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 (BVX), 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.

References:

Legend:
- Lane 1: uninfected control
- Lane 2: A03 accession Musa acuminata (100 ng)
- Lane 3: Musa acuminata cv. Sensation (100 ng)
- Lane 4: Musa acuminata cv. A100 (100 ng)
- Lane 5: Musa acuminata cv. A110 (100 ng)
- Lane 6: Musa acuminata cv. A120 (100 ng)
- Lane 7: Musa acuminata cv. A130 (100 ng)
- Lane 8: Musa acuminata cv. A140 (100 ng)
- Lane 9: Musa acuminata cv. A150 (100 ng)
- Lane 10: Musa acuminata cv. A160 (100 ng)

Determination of BanMMV and BVX by PDO-RT-PCR

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

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

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

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

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) c.c.

PDO-RT-nested PCR (E) and DB-PDO-RT-nested PCR (F) were performed using leaf extracts prepared from accessions Kaojung melinon (E) or Sor (F). Lane 1: undiluted leaf extracts; lanes 2 to 6: 1:10 to 1:100 dilutions, respectively of leaf extract

L: 100 bp DNA ladder (Promega) with indicated marker sizes in kbp.

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