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## INTRODUCTION

- In recent years, practical application of FTA® Classic Card technology has been demonstrated for the detection of viral pathogens and virus derived transgenes (Picard-Meyer, *et al.*, 2007; Ndunguru, *et al.*, 2005; Roy and Nassuth, 2005).
- In this study, we evaluated the usefulness of FTA® cards for the collection, shipment, storage and identification of a broad range of viruses in plants from different countries.
- FTA® Cards contain agents that cause cell lysis and denature proteins, and the nucleic acids are entrapped in the FTA® card matrix and protected from denaturation.

## APPROACH



- Plant samples suspected for viral infections were collected from cassava, chilli pepper, cucumber, tomato, yardlong bean and weed hosts in farmers' fields in India, Indonesia, Nigeria and Uzbekistan.
- Symptomatic plant tissue viz., leaf, stem, petiole and fruit were directly pressed gently on FTA® cards in the field, air dried and shipped to a central location (IAREC, Washington State University, Prosser, WA, USA) under USDA APHIS permit number P526P-07-06707.

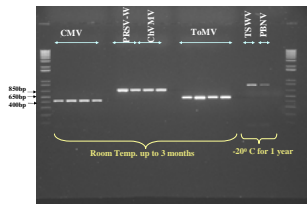
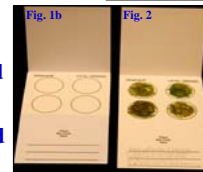
Crop	Country	Virus	Target
Chilli pepper	India	Chilli vein mottle virus (ChVMV)	Partial CI* protein
Snake gourd	India	Papaya ring spot virus-W (PRSV)	Partial CI* protein
Tomato & Chilli pepper	Nigeria	Tomato mosaic virus (ToMV)	Coat protein (CP)
Tomato	USA	Tomato spotted wilt virus (TSWV)	Portion of L RNA
Tomato	Uzbekistan	Impatiens necrotic spot virus (INSV)	Portion of L RNA
Tomato & Chilli pepper	India, Nigeria & Indonesia	Peanut bud necrosis virus (PBNV), Cucumber mosaic virus (CMV)	Portion of L RNA, Coat protein (CP)
Cassava	Nigeria	African cassava mosaic virus (ACMV)	Partial Rep (ACI)
Cassava	Nigeria	East African cassava mosaic (EACMV)	Partial Rep (ACI)
Cassava	Nigeria	East African cassava mosaic cameroon virus (EACMCV)	Partial Rep (ACI)

\*Cylindrical inclusions

## 3-STEP PROTOCOL

A simplified 3-step method described below was optimized for eluting the captured nucleic acids from FTA® Cards (Fig. 1a & b).

- Punch 4-5 discs (2 mm) from circles spotted with samples (Fig. 2) in the FTA® card using Harris Micro Punch (Fig. 3). Transfer discs into an eppendorf tube containing 300µl of Buffer-A (0.015M Na<sub>2</sub>CO<sub>3</sub>, 0.035M NaHCO<sub>3</sub>, pH9.6, 2% PVP-40, 0.2% bovine serum albumin and 0.05% Tween 20).
- Incubate at room temperature for 60min, vortex and transfer 2µl of eluate to 25µl of Buffer-B (0.1M glycine, pH 9.0, 50mM NaCl, 1mM EDTA, 0.5% Triton X-100 and 1% 2-mercaptoethanol).
- Denature at 95°C for 10min, snap cool in ice and take 4 to 5µl for virus detection using either PCR or RT-PCR, depending the type of virus genome. The eluate can be stored for virus detection at a later stage.



0.8% agarose gel showing virus-specific DNA fragments amplified by RT-PCR

FTA® cards spotted with samples can be stored at room temperature or -20°C and used for virus detection at a later stage.

## PCR and RT-PCR

Detection of plant viruses by PCR (Fig. 4) or RT-PCR (Fig. 5, 6, 7, 8 & 9) from total nucleic acids eluted from FTA® cards.

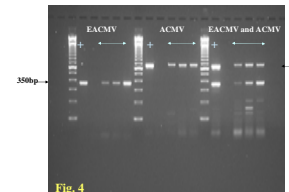


Fig. 4

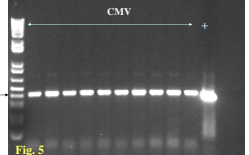


Fig. 5

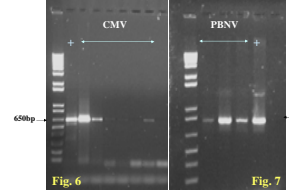


Fig. 6

Fig. 7

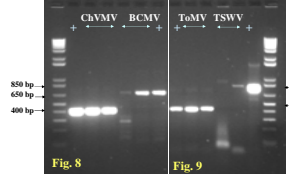


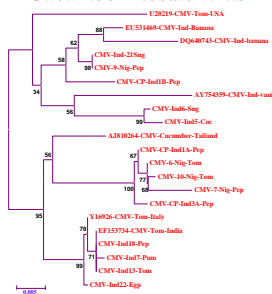
Fig. 8

Fig. 9

## DOWNSTREAM APPLICATIONS

- Virus detection in epidemiological studies
- Molecular analysis and diversity of viruses
- Gene expression and spatial distribution in plants

### A case study of Cucumber mosaic virus



Neighbor-joining phylogenetic tree of partial coat protein gene nucleotide sequence of CMV isolates from different countries

## CONCLUSIONS

- FTA® cards are useful in the diagnosis of a broad range of viruses.
- FTA® cards spotted with samples can be transported and stored at room temperature.
- The protocol for elution and virus detection is simple and rapid.
- Since viral nucleic acids bound to FTA® cards are inactivated, there is no risk of introducing alien pathogens.

## REFERENCES

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## ACKNOWLEDGEMENT

This research was supported by the Agriculture Office within the Bureau for Economic Growth, Agriculture, and Trade (EGAT) of the U.S. Agency for International Development, under the terms of the Integrated Pest Management Collaborative Research Support Program (IPM CRSP) (Award No. EPP-A-00-04-00016-00 to R.A. Naidu) and USAID-Linkage grant from IITA. The opinions expressed herein are those of the authors and do not necessarily reflect the views of the U.S. Agency for International Development.