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DETECTION OF BANANA STREAK VIRUS AND BANANA BUNCHY TOP VIRUS THROUGH THE MULTIPLEX PCR TECHNIQUE

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Banana bunchy top virus (BBTV) and Banana streak virus (BSV) are the most common viruses that cause substantial economic losses in banana cultivation in Sri Lanka. PCR-based detection methods have become popular due to their rapidity and sensitivity. The multiplex PCR technique allows simultaneous detection of more than one target DNA sequence in a single tube in one reaction. The objectives of the current study were to standardize a DNA extraction method for banana leaf tissues, detection of BBTV and BSV through singleplex PCR and development of a multiplex PCR protocol for concurrent detection of the two target viruses. The experimental work was carried out at the Horticultural Crop Research and Development Institute, Gannoruwa.

Modified CTAB and SDS protocols were standardized by analyzing quality and quantity through spectrophotometry and agarose gel electrophoresis. Although absorbance data and electrophoresis profiles exihibited more or less similar results for both protocols, the SDS method yielded better quality DNA in terms of the pellet colour and solubility in TE buffer as compared to those extracted by the CTAB method. Hence, out of two DNA extraction methods, DNA extracted by the CTAB method satisfied the requirements of quality and quantity as template DNA for PCR amplification of BBTV and BSV.

Selected BSV specific primers amplified a fragment of 731 bp corresponding to the conserved domain of reverse transcriptase and RNase H of the BSV genome while BBTV specific primers amplified a region of 1.111 kb size in BBTV DNA component 1. The PCR products of the expected sizes could be consistently amplified and the primers have proven to be specific and sensitive enough to detect BBTV and BSV through singleplex PCR. Multiplex gradient PCR was conducted to optimize the annealing temperature. At an optimum annealing temperature of 61 ^{0}C, under the given PCR conditions (i.e. initial denaturation at 94 ^{0}C for 4 min, 30 cycles consisting denaturation at 94 ^{0}C for 1 min, annealing at 61^{0}C for 30 sec and extension at 72 ^{0}C for 75 sec followed by final extension at 72 ^{0}C for 10 min.), multiplex PCR yielded the fragments specific to BBTV and BSV at a reasonably higher intensity enabling the detection of the two target viruses in a single reaction.

The developed protocol could be applied to screen the samples having mixed infections and the findings will be useful in the virus indexing programs especially with a large number of samples such as *in vitro* production of *Musa* plants.