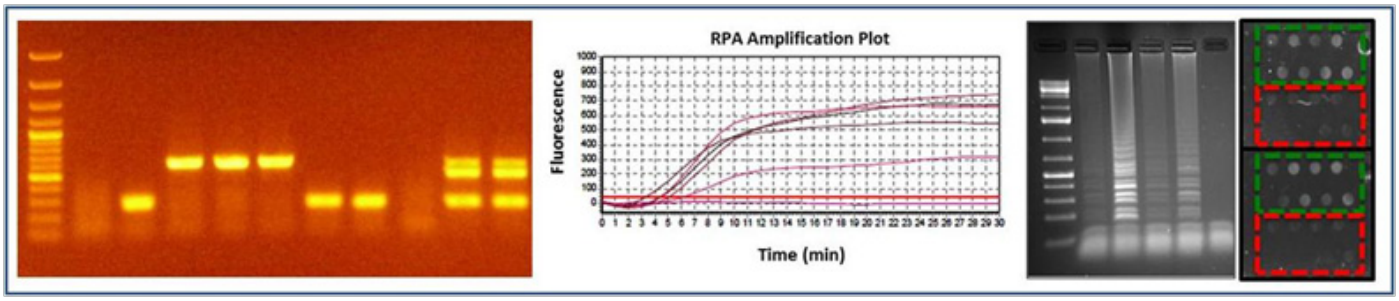
Sensitive and Robust Yam Virus Diagnostic Tools



Introduction

The major viruses infecting yam (*Dioscorea* spp.) in West Africa are *Yam mosaic virus* (YMV, genus *Potyvirus*), *Yam mild mosaic virus* (YMMV, genus *Potyvirus*), and *Cucumber mosaic virus* (genus, *Cucumovirus*). Several variants of yam infecting badnaviruses, generically termed Yam badnaviruses (YBVs), have also been recognized. YBVs genomes are known to be integrated in the host genome (endogenous pararetro viruses [EPRVs]). Simultaneous infection of yam with more than one virus is frequent in the field. YIIFSWA's objective is to establish robust and sensitive diagnostics tools for disease surveillance, virus indexing, and certification.

Mosaic is the common symptom caused by several different viruses infecting yam

Objectives

- Determine virus diversity to identify genome targets for diagnostics
- Understanding integrated yam badnaviruses and development of diagnostics for episomal viruses
- Optimize diagnostic protocols for disease surveillance and virus indexing

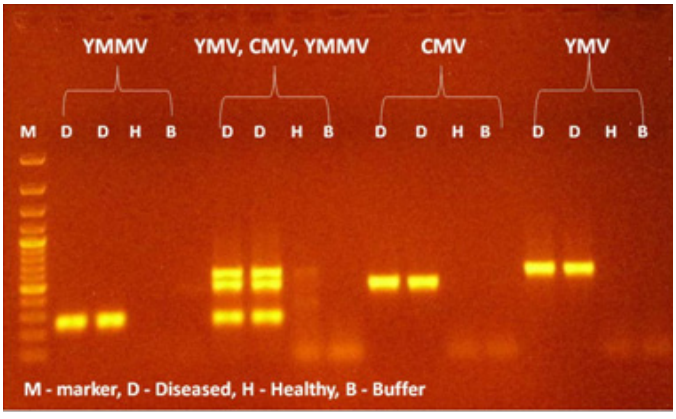
Validate diagnostic tests for virus indexing and certification of seed yams

Results

Samples from virus infected yams collected from ~ 90 locations in Nigeria and Ghana were used for characterizing the coat protein gene of YMV and YMMV; and RNaseH encoding gene of YBVs. Nucleotide sequence information was used to design new oligonucleotide primers for virus detection, which were used in combination with existing primers to develop various diagnostic tests.

Single tube, multiplex RT-PCR assay for simultaneous detection of three RNA viruses

This method was developed to detect the three most prevalent RNA viruses; YMV, YMMV, and CMV. This method has been well established and routinely used for virus indexing and selection of virus-free planting material. Presently, different YMV primer combinations are being tested to further improve the detection sensitivity.

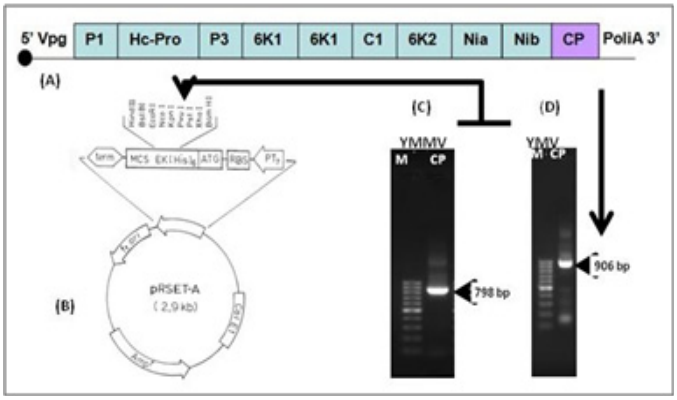


Multiplex RT-PCR products in agarose gel

Antibody-based diagnostic tools for the detection of YMV, YMMV, CMV, and YBV

Antibody-based diagnostic tests, such as ELISA or lateral flow device (LFD), offer simple and convenient choices for virus detection.

Polyclonal sera were produced against the recombinant coat protein peptides of YMV and YMMV expressed in *E. coli*. Tests revealed suitability of this serum for virus detection in Western immuno assay (denature proteins) but not in ELISA. Efforts are continuing to purify native YMV to generate polyclonal antiserum.

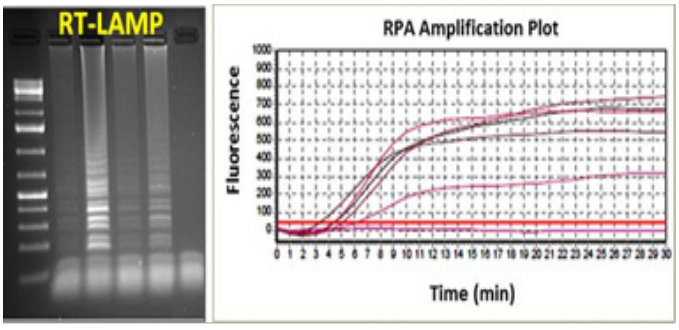


Cloning YMV and YMMV coat protein genes for recombinant peptides. (A) Potyvirus genome organization; (B) expression vector; and coat proteins of YMV (C) and YMMV (D).

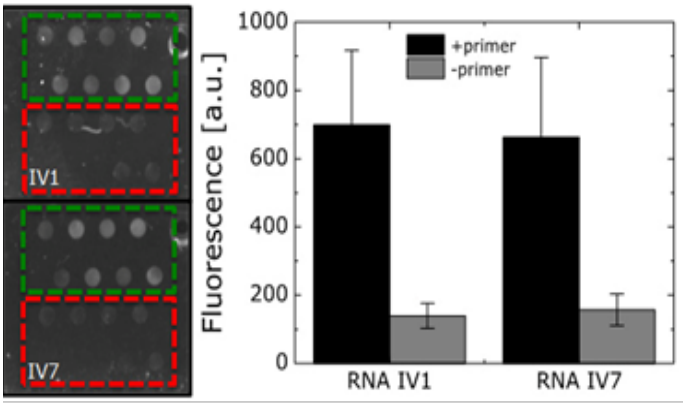
Conserved amino acid domains in YBV coat protein were identified and synthetic peptides were generated for antibody production. These antibodies recognized peptides but not virus particles. Further work is in progress to generate effective antibodies for YBV detection by immuno-capture PCR (IC-PCR). Antibodies for CMV produced previously will be used for tool development.

Diagnostic tools for field detection

Efforts have also been initiated to establish simple, sensitive, and robust diagnostics for quick detection of viruses in the field or on-site. For this, LFD-based tools (antibody-dependent) and isothermal amplification methods such as Recombinase Polymerase Amplification (RPA) are used. Both methods can be performed without a thermal cycler and the reactions can be optimized for field tests. In addition they can be tailored to "chip diagnostics". Early results of chip-based diagnostics for YMV and YMMV detection are promising.



RT-LAMP and RPA assays for YMV detection



Chip diagnostics for YMV and YMMV detection

Diagnostics for yam badnaviruses

Developing a test that can selectively identify episomal YBV infection. IC-PCR is the method of choice, but due to scarcity of antibodies, direct binding PCR (DB-PCR) is being used. DB-PCR, although less robust, is proving to be a reasonable alternative for episomal YBV detection.

Training in application of multiplex RT-PCR

Two training courses were organized for national program partners in application of multiplex RT-PCR for seed yam health testing.

Conclusions

A variety of diagnostic tools are being established for yam virus detection in the lab and field. Multiplex RT-PCR, widely used in YIIFSWA, reduces costs and efforts three fold. Other tools are at various stages of development. Future work will place greater emphasis on simple and robust tools for on-site application, including in fields.

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