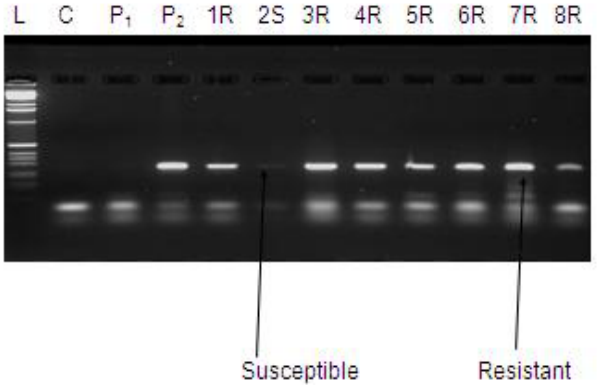

Fig. 4 Agarose gel electrophoretic analysis of PCR amplified product using C42B marker for the F_2 progenies derived from Borno brown x IT97K-499-35. L = 1 kb ladder, C = control without genomic DNA template. P1 = Borno brown, P2 = IT97K-499-35, line 1-6 are resistant with double bands while line 8 with single band is susceptible. R and S indicate resistant and susceptible.

genomic DNA isolated from cowpea leaf tissues. We believe that the application of this technology has the potential to significantly enhanced cowpea breeding program and efficiency of breeder to speed up the process of developing and deploying cowpea *Striga* resistance varieties to farmers. There was a positive significant correlation (0.864) between the two makers and similarity with that of the phenotypic classification (Table 1). This indicates that these markers are efficient in detecting resistance and susceptibility in segregating population and that the marker techniques are valuable.

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 on 3% agarose gel, indicating complete amplification of DNA samples, regardless of the method used. The AccuPower® PCR PreMix yielded the expected result in the F_3 populations tested with primer pair MahSe2.

The results obtained here indicate that it is possible to use this effective technology for sampling and retrieval of DNA from plant tissue and their subsequent molecular analysis for molecular characterization in cowpea for identifying resistance to *S. gesnerioides*. Similar results have been reported in other crop using this procedure for marker genotyping [15, 16]. 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 for *S. gesnerioides* resistance. The benefits of this technology have important implications for improving the efficiency of molecular characterization of cowpea genotypes for resistance to *Striga* in the laboratory especially when working in remote area and in developing countries where access to laboratory facilities, chemicals and equipment are limited. Results obtained from FTA® sampled materials were effective and reproducible in our hands from the three 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 plant tissues.

Table 1 Spearman rank correlation coefficient of marker similarity matrix.

		Phenotype
Borno brown x IT97K-499-35	MahSe2	0.8346 (< 0.0001)
	C42B	0.8382 (< 0.0001)
Borno brown x B301	MahSe2	0.8207 (< 0.0001)
	C42B	0.91437 (< 0.0001)
Borno brown x IT03k-338-1	MahSe2	0.8570 (< 0.0001)
	C42B	0.8847 (< 0.0001)

Correlation values above 0.8 are considered good association [14]. Values in parentheses are level of significance expressed as probability.

4. Conclusion

Results of this study demonstrated that FTA® is effective and sensitive method for sampling, storage and retrieval of genomic DNA from cowpea leaf tissues. The important advantage brought by FTA® technology is the ability to fix and reliably preserve DNA from plant tissue. Benefits of this technology are realized at both the sampling and processing phases. Sampling plant material with FTA® cards is reduced to sample and is thus rapid and uncomplicated. The

ability to store pressed and fixed samples for a long time at ambient temperatures also significantly reduces concerns regarding DNA degradation during storage. The potential number of samples that can be collected within a given time and location is significantly increased compared to traditional methods of using liquid nitrogen, and one no longer needs to depend on storage of rapidly frozen materials for DNA Isolation. Application of this technology has the potential to significantly increase the ability of breeders to bring modern analytical techniques to bear in an effort to introgress durable and stable resistance to *S. gesnerioides* into local germplasm and speed up the process of developing and deploying improved Striga resistant cowpea varieties to farmers.

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