UNIVERSITI PUTRA MALAYSIA

DETECTION AND CHARACTERIZATION OF CITRUS VIROIDS IN MALAYSIA USING REVERSE TRANSCRIPTION- POLYMERASE CHAIN REACTION

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DETECTION AND CHARACTERIZATION OF CITRUS VIROIDS IN MALAYSIA USING REVERSE TRANSCRIPTION- POLYMERASE CHAIN REACTION

BY

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A project report submitted of Faculty of Agriculture, Universiti Putra Malaysia, in fulfilment of the requirement of PRT 4999 (Final Year Project) for the award of the Degree of Bachelor of Agriculture Science.

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CERTIFICATION

This project entitled "Detection and Characterization of Citrus Viroids in Malaysia using Reverse Transcription- Polymerase Chain Reaction" is prepared by Taneswari d/o Murugan 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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Bachelor of Agriculture Science

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……

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ABSTRACT

Citrus belongs to *Rutaceae* family and sub-family *Aurantoidea* originated in Southeast Asia and it is a major fruit crop throughout world. Viroids have been reported in citrus species causing Citrus Exocortis disease, Citrus Cachexia disease, Citrus Leaf Bending disease, Citrus Bark Cracking disease and Citrus Dwarfing disease. These diseases cause a reduction in yield and quality of citrus production. Yet, there is no study or information on citrus viroids in Malaysia. The purpose of this study is to identify and characterize citrus viroid in Malaysia. A total of 33 citrus samples showing leaf bending, petiole necrosis, vein yellowing, and stunting symptoms were collected from Muar, Johor and Kajang, Selangor. RNA was extracted using TELSP buffer and was amplified by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) using six different sets of citrus viroid specific primers. The RT-PCR products fractionated using 2% agarose gel and the viroid positives samples produced expected size of amplicon (200-300 bp). A total of 2 out of 16 samples from Muar and 2 out of 17 samples from Kajang were positive by RT-PCR. Positive RT-PCR products were purified and sent for sequencing. Blast analysis for sequences of all the four PCR positive samples showed high sequence similarity (95 to 99%) with *Citrus bent leaf viroid* (CBLVd) isolate lot3/3-1, complete genome (Genbank: KM214210.1) and the nucleotide size ranged from 192 to 218 nt. The viroid was found in *C. aurantifolia*, *C. hystrix* and *C.sinensis* which showed virus and viroid like symptoms and this is the first report of CBLVd in Malaysia.

ABSTRAK

Citrus tergolong dalam keluarga *Rutaceae* dan sub-keluarga *Aurantoidea* berasal dari Asia Tenggara. Ia merupakan salah satu tanaman buah-buahan yang terbesar di dunia. Viroid juga meyebabkan penyakit dalam spesies citrus seperti Citrus Exocortis, penyakit Citrus Cachexia, penyakit Citrus bent leaf, Citrus Bark Cracking dan penyakit Citrus dwarfing. Penyakit-penyakit ini menyebabkan pengurangan hasil dan kualiti pengeluaran citrus. Namun, tidak ada kajian atau maklumat mengenai citrus viroid di Malaysia. Tujuan kajian ini adalah untuk mengenal pasti dan mencirikan citrus viroid di Malaysia. Sebanyak 33 sampel daun citrus telah dikutip dari Muar, Johor dan Kajang, Selangor berdasarkan simptom terbantut, kelenturan daun, petiol nekrosis, dan urat daun kekuningan. RNA telah diekstrak menggunakan penampan TELSP. RNA yang telah diekstrak telah dianalisis melalui Reverse Transcription-Polymerase Chain Reaction (RT-PCR) menggunakan enam set primer spesifik citrus viroid tertentu. Produk RT-PCR telah dipisahkan menggunakan 2% jel agarose elektroforesis dan positif sampel menghasilkan saiz amplikon yang telah dijangka (200-300 bp). Sejumlah 2 daripada 16 sampel dari Muar adalah positif untuk CBLVd dan hanya 2 daripada 17 sampel dari Kajang CBLVd adalah positif RT-PCR. Positif RT-PCR produk telah dihantar untuk penjujukan. Saiz nukleotida adalah di antara 192-218 nt dan kadar persamaan antara 95-99 % dengan *Citrus bent leaf viroid* (CBLVd) isolate lot3/3-1, genom lengkap (Genbank: KM214210.1) telah dibuktikan melalui analisis BLAST. Viroid ini telah ditemui daripada *C. aurantifolia, C. hystrix* dan *C.sinensis* dan ini adalah laporan pertama CBLVd di Malaysia.

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ABBREVIATION

%	Percentage
^{o}C	Degree Celcius
µg	Microgram
µg/ml	Microgram per milliliter
µl	Microliter
AMV-RT	Avian Myeloblastosis Virus Reverse Transcription
ASVd	*Avocado sunblotch viroid*
bp	Base pair
CA	Chloroform isoAmyl
CBCVd	*Citrus bark cracking viroid*
CBVd	*Citrus bent leaf viroid*
CCVd	*Coconut cadang cadang viroid*
cDNA	Complementary deoxyribonucleic acid
CDVd	*Citrus dwarfing viroid*
CEVd	*Citrus exocortis viroid*
CSVd	*Chrysanthemum stunt viroid*
CtiVd	*Coconut tinangaja viroid*
CVd V	*Citrus viroid V*
CVd VI	*Citrus viroid VI*
Dntp	Deoxyribonucleic triphosphate
EDTA	Ethylenediamine tetra acetic acid
EtBr	Ethidium bromide

g	Gram
HCL	Hydrochloric acid
HSVd	*Hop Stunt viroid*
L	Liter
LiCL	Lithium Chloride
M	Molar
mg	Milligram
mg/Ml	Milligram per milliliter
Ml	Milliliter
min	Minute
Ml	Milliliter
Mm	Millimol
nt	Nucleotide
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PSTVd	*Potato spindle tuber viroid*
PVP	Polyvinylpolypyrrolidone
RNA	Ribonucleic acid
Rpm	Rotation per minute
RT buffer	Reverse transcription buffer
RT-PCR	Reverse transcription polymerase chain reaction
SDDW	Sterile double distilled water
SDS	Sodium dodecyl sulphate
sp	species
TBE	Tris-borate EDTA

UV	Ultraviolet
V	Voltage
v/v	Volume/volume
Vol	Volume

CHAPTER 1

INTRODUCTION

Citrus belongs to family *Rutaceae*, originated in Southeast Asia. It is grown in tropical and subtropical climate and is one of the major fruit crop throughout world. It has high contribution towards human diet. Citrus is a major source of vitamin C with antioxidant properties (Liu, *et al*, 2012). The leading citrus producers in the world are China followed by Brazil, United States, India, Mexico and Spain (FAO, 2013). Malaysia is ranked at 81st among the countries producing citrus in the world. Malaysia produced about 36,450 tonnes of citrus in 2013 (MOA, 2013). The major citrus producing areas in East Malaysia are Sabah and Sarawak while from West Malaysia are Kelantan and Johor.

Citrus production is also affected by viroids. They are the emerging threat to the citrus industry. To date, seven citrus viroids have been detected so far in the citrus. Those viroids are *Citrus Exocortis viroid* (CEVd), *Citrus Bent Leaf viroid* (CBLVd), *Hop Stunt viroid-citrus* (HPSVd-cit), *Citrus Dwarfing viroid* (CDVd), *Citrus Bark Cracking viroid* (CBCVd), *Citrus viroid V* (CVd V) and *Citrus viroid VI* (CVd VI-os) have been distributed in different geographic areas (King *et al*, 2011).

Reported citrus viroids cause Citrus Exocortis disease (CED), Citrus Cachexia disease (CCD), Citrus Leaf Bending disease (CLBD), Citrus Bark Cracking disease (CBCD) and Citrus Dwarfing disease (CDD). Among citrus diseases caused by these viroids, only citrus exocortis and citrus cachexia-xyloporosis are the most devastating and widely distributed (Hadidi *et al*., 2003). These diseases cause a reduction in yield, size of fruit and quality of production (Roistacher, 1991).

Currently, citrus viroid outbreak in Southeast Asia is at a medium stage. It may be due to inadequate research or findings in citrus viroids and diseases caused by them. There is scarcity of information or literature available on citrus viroids in Malaysia. Therefore, monitoring of citrus viroids and their characterization is the need of time. Hence, the study was carried out with the objective of detection and characterization of citrus viroids.

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