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OPTIMIZATION OF PCR CONDITIONS FOR THE IDENTIFICATION OF CUCUMBER GREEN MOTTLE MOSAIC VIRUS

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The average yield potential of the cucumber cultivation in Sri Lanka in 2001 was 13t/ha. Although the crop can be grown throughout the year in the wet-zone and during *Maha* season in the dry-zone, the production volumes are 40–50 % lower due to the infection of a complex of viruses. Cucumber Green Mottle Mosaic Virus (CGMMV) is considered as one of the most destructive viruses. Enzyme Linked Immunosorbent Assay (ELISA) is a serological method for the detection of CGMMV infection in plants. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) can be used as a molecular based diagnostic method to identify viruses. Viruses with very low concentrations can be identified using this method. PCR method is also very specific to the virus, cost effective and highly accurate compared to other methods. PCR conditions have to be optimized to use this method for diagnosis. In this regard, contaminated sap was inoculated to healthy cucumber plants and a preliminary screening was done for CGMMV inoculated plant samples by Double Antibody sandwich ELISA (DAS ELISA) method using a commercially available ELISA test kit. Then the samples with high positive values for ELISA were selected and subjected to molecular diagnosis by RT-PCR. The RNA was extracted by size fractionated silica extraction method and complementary DNA (cDNA) was synthesized under standard conditions (such as 5 µl of 5×Reverse transcriptase buffer, 0.8 µl of dNTPs, 0.1 µl of RNA sin, 0.25 µl of Reverse Transcriptase, 4.85 µl of deionized water, 8 µl of RNA template and 1 µl of reverse primers). First strand synthesis of cDNA was done by providing conditions with 42 ^{0}C/45 min followed by inactivation at 95 ^{0}C for 5 min. The primer pairs 2R, 3F and 4R, 5F were used. Several sets of PCR were carried out changing the PCR mixture and with different annealing temperatures. The optimized PCR mixture for the detection of CGMMV were identified as 2.5 µl of 10×PCR buffer, 1.5 µl of $Mgcl_2$, 1 µl of each primer pairs. The conditions were 94 ^{0}C for 30 s of initial denaturation, 94 ^{0}C for 10 s of denaturation, 57 ^{0}C for 30 s of optimized annealing temperature, 72 ^{0}C for 1 min of extension and 72 ^{0}C for 5 min of final extension.