UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF CITRUS BENT LEAF VIROID (CBLVd) FROM CITRUS AND IT'S HOST RANGE

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CHARACTERIZATION OF CITRUS BENT LEAF VIROID (CBLVd) FROM CITRUS AND IT'S HOST RANGE

BY

NAVINDRRAN VELOO

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

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CERTIFICATION

This project entitled “Characterization of Citrus bent leaf viroid (CBLVd) from citrus and its host range’ is prepared by Navindrran Veloo and submitted to the Faculty of Agriculture in fulfilment of the requirement of PRT 4999 for the award of the Degree of Bachelor of Agricultural Science.

Student’s name: Student‘s signature

Navindrran Veloo ……

Bachelor of Agriculture Science

Certified by,

……

(Assoc. Prof. Dr. Ganesan Vadamalai)

Department of Plant Protection,

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ABSTRACT

Citrus is a flowering plant and shrub that belongs to *Rutaceae* family which encompasses several variety of citrus such as orange, lemon, grape fruits, and limes. Citrus viroids cause devastating impact in the citrus industry by reducing yield and plant health. Geographically, citrus viroids are widely distributed and recently Citrus bent leaf viroid (CBLVd) RNA was detected in Malaysian citrus. Although citrus is an important industry in Malaysia, there is a lack of knowledge on the characterization and host range of CBLVd in Malaysia. The purpose of this study is to identify and characterize CBLVd from citrus and to determine its alternate hosts. Nucleic acid was extracted from the eight citrus plants using TELSP buffer. The extracted RNA was amplified by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) using CBLVd specific primers. The RT-PCR products were separated using 2% agarose gel electrophoresis. RT-PCR products from positive samples were sequenced. Host range studies were conducted by using positive CBLVd samples from the citrus plants and inoculated into the alternate host through sap inoculation. The CBLVd variants characterized in this study showed high similarity with CBLVd isolate JP (AB006734.1) with a nucleotide length of 328nt. The viroid was found in *Citrus microcarpa* and *Citrus maxima* which exhibited virus and viroid like symptoms such vein yellowing, epinasty, midrib necrosis and stunted growth. The sap inoculated hosts tested with molecular assay for the presence of CBLVd but yielded negative result where CBLVd was not able to infect and develop symptoms in tomato, chilli, cucumber, and tobacco via sap inoculation.

ABSTRAK

Sitrus adalah pokok renek berbunga yang tergolong dalam keluarga *Rutaceae* yang merangkumi beberapa jenis sitrus seperti oren, lemon, buah-buahan anggur, dan limau. Viroid Sitrus menyebabkan kesan yang amat buruk kepada pokok sitrus dalam mrngurangkan hasil dan kesihatan pokok. Selain itu, ia merupakan salah satu ancaman kepada industri sitrus. Secara geografi, viroid Sitrus tersebar secara meluas dan baru-baru ini, viroid CBLVd telah dikesan di Malaysia. Sitrus merupakan industri penting di Malaysia.Namun begitu, terdapat kekurangan ilmu pengetahuan dalam menjalankan pencirian serta mengenal pasti perumah yang berpotensi dijangkiti CBLVd. Tujuan kajian ini adalah untuk mengidentifikasi dan mencirikan CBLVd daripada pokok citrus serta mengenal pasti perumah alternatif. Asid nukleik (RNA) diekstrak mengunakan TELSP buffer dan seterusnya diamplifikasi melalui Transkripsi Berbalik-Tindakbalas Rantaian Polimerase (RT-PCR) menggunakan pencetus spesifik CBLVd. Produk RT-PCR dipisahkan melalui proses elektroforesis mengunakan gel agarose 2%. Produk RT-PCR dari sampel yang menunjukan positif untuk CBLVd telah di hantar untuk mendapatkan jujukan. Kajian perumah alternatif untuk CBLVd telah dijalankan mengunakan sample Sitrus yang positif bagi CBLVd. Hasil penganalisaan jujukan menunjukan jujukan CBLVd yang dijumpai dalam kajian mempunyai persamaan yang tinggi dengan CBLVd isolat JP (AB006734.1). Panjang nukleotida CBLVd yang dijumpai adalah 328nt. Viroid ini ditemui pada pokok *Citrus maxima* dan *Citrus microcarpa* yang menunjukkan simptom seperti virus dan viroid, diantara simptom-simptomnya adalah daun bengkok, nekrosis urat daun dan pertumbuhan yang terbantut. Manakala ujian terhadap perumah alternatif menunjukan keputusan yang negatif dimana CBLVd tidak dapat menjangkiti ke dalam tomato, cili, timun, dan tembakau melalui kaedah inokulasi sap.

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ABBREVIATION

%	Percentage
°C	degree Celsius
µg	Microgram
µg/ml	Microgram per millilitre
µl	Microliter
AMV-RT	Avian Myeloblastosis Virus Reverse Transcription
ASVd	Avocado sunblotch viroid
Bp	BASE pair
CA	Choloro isoAmyl
CBCVd	Citrus bark cracking viriod
CBLVd	Citrus bent leaf viroid
CCCVd	Coconut cadang cadang viroid
cDNA	Complementary deoxyribonucleic acid
CDVd	Citrus drawfing 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	Litre
LiCL	Lithium Chloride
M	Molar
Mg	Milligram
Mg/ml	milligram per millilitre
Ml	Milliliter
Mm	Millimol
Nt	Nucleotide
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PSTVd	Potato spindle tuber viriod
PVP	Polyvinylpolypyrrolidone
RNA	Ribonucleic acid

Rpm	Rotation per minute
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 is a flowering plant and shrub that belong to *Rutaceae* family which have been evolved from a small citrus and edible berries over million years ago. Recent research indicated that citrus originated from Australia, New Caledonia and New Guinea. However, earlier research documented that citrus started spreading from Yunnan province of China. Citrus became an important crop in the world with major contribution to culinary and medicine.

Producers of citrus in the world are China, Brazil, United States, India, Mexico, and Spain (FAO, 2013). Malaysia ranked 81 among the global citrus producers and produced 36,450 tonnes of citrus in 2013 (MOA, 2013). In Malaysia, total citrus production per hectare is about 6.4 tonnes, which indicates a good production range because the world average yield ranged from 5.3- 6.7 tonnes (FAO, 2013). The intense producers of citrus in Malaysia are Sabah and Sarawak and in Peninsular Malaysia are Kelantan and Johor. Several types of citrus are cultivated in Malaysia such as *Citrus sinensis* (Limau Manis), *Citrus. maxima* (Limau Bali), *Citrus. hystrix* (Limau Kasturi), Citrus. *reticulate* (Limau Madu) and *Citrus. madurensis* (Limau Kasturi). The major contributors to Malaysian citrus production are from the species of *Citrus. sinensis*, *Citrus. limon* and *Citrus. aurantifolia.*

Citrus production in Malaysia is reducing continuously from the year 2007 after hitting the peak in 2006 with 70,308 tonnes (FAO, 2013). The reduction was assumed mostly due to the pest and disease. Specific management on pest and disease induce more operational cost causing growers to lose interest in citrus growing.

Citrus species are affected by viroids. Viroid is the smallest plant pathogens consist of a short and circular single stranded RNA with 246-401 nt in size. Citrus viroids reduces the yield and plant health, considered as a serious threat to citrus industry. To date, citrus naturally played host to viroids, namely Citrus exocortis viroid (CEVd) and Hop stunt viroid (HSVd or CVd-II) from genus Pospiviroid, Citrus bark cracking (CBCVd or CVd-IV) from genera Cocaviroid, Citrus bent leaf viroid (CBLVd or CVd-I), Citrus dwarfing viroid (CDVd or CVd-III), Citrus viroid V (CVd-V) and Citrus viroid VI (CVd-VI or CVd-OS) from genus Apscaviroid. CBLVd has been detected in Malaysia, according to research CBLVd viroid is widely distributed around the globe. Studies documented that, citrus industry is important in Malaysia yet lack of characterization and host range test of CBLVd in Malaysia. In view of this, the objectives of this study to characterization CBLVd RNA from citrus species and to test the host range of CBLVd.

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