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Detection of *Banana bract mosaic virus* (BBrMV) with different primers by Reverse Transcription Polymerase Chain Reaction (RT-PCR) in 'Embul' Banana (Mysore, AAB)

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Banana bract mosaic virus (BBrMV) is a member of genus *Potyvirus*, and has flexuous rod-shaped virions and a positive sense ssRNA genome. Being the causal agent of banana bract mosaic disease (BBrMD), it imposes a risk in banana plant production through micropropagation. Although double antibody sandwich (DAS) ELISA is widely practiced as a diagnostic method, infection by BBrMV might still be left undetected in the case of a relatively low virion concentration present in the sample, while PCR detection is more reliable. The objective of the study was to determine the sensitivity of using potyvirus degenerate primers and bract specific primers in reverse transcription (RT) and in PCR for the local banana variety, 'Embul' (Mysore, AAB). Leaf samples with BBrMD symptoms, such as chlorotic streaks specific for BBrMV and spindle shaped mosaic patterns on the lower side of the midrib, were collected from the Southern Province. The virus was extracted and the virions were immunocaptured using anti-BBrMV antibodies. cDNA was synthesized for ssRNA of BBrMV using Superscript II reverse transcriptase. cDNA was obtained by two different reactions using Poty1 (oligo dT) or Bract2 (BBrMV specific) primers. Both Poty1 and Bract2 primed cDNA were amplified separately with Bract1 and Bract2 primers by *Taq* DNA polymerase. The cDNA obtained from two reactions were amplified in equal efficiency, confirming the use of either oligo dT or specific primer in RT does not effect the sensitivity of amplification by PCR for BBrMV in 'Embul'. Poty1 primed cDNA was amplified with six combinations of four different primers (Bract1, Bract2, Poty1 and U341) to choose the best primer combination for amplification with *Taq* DNA polymerase. The U341 primer is a degenerate primer designed to the conserved core of the coat protein of all potyviruses. The Bract1 and Bract2 primers (both BBrMV specific) gave the highest intensity of bands in gel electrophoresis, where as U341 and Poty 1 primers gave the lowest. This showed that the degree of degeneracy in primers has an adverse effect on amplification of cDNA by PCR. The use of BBrMV specific primers in PCR has increased the efficiency of PCR technique as a reliable diagnostic method for the virus. The selection of appropriate primers for RT and the correct combination of primers in PCR is crucial for the efficiency of RT -PCR for BBrMV in 'Embul' of Sri Lanka. Although the Poty 1/U341 RT-PCR was less efficient, it will be a useful tool for studying genetic diversity of BBrMV in Sri Lanka, and can also be used in quarantine to screen for new potyviruses such as Abaca mosaic virus, which is presently not found in Sri Lanka.

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