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OPTIMIZATION OF A RNA EXTRACTION PROTOCOL FOR TOMATO LEAF TISSUES AND PCR CONDITIONS TO DETECT RNA TYPE VIRUSES IN CHILLI LEAVES

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Chilli and tomato are important cash crops grown in Sri Lanka. Cucumber Mosaic Virus (CMV) and Tobacco Mosaic Virus (TMV) are major viruses infecting chilli and tomato with RNA genomes. Extraction of RNA from plant tissues of Solanaceae is difficult due to presence of high amounts of polysaccharides, polyphenols and RNase in plant tissues. One of the objectives of the present study was to optimize a RNA extraction protocol for tomato. RNA extraction from tomato leaf tissues was done by three extraction protocols. As an efficient and successful RNA extraction protocol from leaves of tomato, the modified TRIzol reagent method was selected among the three tested protocols. Polymerace Chain Reaction (PCR) based techniques are reliable methods for the detection of RNA type viruses in plant tissues. Optimization of PCR conditions to detect RNA type viruses in chilli was another objective of this study. A gradient RT-PCR was done for the detection of RNA viruses in chilli. Complementary DNA (cDNA) was prepared using random hexamers method. CMV 3 and CMV 5 primer pairs were used to detect CMV in chilli leaf tissues. Optimized PCR conditions for the detection of CMV in chilli were as follows: 94 ^{0}C for 2 minutes, (40 cycles of 94 ^{0}C for 45 seconds, 47.7^{0}C for 45 seconds, 72 ^{0}C for 1 minutes), and 1 cycle of 72 ^{0}C for 10 minutes.