



MOLECULAR DETECTION OF PINEAPPLE MEALYBUG WILT ASSOCIATED VIRUS-1 AND VIRUS-2 BY DUPLEX PCR METHOD

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ABSTRACT

Pineapple (*Ananas comosus* L.) is one of the most important tropical fruit crops grown in Sri Lanka. It is infected with several viruses including Pineapple Mealy bug Wilt Associated Virus-1 (PMWaV-1) and Pineapple Mealy bug Wilt Associated Virus-2 (PMWaV-2). PMWaVs belong to the genus *Ampelovirus* of the family *Closteroviridae*. These two viruses can be detected by using molecular methods. The duplex PCR (Polymerase Chain Reaction) assay proved to be as one of the sensitive and specific as single-target assays and also detected the mixed infections with certainty. The identification of both viruses in a single reaction offers a reduction in both cost and laboratory diagnostic time. PMWaV-1 & PMWaV-2 suspected positive samples were collected from pineapple variety Mauritius. RNA was extracted using silica fractionated method. Primer 225(5'-ACAGGAAGGACAACACTCC- 3') & 226(5'-CGCACAAACTTCAAGCAATC-3') for PMWaV-1 and Primer 223(5'-TCATTGCACTCACTTATCGTTG-3') & 224(5'-CATACGAACTAGACTCATACG-3') for PMWaV-2 were used to amplify cDNA and for PCR. Negative samples which were given negative result from enzyme-linked immunosorbent assay (ELISA) test were used as negative controls. The sizes of the amplified products were 589 bp (base pairs) and 609 bp respectively in gel electrophoresis. Very close bands were observed and it was difficult to detect PMWaV-1 and PMWaV-2 bands separately. Therefore new forward (5'TATTGCCGCACTTAA CGCGA 3') and reverse primer (5' CATCTCACGACACGAGCTG 3') for virus 1 were designed. The length of the primer was 20 and 19 bases in respectively and detectable band in gel profile was 234 bp with three unstable hairpin loops. Primer best annealing temperature was 50 °C. These primers were specific for PMWaV-1. Duplex PCR was a successful method to detect PMWaV-1 and PMWaV-2. Separate bands were observed and it was an easy method to identify the both viruses in a single reaction.

Keywords: Pineapple Mealy bug Wilt Associated Virus-1 and 2, primers, duplex PCR, gel electrophoresis

Acknowledgement: Financial assistance by Plant Virus Indexing Center, Department of Agriculture, Homagama, Sri Lanka