Novel Diagnostic Techniques for Early and Accurate Detection of Plant Pests and Ghana's experience with plant virus diagnostics

By

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Introduction

Plant pests and diseases pose a major threat to the agricultural industry.

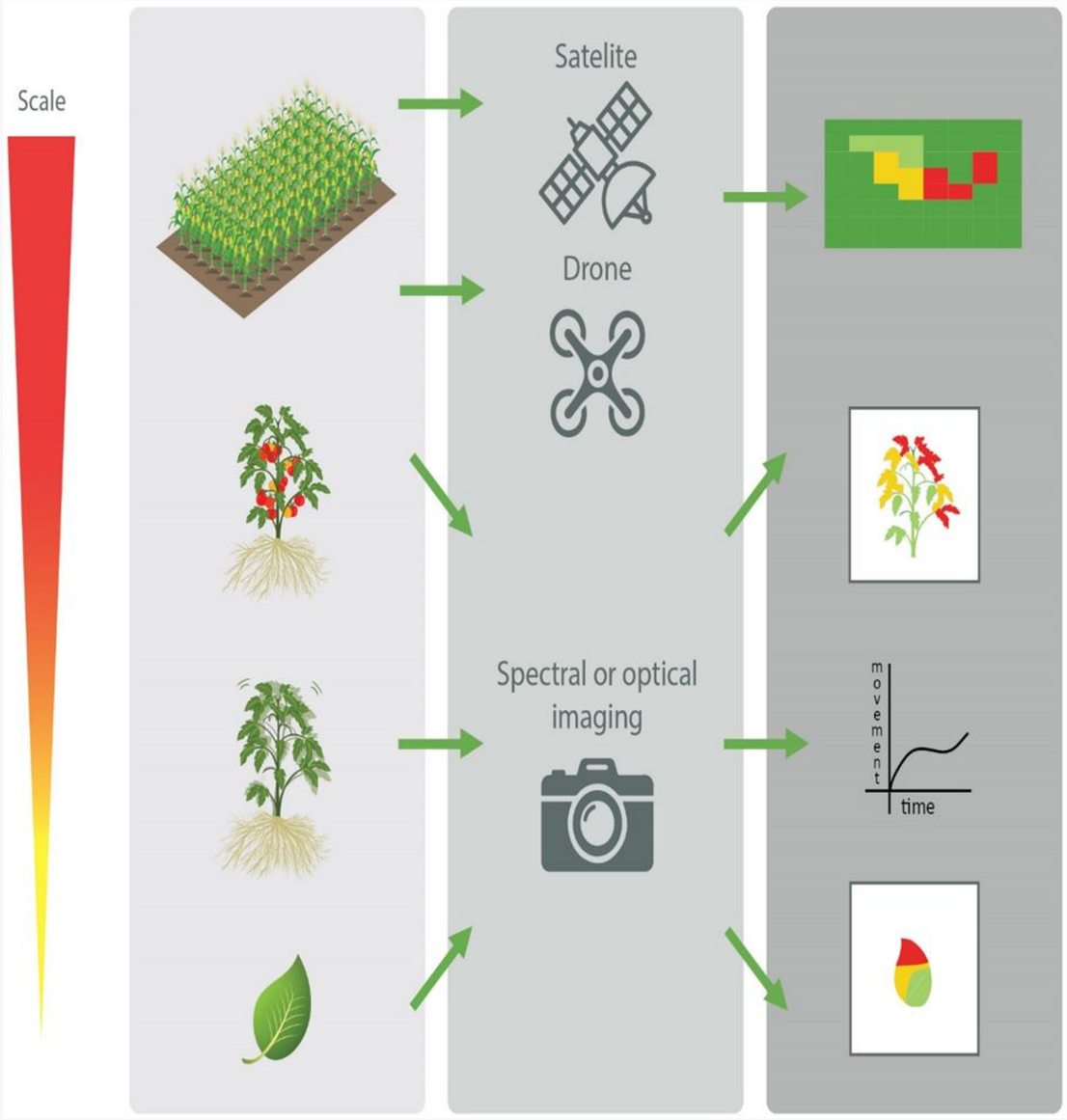
- ✓ Up to 40% of the yield of economically important crops is lost each year due to plant pathogens and pests

Early plant disease detection is important in agriculture to:

- ➢ minimize crop losses
- ➢ prevents the spread of diseases
- ➢ enables effective management strategies to be implemented.

Non-invasive optical and spectral detection methods

- ✓ Upon biotic stresses, plants respond with changes in e.g., chlorophyl content, thermal radiation, and also show subtle plant movement changes.
- ✓ Different advanced spectral methods are currently available to measure such changes in electromagnetic radiation emitted or reflected by the plants
- ✓ With the advent of digitalization, the use of imaging, and optical or spectral techniques in plant disease detection has seen a steady rise.



(Adapted from Singh et al., 2021)

Cultivation-based methods

The method relies on the cultivation and isolation of microorganisms on a:

- ✓ selective or
- ✓ semi-selective growth medium

which allows the growth of the target pathogen, while inhibiting (or reducing) the growth of background microflora

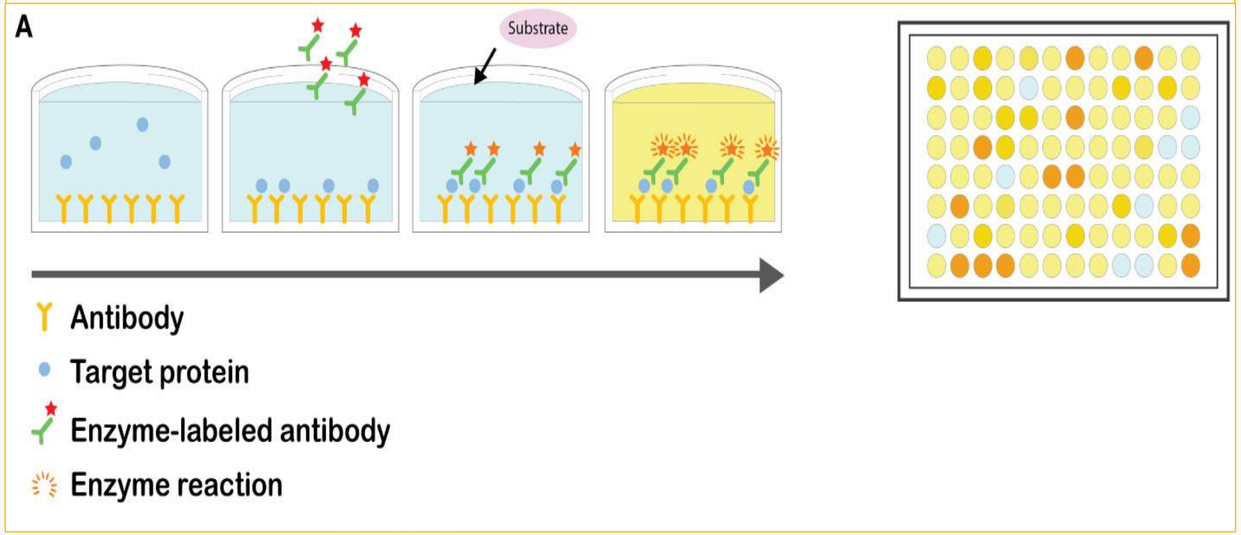
- ➢ Subsequently, the identity of the isolates that grow on the (semi-)selective growth medium needs to be confirmed by
- ✓ morphological
- ✓ microscopical
- ✓ biochemical
- ✓ molecular
- ✓ immunological assays

Immunological methods

Enzyme-Linked Immunosorbent Assay (ELISA):

ELISA uses antibodies to detect pest-specific proteins or antigens in plant samples. It is particularly useful for identifying pest infestations that produce distinctive proteins.

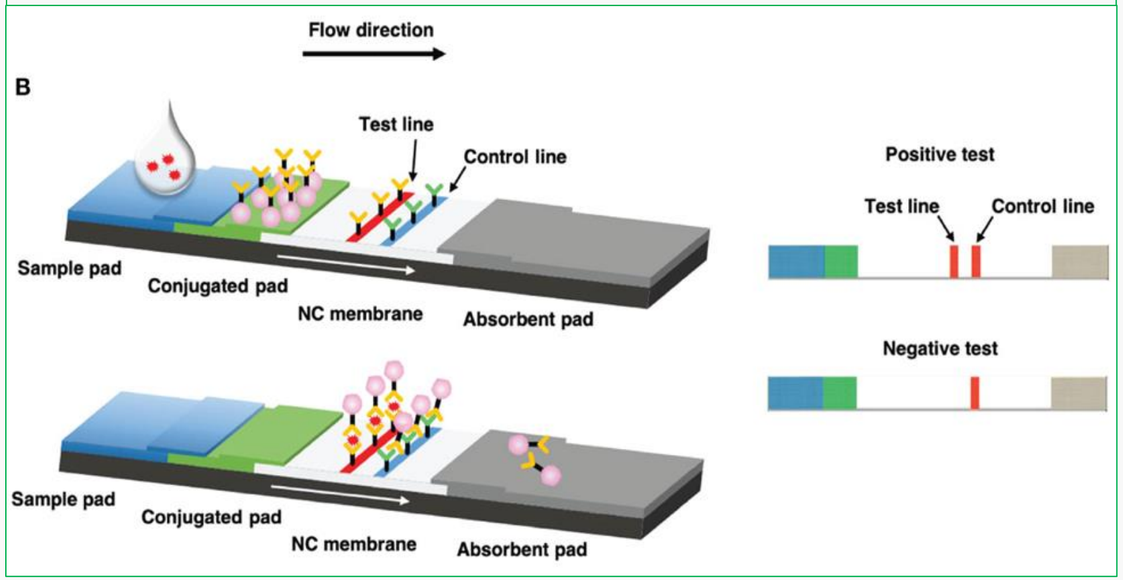
ELISA formats: direct, indirect, sandwich, and competitive ELISA assays



Lateral flow immunoassays (LFIA)

Consist of nitrocellulose membrane strips contained in a plastic receptacle.

The sample is applied on the sample application area and passes through the (primary) antibody conjugate release pad by capillary forces, allowing the antibodies to bind to the target antigen in the sample.



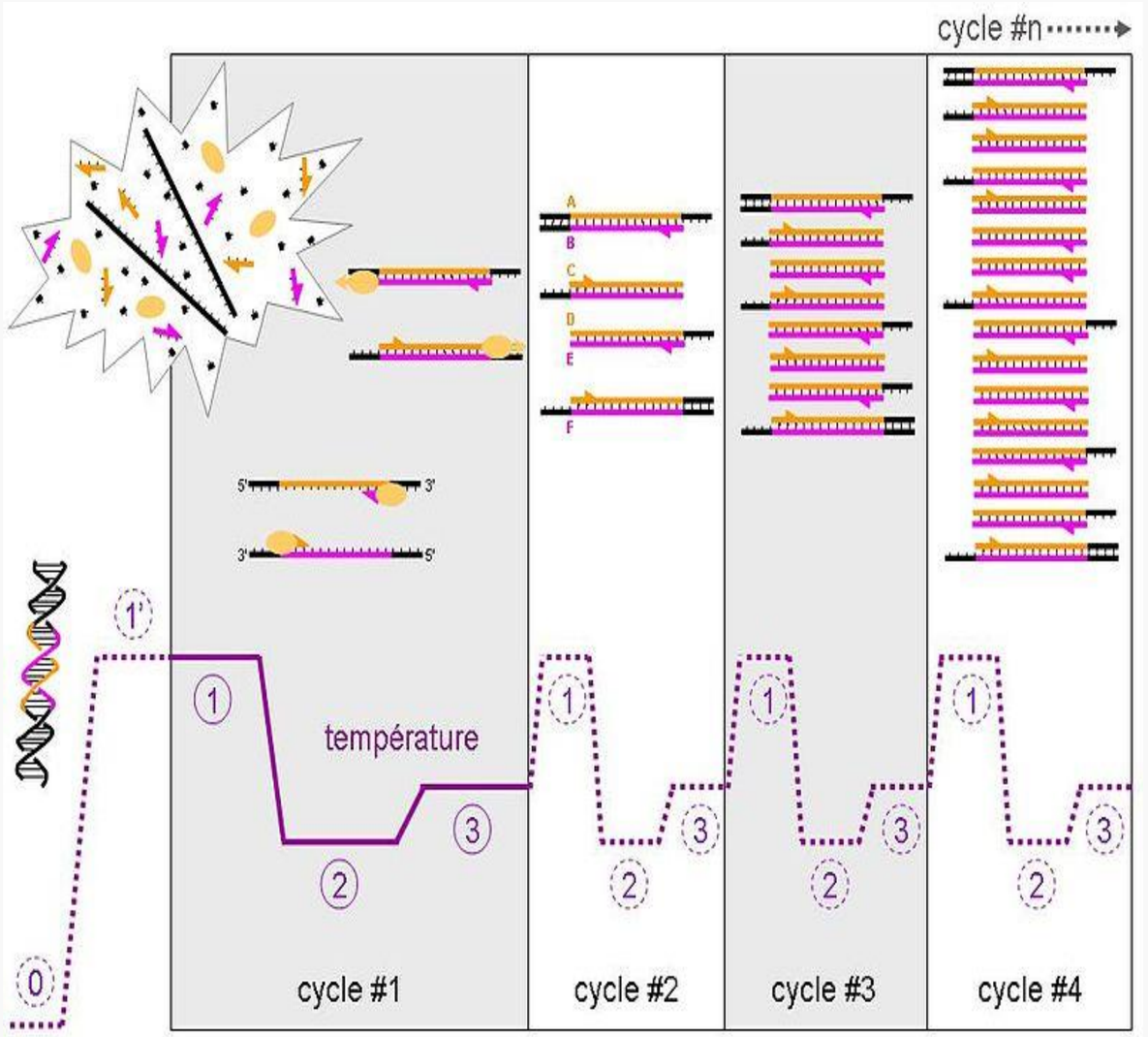
Nucleic acid-based assays

- Nucleic acid (DNA or RNA) sequences make excellent molecular targets for the detection and identification of (pathogenic) microorganisms.

Conventional PCR and variants

- ✓ Polymerase chain reaction (PCR) is a technique used to amplify specific DNA fragments, making use of oligonucleotide primers, a DNA polymerase enzyme, dNTPs and a thermal cycler.

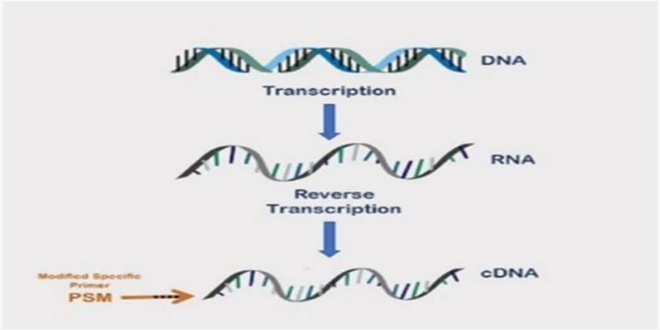
- ✓ Detection of a PCR fragment with the expected size is used to confirm the presence of the target pathogen



Ygonaar (2006)

Variants of PCR

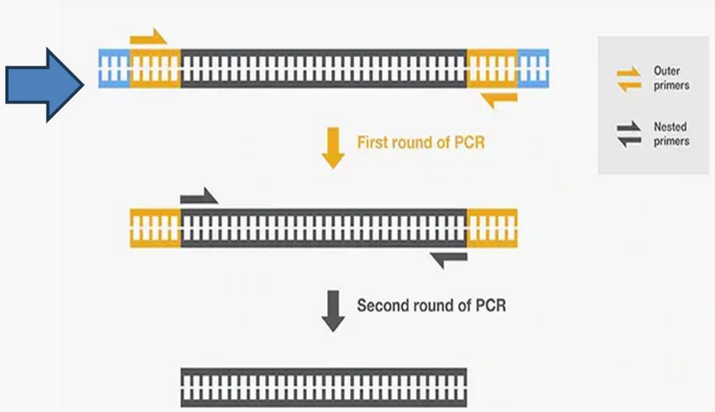
A. Reverse transcriptase PCR (RT-PCR) - used on RNA targets, which is useful for the detection of viable cells and RNA viruses



(Đermić et al. 2023)

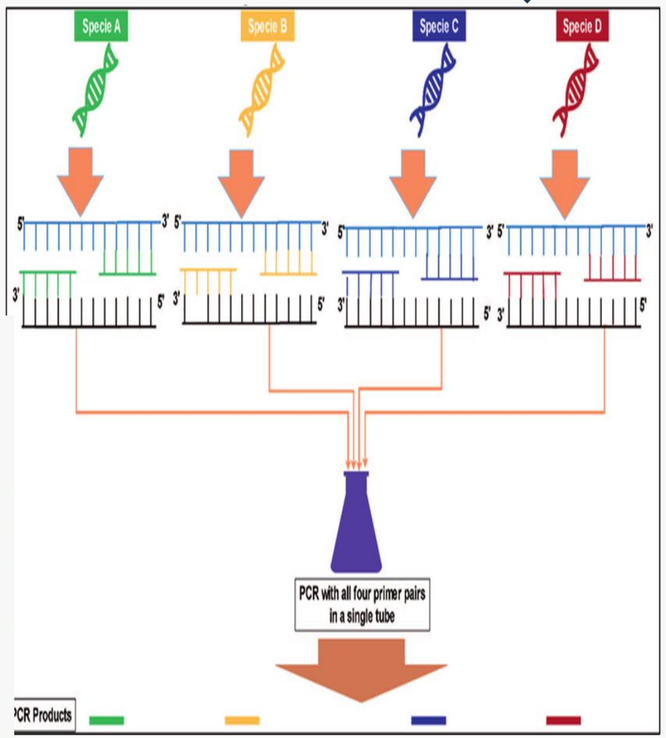
B. Nested PCR (nPCR), which relies on two successive amplification rounds.

- ✓ The first round uses a set of (outer) primers that amplify a larger region of the target DNA.
- ✓ The PCR product of the first round is used as a template in the second amplification round, using primers that anneal to a sequence internal to the sequence amplified by the first primer set (Shen, 2019)



Microbe online, 2024

C. Multiplex PCR utilizes two or more primers sets that are designed to target different genetic sequences within the same PCR reaction.



(Aklilu., 2022)

Quantitative PCR

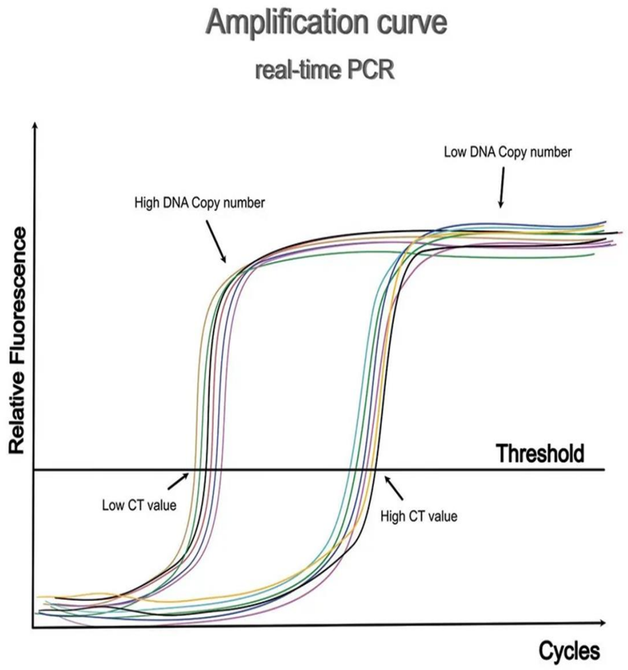
- ✓ Quantitative PCR (qPCR), also referred to as real-time PCR, operates on the same working principle as conventional PCR
- ✓ The increased sensitivity makes it a valuable tool for early detection of pathogens, even before disease symptoms are visible

➢ Intercalating dye (SYBR Green)

- ✓ Bind to DNA non-specifically
- ✓ Emit detectable fluorescence following intercalation with newly synthesized DNA

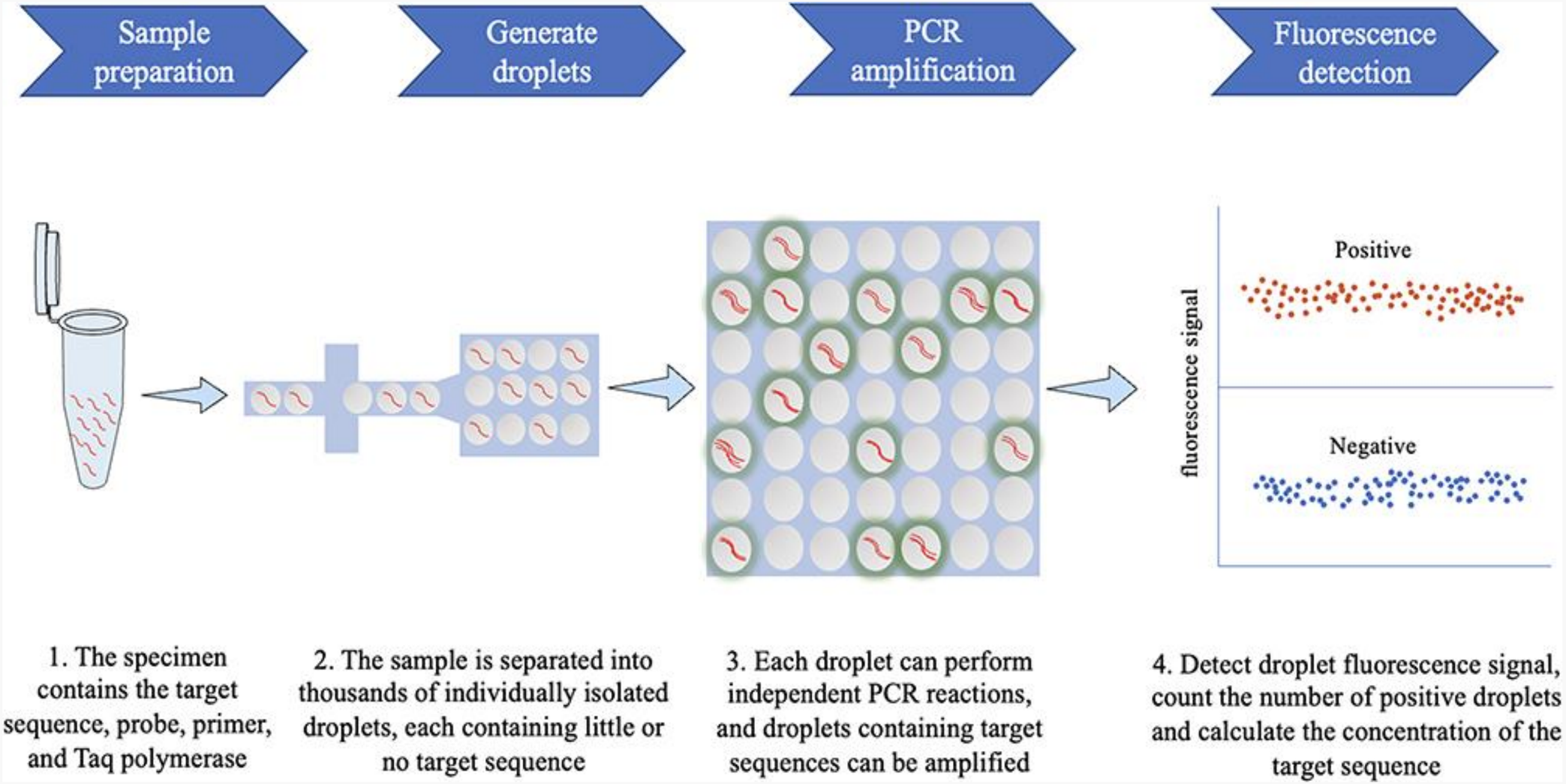
➢ Hydrolysis probes (TaqMan™ probe)

- ✓ The probes are highly specific



(Biswas, 2024)

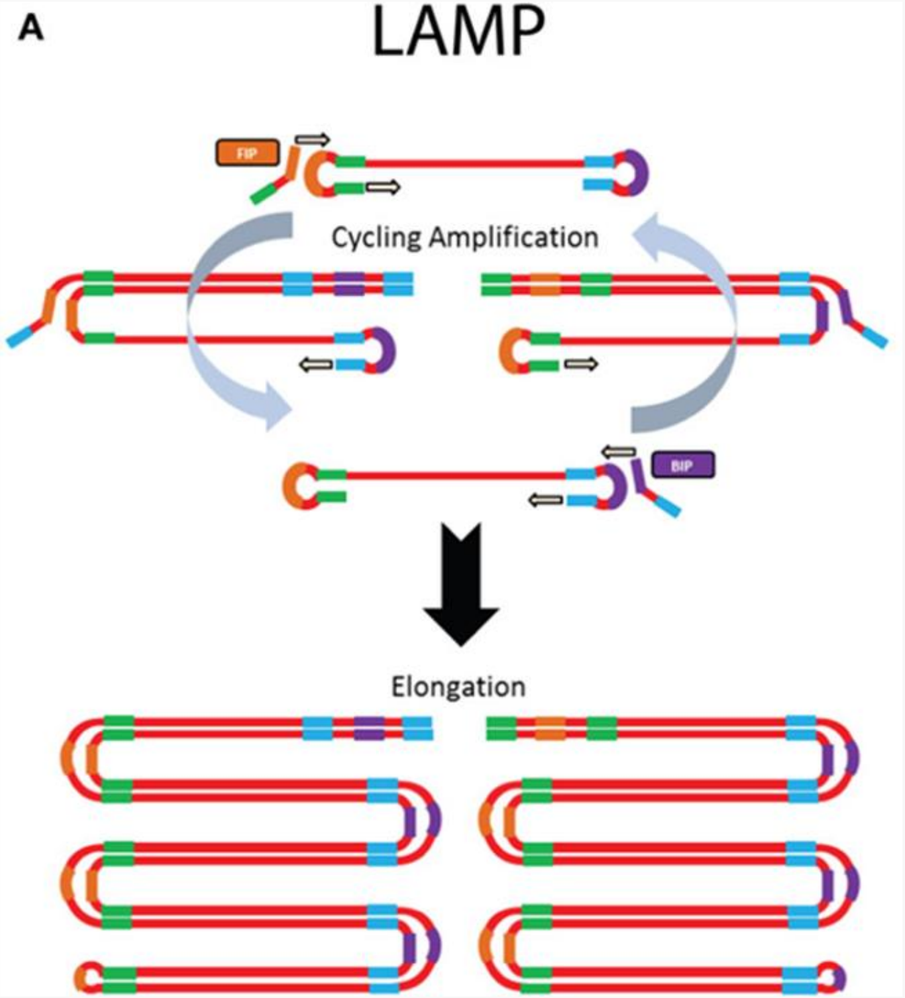
Digital droplet PCR



Fan et al., 2022

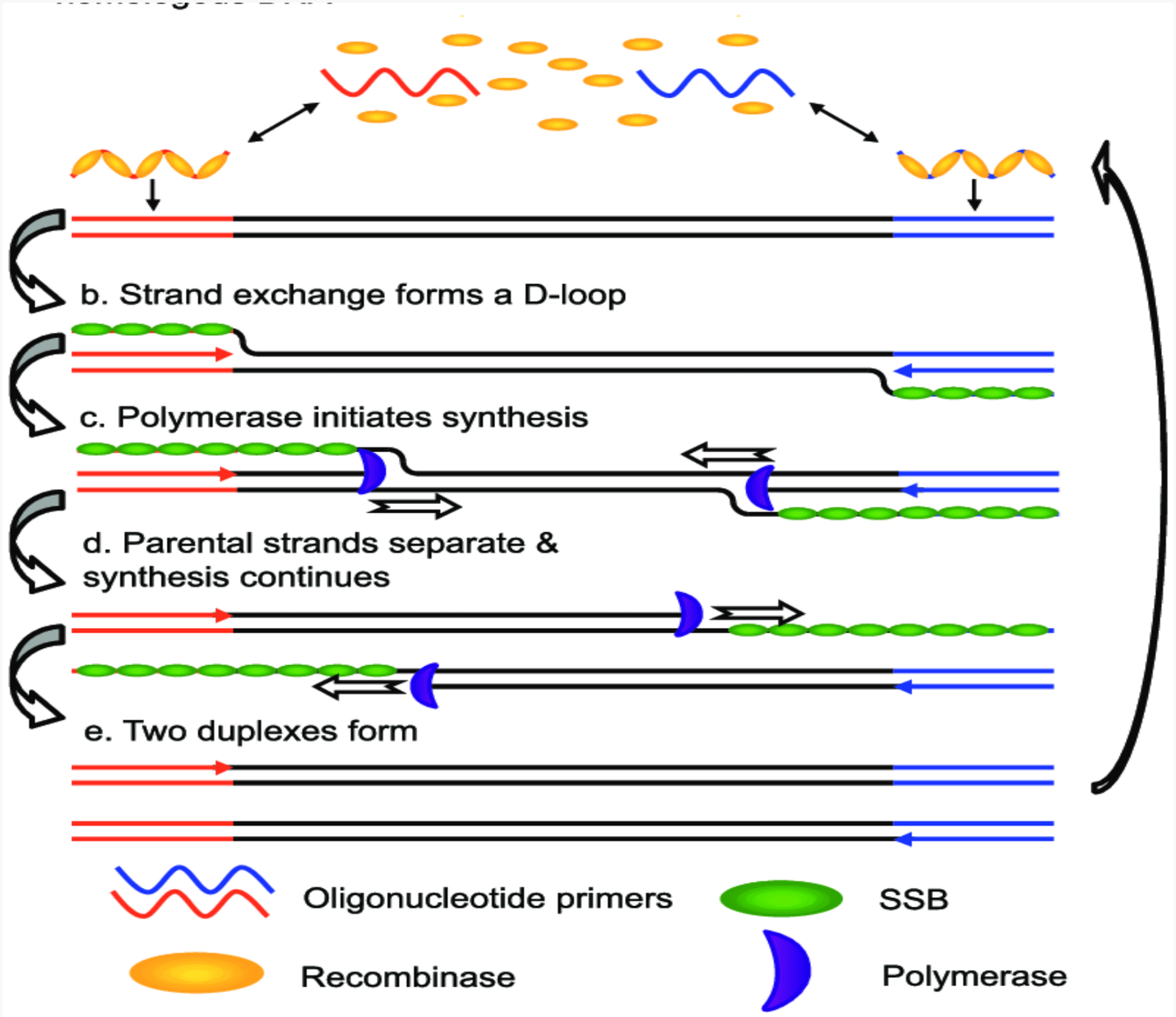
Loop-mediated isothermal amplification

- ✓ LAMP is a nucleic acid amplification technique with a simple workflow that is used to detect DNA or RNA inputs.
- ✓ The technique relies on the utilization of at least four primers, two inner and two outer primers, in combination with a strand-displacing DNA polymerase to allow rapid amplification of a specific sequence at one temperature.
- ✓ It is an ideal technique for field, point-of-care, or low-resource settings because sophisticated molecular diagnostic equipment is not required.



Recombinase polymerase amplification

The three core proteins, recombinase, single-strand DNA binding protein (SSB) and strand-displacing polymerase enable PCR-like DNA amplification without the need for thermal cycling or an initial chemical or thermal melting step.



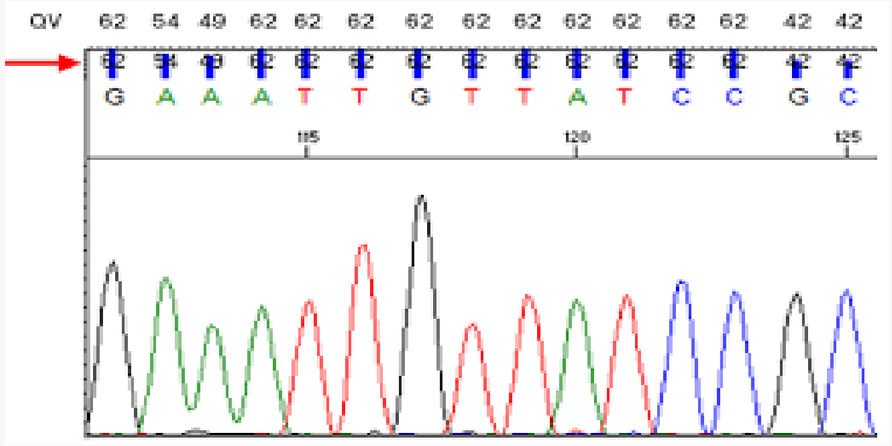
Boyle,et al. 2014

Nucleic acid sequencing methods

Sequencing specific genetic markers and comparing the resulting sequence(s) to a reference sequence, enables the identification of a pathogens.

Amplicon sequencing

- ✓ Involves sequencing of an amplified gene
- ✓ The identity of the organisms is determined by comparing the DNA sequence of the amplified marker genes with a suitable reference database.



A chromatogram showing DNA sequence from Sanger sequencing

(Azenta Life Sciences)

Next Generation Sequencing/High Throughput Sequencing

The basic next-generation sequencing process includes fragmenting DNA/RNA into multiple pieces, adding adapters, sequencing the libraries, and reassembling them to form a genomic sequence.

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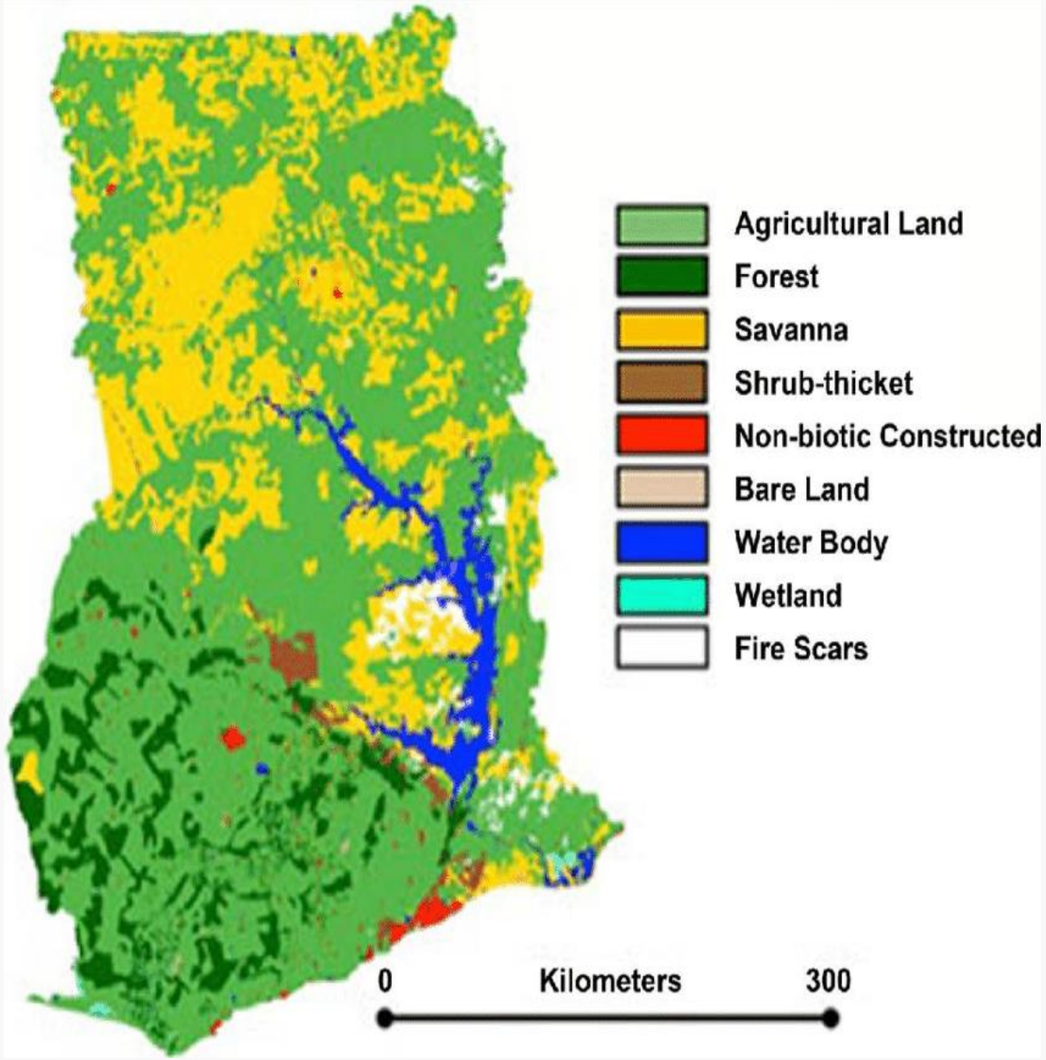
Ghana’s experience with plant virus’s diagnostics

- ✓ Ghana, country of western Africa, situated on the coast of the Gulf of Guinea.

Capital: Accra
Population: (2024 est.) 32,823,000
Official Language: English

- ✓ Agricultural is the backbone of the Ghanaian economy. It is Ghana's most important economic sector, employing more than half of the population on a formal and informal basis.

- ✓ The economy is a mixture of private and public enterprise. About three-fifths of the GDP is derived from the services sector, agriculture contributes almost one-fifth, and industry about one-fourth.



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BNARI was established in 1993

Research and Technology transfer institute of the Ghana Atomic Energy Commission (GAEC

Seven (7) Research Centres

- ✓ **Plant Disease Research Centre (BTC)**
- ✓ Biotechnology Centre (BTC**)**
- ✓ Radiation Technology Centre (RTC)
- ✓ Nuclear Agriculture Research Centre (NARC)
- ✓ Soil and Environmental Sciences Research Centre (SESRC)
- ✓ Radiation Entomology and Pest Management Centre (REPMC)
- ✓ Socio-economic and Commercialization Centre (SECC)

- The plant virus situation in Ghana has raised concerns, particularly those affecting staple crops like cassava, yams, cowpea, tomato okra and maize.

- Efforts to combat plant viral diseases include the development of resistant crop varieties, improved vector management strategies, and farmer education programs.

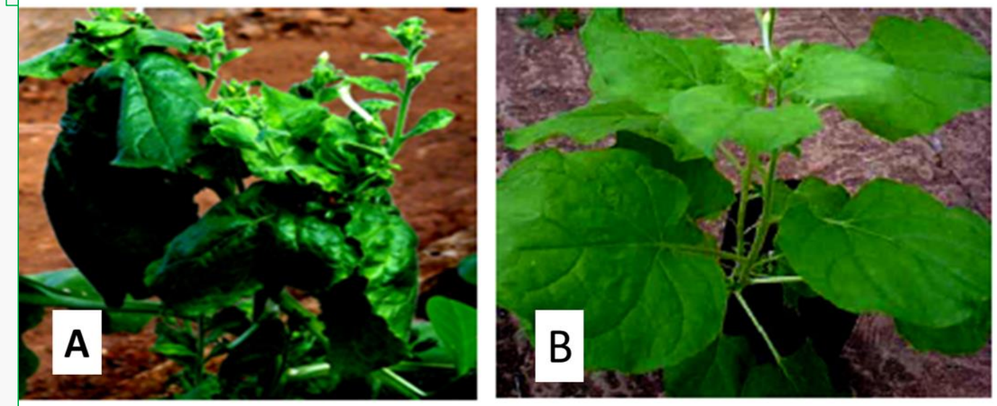
- Collaboration between government, research institutions, and international organizations is crucial for addressing these challenges effectively.

In Ghana, plant virus diagnostics uses a range techniques:

- ✓ Biological
- ✓ Serological
- ✓ Molecular

Spread of African cassava mosaic virus from cassava (*Manihot esculenta* Crantz) to physic nut (*Jatropha curcas* L.) in Ghana
Journal of Phytology 2012, 4(1): 31-37

- Biological (mechanical sap inoculation)
- Serological (ELISA)

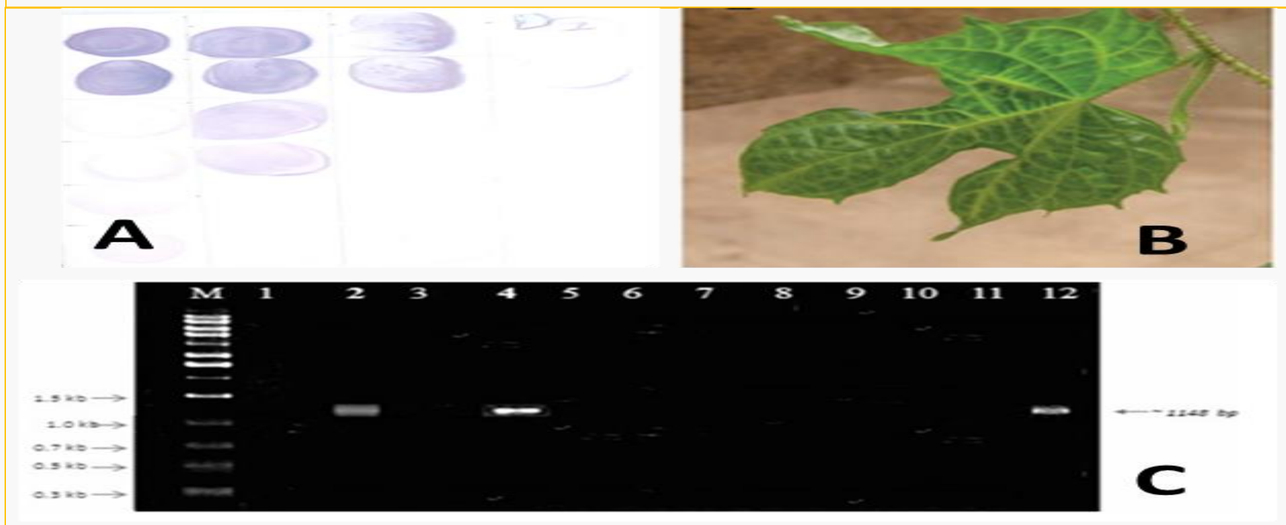


A. Symptomatic *Nicotiana benthamiana* B: Healthy uninoculated N. benthamiana.

Incidence of sweet potato viruses in the coastal savannah agro-ecological zone of Ghana
Journal of Plant Pathology (2015), 97 (1), 109-117

- Nitrocellulose membrane ELISA (NCM-ELISA)
- Grafting
- PCR

Viruses detected: (SPFMV, 85%), (SPMSV, 55%), (SPCV, 45%), (SPCFV, 30%), (SPVG, 20%), (SPMMV, 5%), (SPCSV, 1.67%) (CMV, 1.67%). PCR detected SPLCV



A — NCM ELISA, **B** — Graft inoculation, **C** - PCR

Varietal Response to Groundnut Rosette Disease and the First Report of Groundnut ringspot virus in Ghana

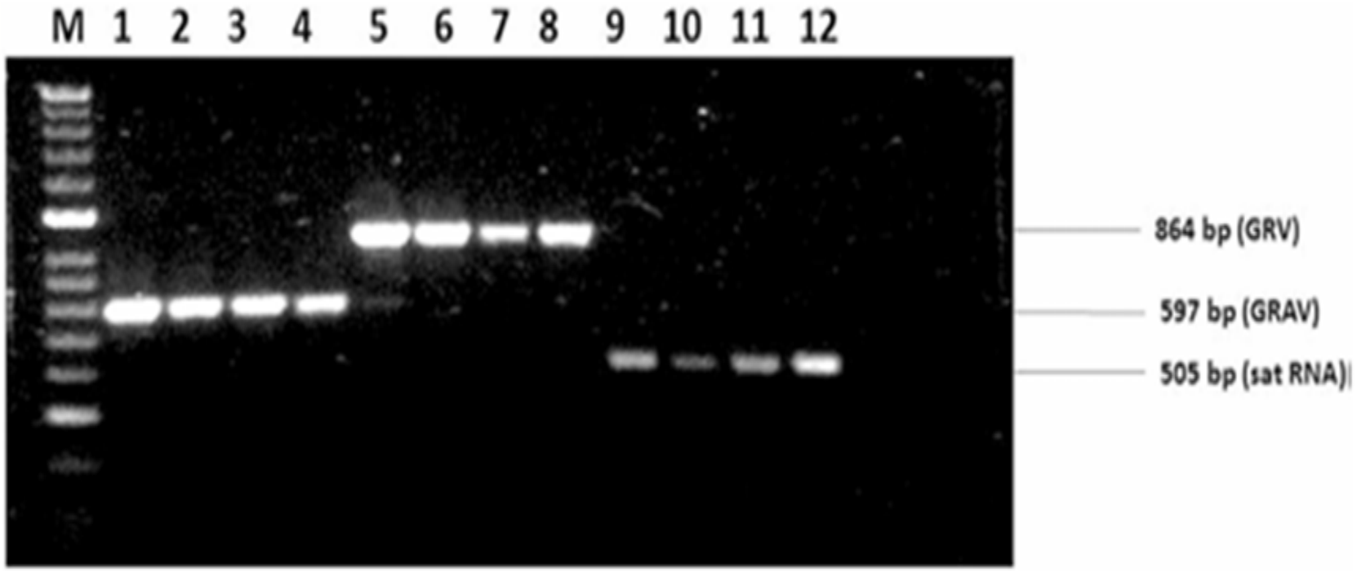
- Serological (ELISA)
- PCR
- Amplicon sequencing

Detection of TYLCV in Ten Genotypes of Tomato (*Solanum* spp L.) using Serological and Molecular Techniques in a Coastal Savanna Zone of Ghana.
Journal of Natural Sciences Research. 5. 17-24.

- ELISA and PCR

Assessing sequence diversity of Groundnut rosette disease agents and the distribution of Groundnut rosette assistor virus in major groundnut-producing regions of Ghana
Trop. plant pathol. (2017) 42:109–120

- Nitrocellulose membrane ELISA (NCM-ELISA)
- PCR
- Amplicon sequencing



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First report of Pineapple mealybug wilt associated virus-2 infecting pineapple in Ghana
New Disease Reports (2020) 41, 9

- RT-PCR
- Amplicon sequencing

Next generation sequencing elucidates cacao badnavirus diversity and reveals the existence of more than ten viral species.
Virus Res. 2018, 244, 235–251

- High-Throughput Sequencing (HTS)

Disease reaction of cacao progenies following inoculation with the cacao swollen shoot Togo B virus (CSSTBV) under field conditions.
Trop. Plant Pathol. **2023**, 48, 703–712.

- ELISA and PCR

Inconsistent PCR detection of Cacao swollen shoot virus (CSSV) is linked to the occurrence of different variants across the cocoa regions of Ghana.
J. Virol. Methods **2021**, *300*, 114400.

- PCR

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First report of Pineapple mealybug wilt associated virus-2 infecting pineapple in Ghana
New Disease Reports (2020) 41, 9

- RT-PCR
- Amplicon sequencing

Other viruses

Pepper viruses:

Pepper veinal mottle virus (PVMV), Tobacco mosaic virus (TMV), Cucumber mosaic virus (CMV) and Pepper mild motle virus (PMMV)

✓ All detected by ELISA

Complete genome sequencing of two causative viruses of cassava mosaic disease in Ghana
Acta virologica 56: 305 – 314, 2012

- PCR
- Sanger Sequencing

Okra Viruses:

Okra mosaic virus (OkMV)
Okra yellow vein mosaic virus (OYVMV)
Detected by ELISA

Okra leaf curl virus (OLCV)

✓ *Detected by PCR*

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Ongoing Activities at the PDRC:

- Nationwide surveillance for Banana bunchy top virus disease
  - Virus detection method: Conventional PCR
  - Collaboration between BNARI, PPRSD and CABI, UK.

- Surveillance for Cassava mosaic geminiviruses in five cassava-growing regions in Ghana
  - Virus detection method: Conventional PCR and Next generation sequencing technique
  - Collaboration between BNARI and the IAEA

Some equipment at the PDRC

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IPPC
International Plant Protection Convention