Detection of *Banana mild mosaic virus* (BanMMV) and Banana virus X (BVX) by PDO RT PCR

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Viruses are important constraints to the movement and propagation of plant germplasm, especially for vegetatively propagated crops such as banana and plantain. Their control relies primarily on the use of virus-free plant material, whose production and certification requires sensitive and reliable detection methods.

Two members of the family *Flexiviridae* infect *Musa* spp : *Banana mild mosaic virus* (BanMMV) and Banana virus X (BVX). BanMMV causes mild leaf chlorosis symptoms in single infection but mixed infections with *Cucumber mosaic virus* (CMV) or *Banana streak virus* (BSV) can lead to important leaf necrosis. BanMMV transmission occurs mainly through vegetative propagation although recent data showed that plant-to-plant transmission occurs at a low rate [1]. BanMMV has therefore become an important constraint to the conservation, movement and exchange of *Musa* germplasm. Banana virus X was recently identified in Guadeloupe [2]. No symptom can be associated to the presence of this virus and no prevalence study could be carried out due to the lack of a detection method. Therefore, an existing polyvalent reverse transcription degenerate oligonucleotide PCR assay (PDO RT-PCR, [3]) was adapted to the specific detection of BanMMV and BVX from banana plants [4].

Polyvalent degenerate oligonucleotides (PDO) containing inosine residues were found to be well suited to the detection of BanMMV, despite its high molecular diversity [1], but not to that of BVX, for which species-specific primers were designed. 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. Although the anti-BanMMV antiserum could recognize to some extent BVX viral 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.

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