

Diagnosis of viral diseases of banana and plantain

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The importance of efficient diagnosis for the detection of viruses

- Accurate and sensitive diagnosis is essential for controlling diseases
- Only certified virus-free germplasm can be exchanged
- Efficient detection methods are essential for the production of certified and safe *Musa* germplasm.
- Such methods have been set up and optimized for the detection of BSV and BanMMV
- Similarly efficient methods exist for the detection of other viruses infecting *Musa*.

Detection of viruses infecting *Musa* spp

Virus	Detection method
BBTV	ELISA, PCR
CMV	ELISA, RT-PCR
▶ BSV	IC-PCR
BBrMV	ELISA, RT-PCR
▶ BanMMV	IC-RT-nested PCR
▶ BVX	DB-RT-nested PCR

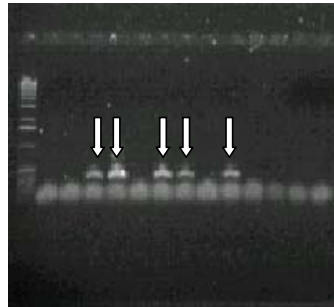
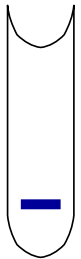
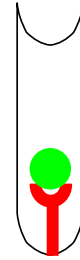
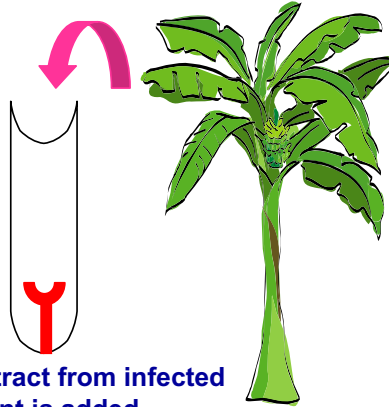
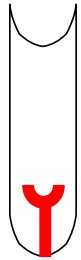
Detection of viruses infecting *Musa* spp

- ELISA : BBTV, CMV, BBrMV



- PCR : BBTV
- RT-PCR : CMV, BBrMV
- IC-PCR : BSV
- M-IC-RT-nested PCR : BanMMV
- M-DB PCR : BVX

Immunocapture – PCR (IC-PCR) : how does it work?



1

Microtubes are coated with specific antibodies

2

Extract from infected plant is added

3

Viral particles are trapped by the antibodies

4

Heat treatment denatures viral particles : genomic DNA becomes accessible for PCR

5

PCR products are analysed by electrophoresis

Detection of BSV by multiplex immunocapture PCR (M-IC-PCR)

- polyclonal antiserum for immunocapture (AGDIA)
- species-specific primers (BSV-OI, BSV-Gf, BSV-Im, BSV-Mys, BSV-Cav, BSV-Vn) or degenerate primers for PCR
- optimized protocol for avoiding false positives due to integrated seque



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Improved detection of episomal *Banana streak viruses* by
multiplex immunocapture PCR

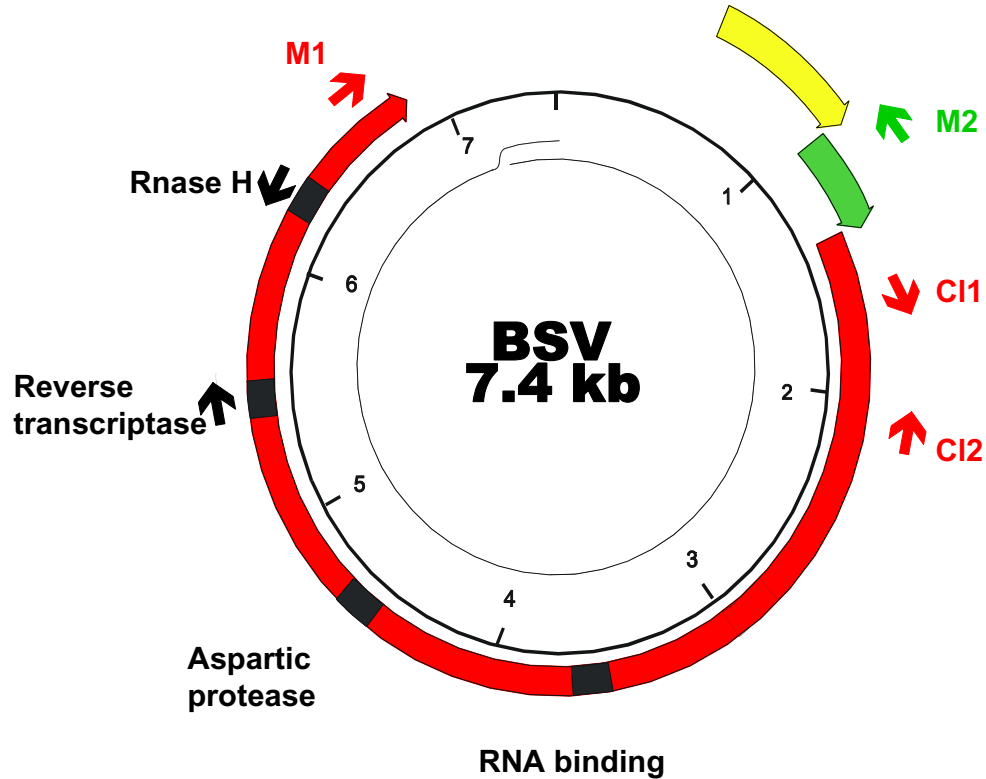
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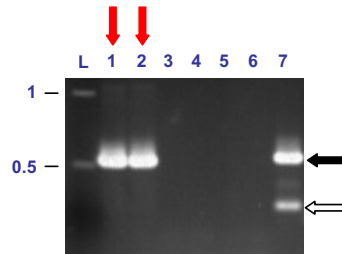
^b CIRAD, UPR75, Station de Neufchâteau, F-97130 Capesterre Belle-Eau, Guadeloupe



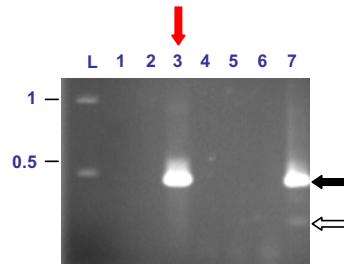
Detection of BSV by M-IC-PCR choice of primers



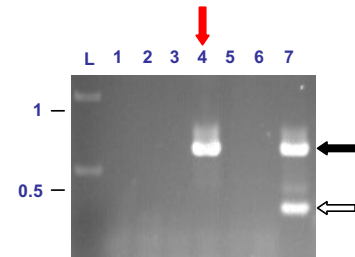
Detection of BSV by M-IC-PCR



BSOLV



BSGFV



BSMysV

Detection of BanMMV by multiplex immunocapture reverse transcription nested PCR (M-IC-RT-nested PCR)

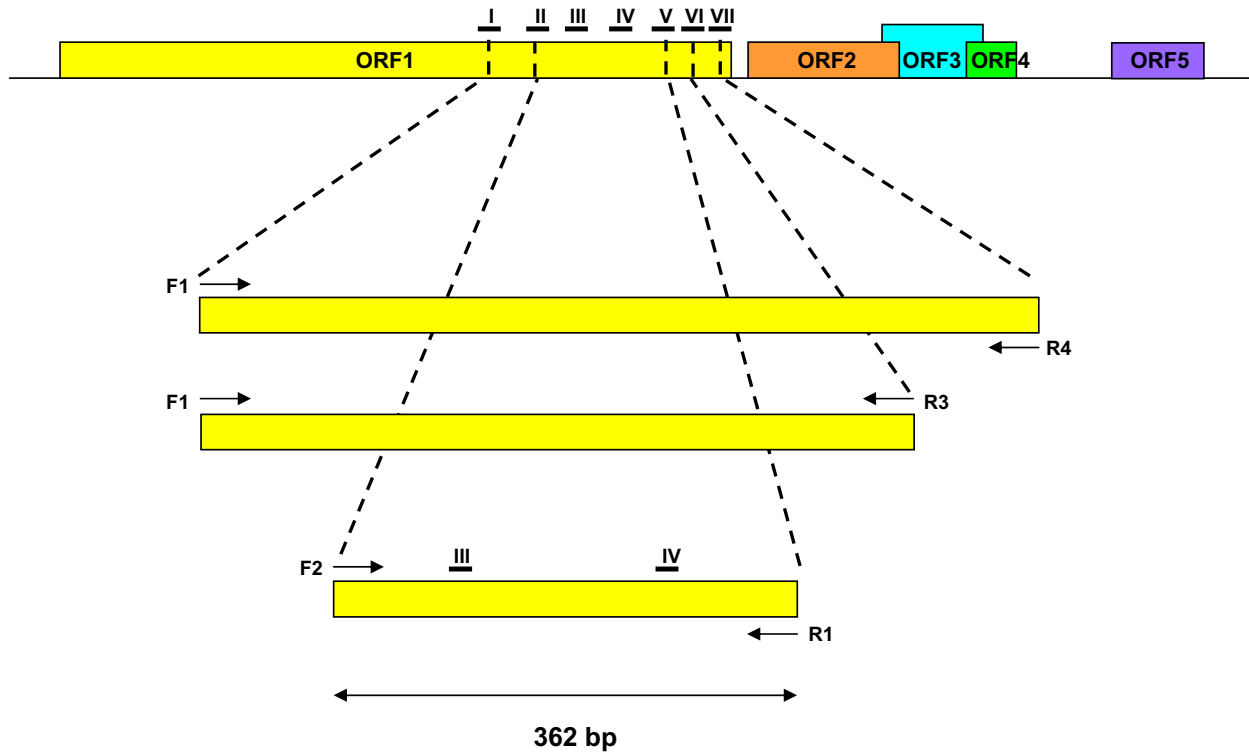
- polyclonal antiserum for immunocapture
- inosine-containing degenerate primers for RT-PCR and nested PCR

Detection of *Banana mild mosaic virus* and Banana virus X by polyvalent degenerate oligonucleotide RT-PCR (PDO-RT-PCR)

Pierre-Yves Teycheney, Isabelle Acina, Benham E.L. Lockhart and Thierry Candresse

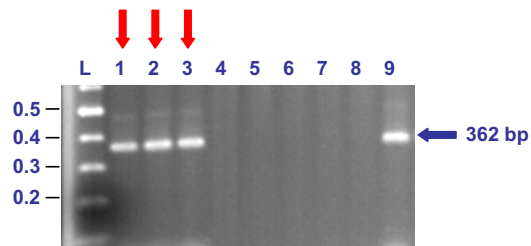
J. Virol. Meth., in press

Multiplex immunocapture reverse transcription nested PCR



Detection of BanMMV by IC-PDO-RT-nested PCR

- polyclonal antiserum for immunocapture
- degenerate primers targeting the RdRp domain for RT-PCR and nested PCR



Detection of BVX by DB-PDO-RT-nested PCR

Arch Virol (2005) 150: 1715–1727
DOI 10.1007/s00705-005-0567-0

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Molecular characterization of banana virus X (BVX), a novel member of the *Flexiviridae* family

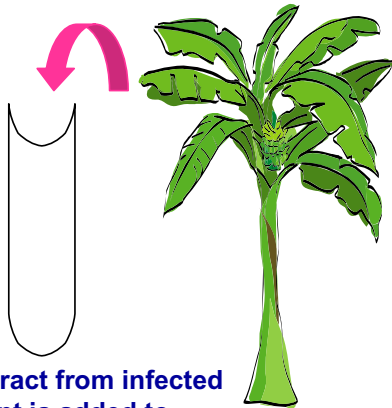
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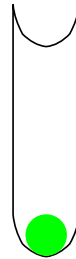
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Direct binding PCR (DB-PCR)



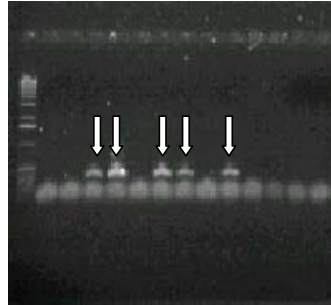
1 Extract from infected plant is added to uncoated tube



2 Viral particles bind to the walls



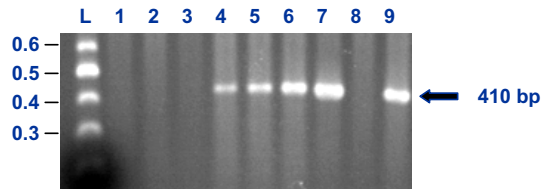
3 Heat treatment denatures viral particles : genomic DNA becomes accessible for PCR



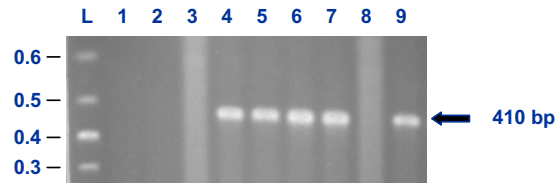
4 PCR products are analysed by electrophoresis

Detection of BVX by DB-PDO-RT-nested PCR

- RT-nested PCR from purified RNA



- DB-RT-nested PCR



Full comprehensive virus indexing procedure for the production of virus-free certified vitroplants

