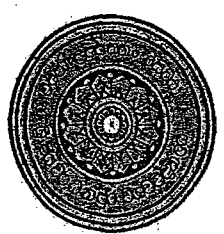


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## MOLECULAR DETECTION OF PINEAPPLE MEALY BUG WILT ASSOCIATED VIRUS-1 AND VIRUS-2 BY USING DUPLEX PCR METHOD

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Pineapple (*Ananas comosus* L.) is one of the most important tropical fruits. At present the crop is widely grown throughout the tropical and sub-tropical regions in the world including Sri Lanka. Pineapple is infected with several viruses among that Pineapple Mealy bug Wilt Associated Virus-1 (PMWaV-1) and Pineapple Mealy bug Wilt Associated Virus-2 (PMWaV-2) are very common in Sri Lanka. PMWaVs belong to the genus *Ampelovirus* of the family *Closteroviridae*. The presence of these two viruses can be detected by using molecular methods. The duplex PCR assay proved to be as 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 & PMWaV2 suspected positive samples were collected from pineapple variety Mauritius. RNA was extracted using silica fractionated method. Primer 225 & 226 for PMWaV-1 and Primer 223 & 224 for PMWaV-2 were used to amplify cDNA and PCR. The size of the amplified product was respectively 589bp (base pairs) and 609bp in gel electrophoresis. Very close bands were observed and it was difficult to detect PMWaV-1 and PMWaV-2 bands separately. Sequencing data was use to designed new primer for PMWaV1. Duplex PCR was done for PMWaV-1 and PMWaV-2. PCR was done using newly designed primer and the size of the amplified product was around 700bp. In duplex PCR, products were not amplified because of the formation of primer dimers (hetero dimers) between PMWaV-1 primers and PMWaV-2 primers. New primer was designed again and amplification size of the product was 234bp and duplex was done using two primer pairs including new primer. Separate bands were observed and it was easy method to identifying the both two viruses in a single reaction.

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**Keywords:** Pineapple Mealy bug Wilt, primer, PCR, gel electrophoresis, duplex