



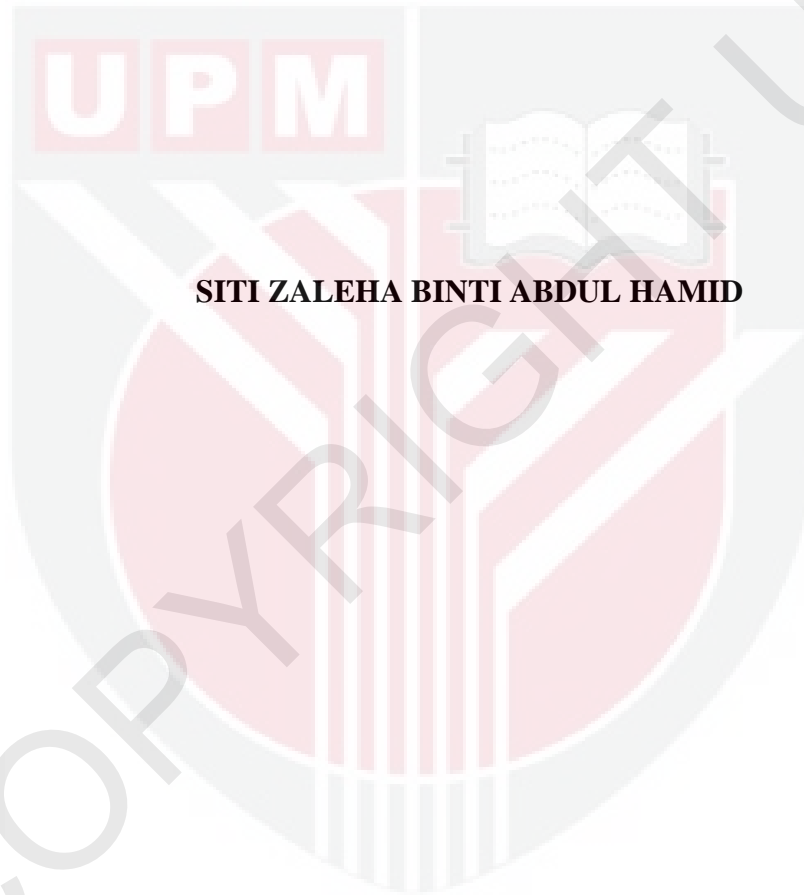
**UNIVERSITI PUTRA MALAYSIA**

**DETECTION AND CHARACTERIZATION OF *Coleus blumei viroid*  
(CbVd)**

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**FP 2016 62**

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**FACULTY OF AGRICULTURE  
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**2015/2016**

**DETECTION AND CHARACTERIZATION OF *Coleus blumei* viroid (CbVd)**

**BY**

**SITI ZALEHA BINTI ABDUL HAMID**

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

**FACULTY OF AGRICULTURE**

**UNIVERSITI PUTRA MALAYSIA**

**SERDANG, SELANGOR DARUL EHSAN**

**2015/2016**

## CERTIFICATION

This project entitled “Detection and Characterization of *Coleus blumei viroid* (CbVd)” was prepared by Siti Zaleha Binti Abdul Hamid and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 for the award of Degree of Bachelor of Agriculture Science.

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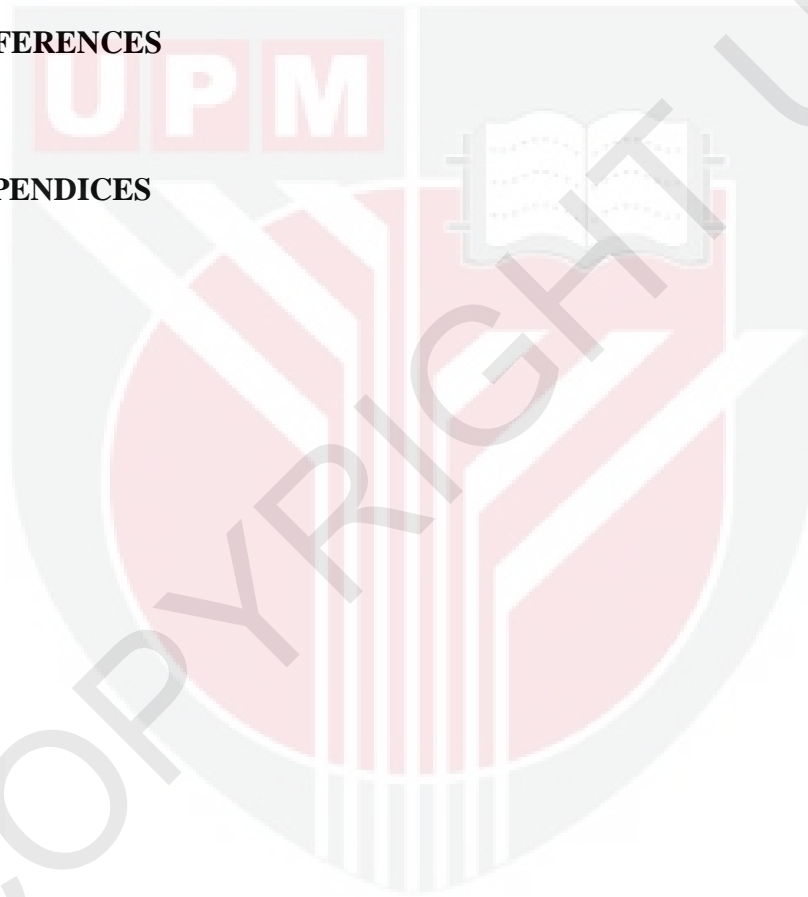
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## ABBREVIATION

%	percentage
°C	Degree Celcius
µg	Microgram
µg/mL	Microgram per milliliter
µl	Microliter
Acryl	Acrylamide
AMV-RT	Avian Myeloblastosis Virus Reverse Transcriptase
APS	Ammonium Persulphate
bis	bisacrylamide
bp	base pair
CA	Chloroform isoAmyl
cDNA	Complementary deoxyribonucleic acid
DDW	Double distilled water
dNTP	Deoxyrebonucleic triphosphate
EtBr	Ethidium Bromide
EtOH	Ethanol
g	Gram
HCl	Hydrochloric acid
kb	Kilobase
L	Liter
M	Molar
mA	miliAmpere
mg	milligram
mg/mL	Miligram per milliliter
MgCl	Magnesium chloride

min	minute
mL	milliliter
mM	milimol
NaBH <sub>4</sub>	Sodium borohydrate
NaCl	Sodium chloride
NaOH	Sodium hydroxide
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 minute
RT	Reverse Transcription
RT buffer	Reverse Transcription Buffer
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SDDW	Sterile double distilled water
sp	species
TBE	Tris-borate EDTA
TEMED	N, N, N'-N'-Tetranethylenediamine
UV	ultraviolet light
V	Voltage
v/v	Volume/volume
vol	volume
w/w	Weight/weight

## ABSTRACT

*Coleus blumei* is an ornamental plant of the family *Lamiaceae* which originated from Indonesia and currently widely distributed worldwide. *Coleus* cultivars are popularly grown as garden plants and used for landscape. This ornamental can be infected by several viroids of the genus *Coleoviroid*. *Coleus blumei* viroid (CbVd) is a viroid in family *Pospiviroid* which have been reported to cause disease in *Coleus blumei* worldwide. In Malaysia, sequences of CbVd-1 and CbVd-5 have been reported in *Coleus* species but more researches are needed to study the sequence variation and circularity of the RNA. The objectives of the study are to characterize CbVd from *Coleus* by using Reverse Transcription Polymerase Chain Reaction (RT-PCR), to sequence and to prove the circularity of the RNA by Two Dimensional Polyacrylamide Gel (2D-PAGE). A total of 14 samples of *Coleus* with 11 samples showing no symptoms and 3 samples showing CbVd-like symptoms were collected around UPM. RT-PCR analysis of nucleic acid extracted from the *Coleus* samples showed that 6 out of the 14 *Coleus blumei* samples produced an amplicon of the expected size of range between 211 to 257 of CbVd. Sequencing of the amplicon confirmed the presence of *Coleus blumei* viroid 1 (CbVd-1) and *Coleus blumei* viroid 5 (CbVd-5) variants in positive samples. The study showed 96 to 99 % sequence similarity of positive samples with the reference; *Coleus blumei* viroid 1 (CbVd-1) (249nt) CbVd-K complete genome (Genbank: EU410620.1) and *Coleus blumei* viroid 1 (CbVd-1) CbVd-Chi complete genome (Genbank: DQ178399.1) with variants ranged from 211 to 240 nt. Positive samples also showed 84 to 91 % sequence similarity with *Coleus blumei* viroid 5 (CbVd-5) (274nt) clone 1, complete genome (Genbank : FJ151370.1) with variants

range from 241 to 257 nt. The detected variants of CbVd-1 and CbVd-5 were from symptomatic and non-symptomatic *Coleus sp.* Meanwhile, 2D-PAGE produced negative results after few trials. Therefore, the circularity of the CbVd RNA cannot be confirmed in this study.



## ABSTRAK

*Coleus blumei* merupakan tumbuhan hiasan dalam keluarga *Lamiacea* yang berasal dari Indonesia dan kini diedarkan secara meluas di seluruh dunia. Kultivar *Coleus* popular ditanam sebagai tumbuhan taman dan digunakan untuk landskap. Hiasan ini boleh dijangkiti oleh beberapa viroid genus *Coleoviroid*. *Coleus viroid blumei* (CbVd) adalah viroid dalam keluarga *Pospiviroid* yang telah dilaporkan menyebabkan jangkitan penyakit pada *Coleus blumei* di seluruh dunia. Di Malaysia, varian CbVd-1 dan CbVd-5 telah dilaporkan dalam spesies *Coleus* tetapi lebih banyak kajian diperlukan untuk mengkaji variasi urutan dan kebulatan RNA ini. Objektif kajian ini adalah untuk mencirikan CbVd dari *Coleus* dengan menggunakan Reverse Transcription Polymerase Chain Reaction (RT-PCR) dan penjujukan dan untuk membuktikan kebulatan RNA dengan penjujukan Dua Dimensi Polyacrylamide Gel (2D-PAGE). Sebanyak 14 sampel tumbuhan *Coleus* yang mana 11 sampel yang tidak mempunyai gejala dan 3 sampel dengan gejala CbVd telah dikumpulkan dari sekitar UPM. Analisis RT-PCR asid nukleik yang diekstrak daripada sampel *Coleus* menunjukkan bahawa 6 daripada 14 sampel *Coleus blumei* menghasilkan saiz amplicon antara 211-257 daripada pelbagai varian CbVd. Aturan amplicon telah mengesahkan kehadiran varian *Coleus blumei viroid 1* (CbVd-1) dan *Coleus blumei viroid 5* (CbVd-5) dalam sampel positif. Kajian menunjukkan 96 hingga 99% urutan persamaan sampel positif dengan *Coleus blumei viroid 1* (CbVd-1) (249nt) CbVd-K genom lengkap (GenBank: EU410620.1) dan *Coleus blumei viroid 1* (CbVd-1) CbVd-Chi genom lengkap (GenBank: DQ178399.1) dengan saiz varian adalah di antara 211-240 nt. Sampel positif juga menunjukkan 84-91%



urutan persamaan dengan *Coleus blumei viroid 5* (CbVd-1) (274nt) klon 1, genom lengkap (GenBank: FJ151370.1) dengan saiz varian berkisar 241-257 nt. Varian CbVd-1 dan CbVd-5 yang dikesan ialah dari gejala dan bukan gejala *Coleus sp.* Sementara itu 2D-PAGE menunjukkan hasil negatif selepas beberapa percubaan. Oleh itu, kebulatan daripada CbVd RNA tidak dapat disahkan.



## CHAPTER 1

### INTRODUCTION

*Coleus blumei* is an important ornamental plant of family *Lamiacea* which originated in Indonesia and currently distributed around the worldwide. Coleus consists of brightly color foliage. It varies in leaf color, leaf patterns and leaf shapes. The color of foliage changing varies from red, maroon, dark green, orange green, purple, brown, violet, purple, dark green and other colors. Coleus are popularly grown in Malaysia for beautification, home garden and landscape purposes. It can be propagated through seed and cutting (Faucon, 2005).

*Coleus blumei* is susceptible to *Coleus blumei viroid* (CbVd). CbVd is classified in family *Pospiviroidae* under genus *Coleviroid*. CbVd has been reported infecting *Coleus blumei* species all around the world and the first reported CbVd infecting *Coleus blumei* was in Brazil in a commercially field grown yellow Coleus in Brazil (Fonseca *et al.* 1989). The transmissions of CbVd are through mechanical, graft inoculation and seed.

*Coleus blumei* in Indonesia has been reported to be infected with *Coleus blumei viroid 5* (Jiang *et al.* 2013). Since the planting materials of Coleus are imported from Indonesia, the transmission of CbVd can cause a threat to Coleus in Malaysia. *Coleus blumei viroid 1* (CbVd-1) and *Coleus blumei viroid 5* (CbVd-5) have been reported in

Malaysia (Najwa, 2014). However, there is still lack of knowledge in sequence variation and circularity of the viroid RNA. Therefore, the objectives of this study are to characterize CbVd from Coleus by using RT-PCR, to sequence and determine the circularity of the RNA by 2D-PAGE.



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