UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF Coleus blumei viroid 5 (CbVd-5) FROM Coleus blumei IN MALAYSIA

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CHARACTERIZATION OF *Coleus blumei viroid 5* (CbVd-5) FROM *Coleus blumei* IN MALAYSIA

BY

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CERTIFICATION

The project report attached here entitled:

"Characterization of *Coleus blumei viroid 5* (CbVd-5) from *Coleus blumei* in Malaysia"

is prepared by Nurnajwa binti Zulkifli and submitted to the Faculty of Agriculture In Partial Fulfillment Of The Requirement Of PRT 4999 (Final Year Project) For Award Of The Degree of Bachelor of Agriculture Science is hereby accepted.

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ABSTRACT

Coleus blumei is an ornamental plant in the family *Lamiaceae* which originated from Indonesia. It produces many tiny beautiful flowers and has colorful of kaleidoscopic leaves with nettle-like edges and bronze-colored leaves which popularly used for landscape. However, it is known to be infected by viroids which is the smallest plant pathogen. *Coleus blumei viroid* (CbVd) is a member of family *Pospiviroid* which have been reported to cause serious problem in *Coleus blumei* species in all over the world. Its infection can be either asymptomatic or result in symptoms including dwarfing, chlorosis or purple pigmentation on leaves margin depending on the cultivar. *Coleus blumei* is susceptible to infection by six CbVd genotypes (genus, *Coleviroid*; family, *Pospiviroid*), CbVd-1 to CbVd-6. In Malaysia, only CbVd-1 has been discovered and the presence of other CbVd viroids has not been reported yet. However, CbVd-5 has been reported in Indonesia and China. The objective of this study are to detect and characterize CbVd-5 in *Coleus blumei* using reverse transcription polymerase chain reaction (RT-PCR) and two dimensional gel electrophoresis (2D-PAGE) and sequencing. 32 samples of *Coleus blumei* showing viroid-like infection symptoms and non-symptomatic plants were collected around Selangor, Kedah and Melaka. Total nucleic acid were extracted and the presence of CbVd-5 was detected by RT-PCR using CbVd-5 specific primers. RT-PCR analysis showed that 10 out of 32 *Coleus blumei* were positive CbVd-5. All RT-PCR positive samples produced an amplicon that ranged 250-300 bp on 1.5% agarose gel. Three out of 10 samples positive with CbVd-5 were samples showing viroid infection-like symptom in which, one from Selangor, and two from Kedah. Meanwhile, seven out of 10 samples positive with CbVd-5 were non-symptomatic samples in which two from Selangor, one form Kedah and four from Melaka. Sequencing of the amplicons from the positive samples showed variants of CbVd-5 with 98-99% similarity to *Coleus blumei viroid 5* clone 1, complete

genome (Genebank:FJ151370.1). Sequence analysis of clones for Selangor sample did not yield good sequencing result. The circularity of the CbVd RNA by 2D-PAGE was not able to be proven. However, the presence of CbVd-5 in *Coleus blumei* species in Malaysia was confirmed.

ABSTRAK

Coleus blumei adalah tumbuhan hiasan dalam keluarga *Lamiaceae* yang berasal dari Indonesia. Ia menghasilkan banyak bunga yang halus dan mempunyai berwarna-warni daun kaleidokop seperti netel dan daun berwarna gangsa yang popular digunakan untuk landskap. Walau bagaimanapun, ia dikenali sebagai tumbuhan yang dijangkiti viroid yang merupakan patogen tumbuhan yang paling kecil. *Coleus blumei viroid* (CbVd) adalah ahli keluarga *Pospiviroidae* yang telah dilaporkan menyebabkan masalah yang serius dalam spesies *Coleus blumei* di seluruh dunia. Jangkitan ini boleh sama ada asimptomatik atau mengakibatkan symptom termasuk bantut, daun berwarna kuning atau pigmentasi ungu pada margin daun mengikut kultivar. *Coleus blumei* mudah terdedah kepada enam jenis jangkitan CbVd (genus, *Coleviroid*; keluarga, *Pospiviroid),* CbVd-1 sehingga CbVd-6. Di Malaysia, hanya CbVd-1 telah ditemui dan kehadiran viroid CbVd lain tidak pernah dilaporkan lagi. Walau bagaimanapun, CbVd-5 telah dilaporkan di Indonesia dan China. Objektif kajian ini adalah untuk mengesan dan mencirikan CbVd-5 dalam *Coleus blumei* menggunakan RT-PCR dan dua dimensi gel elektroforesis (2D-PAGE) dan penjujukan. 32 sampel *Coleus blumei* yang terdiri daripada tumbuhan yang menunjukkan simptom dan tidak dikumpulkan sekitar Selangor, Kedah dan Melaka. Asid nukleik diekstrak dan kehadiran CbVd-5 telah dikesan oleh RT-PCR menggunakan pencetus CbVd-5. Analisis RT-PCR menunjukkan bahawa 10 daripada 32 *Coleus blumei* adalah positif CbVd-5. Semua sampel RT-PCR yang positif menghasilkan amplikon yang antara 250-300 bp pada 1.5% gel agarose. Tiga daripada 10 sampel positif dengan CbVd-5 adalah sampel menunjukkan simptom iaitu, satu dari Selangor, dan dua dari Kedah. Sementara itu, tujuh daripada 10 sampel positif dengan CbVd-5 adalah sampel yang tidak menunjukkan sebarang simptom di mana dua dari Selangor, satu dari Kedah dan empat dari Melaka. Aturan amplikon dari sampel positif menunjukkan varian CbVd-5

dengan 98-99% persamaan dengan *Coleus blumei viroid 5* klon 1, genom lengkap (bank gen: FJ151370.1). Analisis turutan klon bagi sampel Selangor tidak menghasilkan hasil turutan yang baik. Kebulatan daripada CbVd RNA menggunakan 2D-PAGE tidak dapat dibuktikan dalam kajian ini. Walau bagaimanapun, kehadiran CbVd-5 dalam spesies *Coleus blumei* di Malaysia telah disahkan

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ABBREVIATION

%	percentage
°C	Degree Celsius
µg	Microgram
µg/mL	Microgram per milliliter
µl	Microliter
Acryl	Acrylamide
AMV-RT	Avian Myeloblastosis Virus Reverse Transcription
APS	Ammonium Persulphate
bis	bisacrylamide
bp	base pair
CA	Choloroform isoAmyl
cDNA	Complementary deoxyribonucleic acid
DDW	Double distilled water
dNTP	Deoxyribonucleic triphosphate
EtBr	Ethidium Bromide
g	Gram
HCl	Hyrochloric acid
kb	Kilobase
L	Liter

M	Molar
mg	milligram
mg/mL	milligram per milliliter
MgCl	Magnesium chloride
min	minute
mL	milliliter
mM	millimol
Na_2EDTA	di-Sodium Ethylenediamine tetra acetic acid
NaAc	Sodium acetate
$NaBH_4$	Sodium borohydrate
NaCl	Sodium chloride
NaOH	Sodium hydoxide
nm	Nanometer
nt	Nucleotides
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PSTVd	Potato tuber spindle viroid
RNA	Ribonucleic acid
R-PAGE	Return Polyacrylamide Gel Electrophoresis
rpm	Rotation per time
RT	Reverse Transcription

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
TEMED	N,N,N’-N’-Tetranethylenediamine
UV	Ultraviolet light
V	Voltage
v/v	Volume/volume
Vol	Volume

CHAPTER 1

INTRODUCTION

Coleus blumei is an ornamental plant of family *Lamiaceae* originated from Indonesia and currently found throughout the world. Coleus varies in leaf color, patterns and shapes. It consist of brightly colored foliage which is nettle-like shape and varies from red, maroon, dark green, orange green, purple, brown and pink color. Coleus are popularly grown in Malaysia for beautification, home garden, and landscape purposes. *Coleus blumei* can be propagated either from their seed or stem cutting (Faucon,2005)

Coleus blumei is susceptible to *Coleus blumei viroid* (CbVd). CbVd is viroid of genus *Coleviroid*, and classified in the family *Pospoviroide*. CbVd was first reported infecting yellow Coleus in Brazil and currently infecting Coleus species all over the world (Fonseca *et al*. 1989). There are six main variants of (CbVd) that have been reported to cause disease problem to Coleus species which is CbVd-1 to CbVd-6). The transmissions of CbVd can be through mechanical, graft inoculation and Coleus seeds.

In Indonesia, *Coleus blumei viroid* 5 (CbVd-5) has been reported to cause infection in Coleus (Jiang *et al*.2013). CbVd can cause a potential threat to *Coleus blumei* in Malaysia due to the planting material of Coleus are mainly imported from Indonesia. The presence of CbVd-1 and CbVd-5 has been reported in Malaysia (Najwa,2014). However , there is still lack of knowledge

in sequence variation and circularity of the viroid RNA and need to carry out in order to confirm the presence of *Coleus blumei viroid 5* (CbVd-5) in *Coleus blumei* in Malaysia.

1.1 Objective

To detect and characterize *Coleus blumei viroid 5* from *Coleus blumei* showing viroid-like infection symptoms in Malaysia by using RT-PCR, sequencing and determining the circularity of the RNA by 2D-PAGE.

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