



UNIVERSITI PUTRA MALAYSIA

***DETECTION OF CITRUS VIROIDS BY MULTIPLEX REVERSE
TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)***

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TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)

BY

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A project report submitted of Faculty of Agriculture, Universiti Putra Malaysia, in fulfilment of the requirement of PRT4999 (Final Year Project) for the award of the Degree of Bachelor of Agriculture Science.

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CERTIFICATION

This project title is "Detection Of Citrus Viroids By Multiplex Reverse Transcription Polymerase Chain Reaction (RT-PCR)" prepared by Nor Athirah binti Roslin and submitted to the Faculty of Agriculture in fulfilment of the requirement of PRT4999 for the award of the Degree of Bachelor of Agriculture Science.

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ABSTRACT

Citrus from family Rutaceae is a perennial plant that found in subtropical and tropical regions including Malaysia. One of the problems in citrus is the infection of viroids such as *Citrus exocortis viroid* (CEVd), *Citrus bent leaf viroid* (CBLVd), *Citrus viroid III* (CVd-III) and *Citrus viroid IV* (CVd-IV). Symptoms of viroid infections are bark shelling, leaf necrosis, bending of leaf margin and dwarfing. CBLVd has been reported in Malaysia which is associated with yield loss. However, symptoms are not enough to prove that particular viroid is the causes of that infection. Multiplex RT-PCR has been reported to detect multiple viruses and viroids in plants. Multiplex RT-PCR was used in this study to detect the citrus viroids simultaneously in citrus samples. Citrus samples showing viroid infection like symptoms was taken; 16 samples from Kajang and 10 samples from UPM, Selangor, 6 samples from Jaya Gading, 2 samples from Sri Damai, and 4 samples from Semambu Kuantan, Pahang and 25 samples from Bertam Jaya, Melaka. The RNA extract from all 63 leaf samples was used for the amplification of the viroids using 4 different primers sets using multiplex RT-PCR. The analysis showed 10 out of 63 samples (1 sample from UPM, Selangor and 9 samples from Bertam Jaya, Melaka) produced amplicons at 200-300 bp. The other 53 samples (16 samples from Kajang, 9 samples from UPM, Selangor, 6 samples from Jaya Gading, 2 samples Sri Damai, and 4 samples from Semambu Kuantan, Pahang) showed negative results with no amplicon produced. These positive results were obtained from *C. hystrix* from UPM, Selangor and *C. microcarpa* from Bertam Jaya, Melaka. RT-PCR analysis of 9 positive results produced amplicon approximately 200-300 bp by specific primer set, CV-I-cp and CV-I-hm. Sequencing analysis for four samples (1 sample from UPM,

Selangor and 3 samples from Melaka) showed high sequence similarity with *Citrus bent leaf viroid* Ia genomic RNA, isolate: Jp, complete genome (GenBank: AB006734.1) ranged from 91-100%. No other citrus viroids were detected in this experiment using the multiplex RT-PCR.



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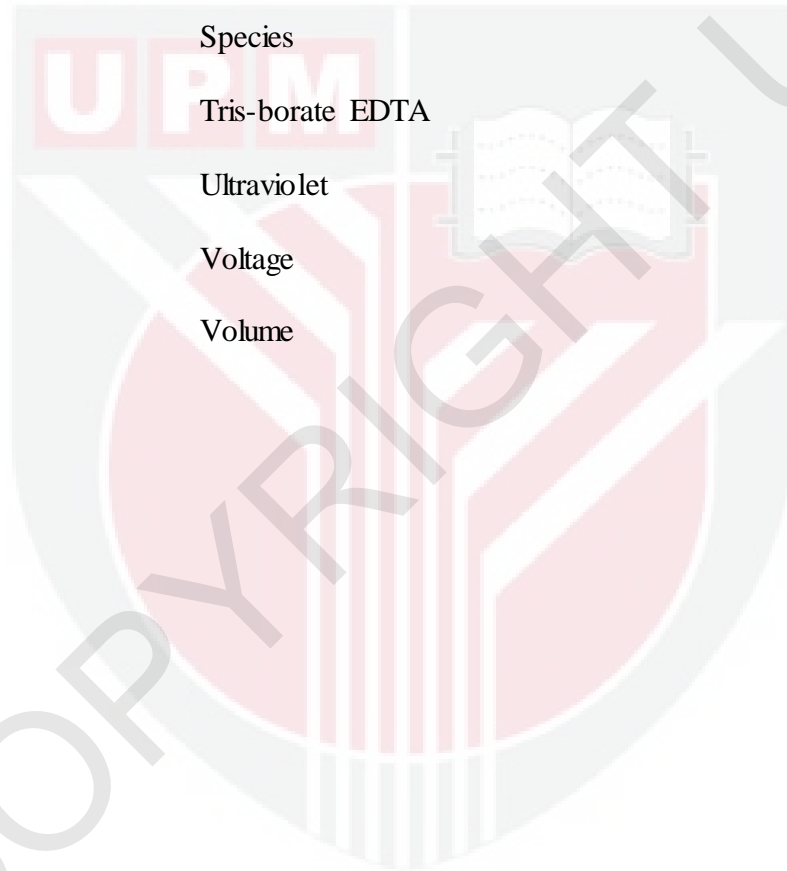
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ABBREVIATIONS

| | |
|--------|--|
| % | Percentage |
| °C | Degree Celcius |
| µl | Microliter |
| µg | Microgram |
| µg/ml | Microgram per millilitre |
| AMV-RT | Avian Myeloblastosis Virus Reverse Transcription |
| bp | Base pair |
| CA | Chloroform isoAmyl |
| cDNA | Complementary deoxyribonucleic acid |
| CEVd | <i>Citrus exocortis viroid</i> |
| CVd V | Citrus viroid V |
| CVd VI | Citrus viroid VI |
| dNTP | Deoxyribonucleic triphosphate |
| EDTA | Ethylenediamine tetra acetic acid |
| EtBr | Ethidium bromide |
| EtOH | Ethanol |
| G | Gram |
| L | Liter |
| M | Molar |
| Mg | Miligram |
| Min | Minute |
| Nt | Nucleotide |

| | |
|--------|---|
| NaAc | Sodium acetyl |
| PAGE | Polyacrylamide gel electrophoresis |
| PCR | Polymerase chain reaction |
| RNA | Ribonucleic acid |
| Rpm | Rotation per minute |
| RT-PCR | Reverse transcription polymerase chain reaction |
| SDDW | Sterile double distilled water |
| Sp | Species |
| TBE | Tris-borate EDTA |
| UV | Ultraviolet |
| V | Voltage |
| Vol | Volume |



CHAPTER 1

INTRODUCTION

Citrus (*Citrus L.* from Rutaceae) is one of the important fruit crops; which originated in Southeast Asia (Nicolosi, 2007). It is a perennial plant commonly found in the subtropical and tropical areas including Malaysia. In Malaysia, many citrus species have been found such as *Citrus hystrix*, *Citrus maxima*, *Citrus microcarpa*, *Citrus aurantifolia*, and *Citrus sinensis*. Major region of citrus production are in China and Mediterranean, followed by Brazil, USA, India, Mexico and Nigeria (RLF). While Malaysia is ranked 83rd in the world in citrus production with 36,450 million tonnes in 2013 (Knoema, 2013).

The citrus production is affected by biotic or abiotic factors. Some of the reported citrus diseases are caused by bacteria, fungi, virus and spiroplasma. Citrus production is also affected by viroids. They are a threat to the citrus industry. There are about six viroid found in citrus plants from family *Pospiviroidae*, *Citrus exocortis viroid* (CEVd, genus *Pospiviroid*), *Citrus bent leaf viroid* (CBLVd, genus *Apscaviroid*), *Hop stunt viroid* (HSVd, genus *Hostuviroid*), *Citrus viroid III* (CVd-III, genus *Apscaviroid*), *Citrus viroid IV* (CVd-IV, genus *Cocadviroid*) and *Citrus viroid OS* (CVd-OS) (Takao et al., 2002; Lin et al., 2015).

Recently, CBLVd has been reported in Malaysia; however symptoms of other citrus viroids have been observed (Taneswari, 2015). The symptoms of CEVd infection include bark shelling, scaling of the rootstock, stunting, cracking and browning of the underside of the veins. While for CBLVd, the leaf margin will bend downwards or inwards and dwarfing. However, these symptoms alone are insufficient to prove that particular viroid is the cause of the infection. In addition, occurrence of multiple infections of the citrus viroids has been reported, thus making the detection of these viroids difficult. These diseases cause economic losses and reduce yields in citrus production. Therefore, early detection of these viroids are important to develop a better disease management strategy. Multiplex RT-PCR has been previously reported to detect multiple viruses and viroids in vegetable and citrus (Ito et al., 2002). In view of this, this study was carried out with the following objectives:

- 1) To detect citrus viroids simultaneously using Multiplex Reverse Transcriptase Polymerase Chain Reaction.
- 2) To characterize the citrus viroids by cloning and sequencing.

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