DETECTION OF *BANANA MILD MOSAIC VIRUS* AND BANANA VIRUS X BY POLYVALENT DEGENERATE OLIGONUCLEOTIDE REVERSE TRANSCRIPTION PCR (PDO-RT-PCR)

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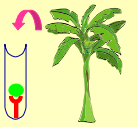
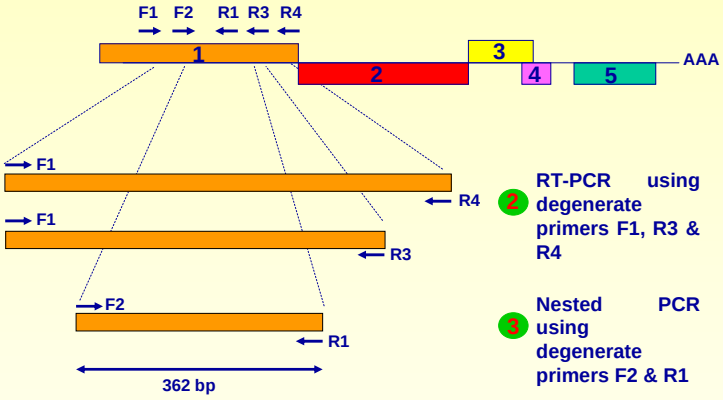
An existing polyvalent degenerate oligonucleotide RT-PCR (**PDO-RT-PCR**) assay [1] was adapted for the **detection** of ***Banana mild mosaic virus* (BanMMV)** and **Banana virus X**, two *Flexiviridae* infecting *Musa* spp. PDO inosine-containing primers were found to be well suited for the detection of BanMMV, despite its high sequence variability [2], but not for that of the highly conserved BVX [3], for which species-specific primers were therefore designed. The sampling and sample processing steps were optimized in order to avoid nucleic acid purification prior to the reverse transcription step. A **polyclonal anti-BanMMV antiserum** was raised and successfully used for the immunocapture (IC) of BanMMV viral particles from leaf extracts, leading to the development of a **PDO-IC-RT-nested PCR assay** [4]. Although the anti-BanMMV antiserum could to some extent recognize BVX particles, **direct binding (DB)** was shown to be a more efficient method for processing BVX-infected samples and a **PDO-DB-RT-nested PCR assay** was developed for the detection of BVX from leaf extracts [4]. This work completes existing PCR-based detection of other viral species infecting *Musa* spp. [5, 6] and will benefit movement and propagation of *Musa* germplasm, for which viruses are important constraints.

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

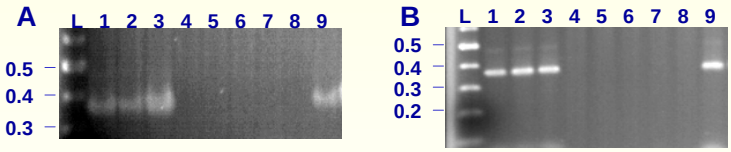


1 Viral particles from leaf extracts are immunocaptured on anti-BanMMV antibody-coated polypropylene microtubes



2 RT-PCR using degenerate primers F1, R3 & R4

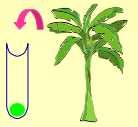
3 Nested PCR using degenerate primers F2 & R1



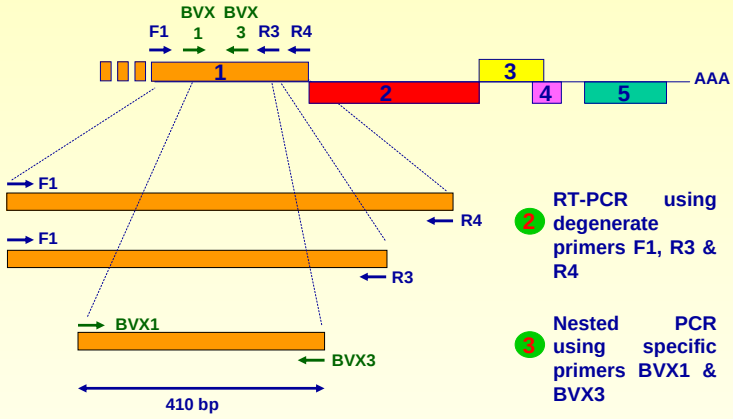
Detection of BanMMV by PDO-RT-nested PCR (A) and PDO-IC-RT-nested PCR(B)

RT-PCR reactions were carried out on 50 ng of total RNAs purified from leaf tissues (A) or on immunocaptured viral particles (B) from leaf samples of plants from *Musa* accessions Moto Ebanga (lane 1), Kwa (lane 2), Kelong mekintu (lane 3), Som (lanes 4, 5 and 6) and Buitenzorg (lane 7).
Lane 8 : uninfected control.
Lane 9 : 10 ng of plasmid DNA containing part of the BanMMV genome used as a positive control for nested PCR.
L: 100 bp DNA ladder (Promega) with indicated marker sizes in kbp.

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

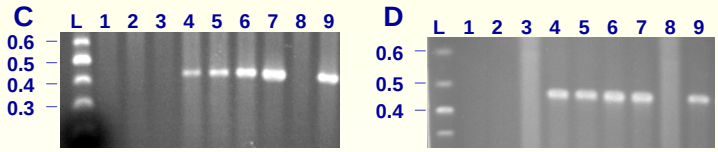


1 Viral particles from leaf extracts are directly bound on polypropylene microtubes



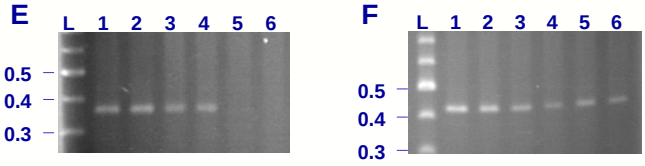
2 RT-PCR using degenerate primers F1, R3 & R4

3 Nested PCR using specific primers BVX1 & BVX3



Detection of BVX by PDO-RT-nested PCR (C) and PDO-DB-RT-nested PCR(D)

RT-PCR reactions were carried out on 50 ng of total RNAs purified from leaf tissues (A) or on directly trapped viral particles (B) from leaf samples of plants from *Musa* accessions Moto Ebanga (lane 1), Kwa (lane 2), Kelong mekintu (lane 3), Som (lanes 4, 5 and 6) and Buitenzorg (lane 7).
Lane 8 : uninfected control.
Lane 9 : 10 ng of plasmid DNA containing part of the BanMMV genome used as a positive control for nested PCR.
L: 100 bp DNA ladder (Promega) with indicated marker sizes in kbp.



Sensitivity thresholds of IC-PDO-RT-nested PCR for the detection of BanMMV (E) and DB-PDO-RT-nested PCR for the detection of BVX (F) IC-PDO-RT-nested PCR (E) and DB-PDO-RT-nested PCR (F) were performed using leaf extracts prepared from accession Kelong mekintu (E) or Som (F). Lane 1 : undiluted leaf extracts; lanes 2 to 6 : 1:10 to 1:100,00 dilutions, respectively of leaf extract
L: 100 bp DNA ladder (Promega) with indicated marker sizes in kbp.

References : [1] Foissac X, Svanella-Dumas L, Gentit P, Dulucq M.-J, Marais A, Candresse T, 2005. *Phytopathology* **95**: 617-625; [2] Teycheney P.-Y, Laboureau N, Iskra-Caruana, M.-L, Candresse, T, 2005. *J. Gen. Virol.* **86**: 3179-3187; [3] Teycheney, P.-Y, Marais, A, Svanella-Dumas L, Candresse T, 2005. *Arch. Virol.* **150**: 1715-1727; [4] Teycheney P.-Y, Acina I, Lockhart B.E.L, Candresse T, 2007. *J. Virol. Meth.* (in press); [5] Sharman M, Thomas J. E, Dietzgen R. G, 2000. *J. Virol. Meth.* **89:** 75-88; Le Provost G, Iskra-Caruana M.-L, Acina I, Teycheney P.-Y, 2006. *J. Virol. Meth.* **137**: 7-13.