UNIVERSITI PUTRA MALAYSIA

DETECTION, CHARACTERIZATION AND PATHOGENICITY OF *Coleus blumei viroid* IN *Coleus blumei* BENTH. IN PENINSULAR MALAYSIA

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FP 2019 26

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Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

January 2019

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman: Associate Professor Ganesan Vadamalai, PhD
Faculty: Agriculture

Plectranthus scutellarioides or synonym known as *Coleus blumei* is a popular ornamental plant in Malaysia grown for beautification as garden plants and mostly used for landscape due to their brightly colour foliage. However, this plant is susceptible to infection of six variants of *Coleus blumei viroid* (CbVd-1 to CbVd-6) of genus *Coleviroid* from family *Pospiviroidae.* CbVd is distributed worldwide, but yet to be reported in Malaysia. Inadequate information on viroid disease in ornamental plant species, specifically *C. blumei* in Malaysia is of concern for the landscape industry. In view of this, the objectives of this study were (a) to detect and characterize of CbVd from *C. blumei* benth. in Peninsular Malaysia using RT-PCR, cloning and sequencing, (b) to study the pathogenicity of CbVd-1 and CbVd-5 on *C. blumei* cultivar 'Dipt in wine'. Altogether, 49 samples of *C. blumei* showing viroid-like symptoms such as stunted growth, faded leaf colour and reduction in leaf size and non-symptomatic consists mixed cultivars were sampled from Perlis, Kedah, Perak, Selangor, Melaka, Kelantan and Johor. RT-PCR was used to detect CbVd using three sets of CbVd primers (universal CbVd primer, specific CbVd1K and specific CbVd-5 primer). Results of molecular detection by RT-PCR showed that 47 of 49 samples were positive for CbVd (CbVd universal primer), 45 samples were positive with CbVd-1 (CbVd1K primer) and 41 samples were positive with CbVd-5 (CbVd-5 primer). Meanwhile, mixed infection of both CbVd-1 and CbVd-5 variants were detected in 40 out of 49 collected samples. All nucleotides of CbVd-1 and CbVd-5 positive samples were 249-250 nt and 274 nt in length. Sequence analysis of both CbVd-1 (MF176948-MF176951) and CbVd-5 clones (MF176952-MF176955) revealed 99% to 100% sequence similarity to CbVd-1 clone 1, complete genome (GenBank Accession No. DQ178399.1) and Korea (GenBank Accession No. EU410620.1) and CbVd-5 clone 1, complete genome (GenBank Accession No. FJ151370.1) from China. Results from phylogenetic analysis of the Malaysian CbVd-1 with isolates from Korea, India, China, Brazil and Germany showed that the Malaysian isolates formed same clade with Korea (EU410620), India (AB740017 and AB740018), China (DQ178397- DQ178399) and Brazil (X69293) meanwhile CbVd-

5 isolates with isolates from China (FJ151370-FJ151372 and NC012127) and Japan (LC068970). Pathogenicity study showed that *C. blumei* cultivar 'Dipt in wine' seedlings inoculated with leaf sap and plasmid containing a CbVd-1 (CbVd-1_J1MY) and CbVd-5 insert (CbVd-5_R3MY) expressed only leaf colour fading symptom meanwhile inoculated seedlings with leaf sap and plasmid containing combination of both CbVd variants (CbVd-1_J1MY and CbVd-5_R3MY) showed leaf colour fading and stunting over 12 weeks observation period. CbVd-1, CbVd-5 and combination of both variant were detected in *C. blumei* cultivar 'Dipt in wine' seedlings between 3 to 9 weeks post-inoculation by RT-PCR. Sequencing of the amplicons confirmed the presence of CbVd-1, CbVd-5 and mixed infection with more than 95% sequence similarity with CbVd-1 (CbVd-1_J1MY) and CbVd-5 variants (CbVd-5_R3MY) that were used for the inoculation. In summary, CbVd-1 and CbVd-5 were present in *C. blumei* in Peninsular Malaysia and they were able to replicate in *C. blumei* cultivar 'Dipt in wine'. This is the first report of CbVd-1 and CbVd-5 in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGESANAN, PENCIRIAN DAN KEPATOGENAN *Coleus blumei viroid* PADA *Coleus blumei* BENTH. DI SEMENANJUNG MALAYSIA

Oleh

NURUL NAJWA BINTI CHE ROSLAN

Januari 2019

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Plectranthus scutellarioides atau lebih dikenali sebagai *Coleus blumei* merupakan tumbuhan hiasan yang popular ditanam di Malaysia untuk tujuan keindahan sebagai tumbuhan taman dan kebanyakannya digunakan untuk landskap disebabkan daunnya yang berwarna terang. Walau bagaimanapun, tumbuhan ini terdedah kepada jangkitan enam varian *Coleus blumei viroid* (CbVd-1 hingga CbVd-5) daripada genus *Coleviroid* dari keluarga *Pospiviroidae.* CbVd telah tersebar di seluruh dunia, namun belum dilaporkan di Malaysia. Maklumat yang tidak mencukupi mengenai penyakit viroid dalam spesis tanaman hiasan, khususnya *C. blumei* menjadi kebimbangan terhadap industri landskap di Malaysia. Oleh yang demikian, objektif kajian ini adalah (a) Untuk mengesan dan mencirikan CbVd dari *C. blumei* benth. di Semenanjung Malaysia menggunakan RT-PCR, pengklonan dan penjujukan, (b) Untuk menguji kepatogenan CbVd-1 dan CbVd-5 pada anak benih *C. blumei* kultivar Dipt in wine'. Kesemuanya 49 sampel *C. blumei* menunjukkan gejala jangkitan viroid seperti pertumbuhan terbantut, pudar warna daun dan pengurangan saiz daun serta tidak menunjukkan sebarang gejala jangkitan viroid, yang terdiri daripada pelbagai jenis kultivar telah disampel daripada Perlis, Kedah, Perak, Selangor, Melaka, Kelantan dan Johor. RT-PCR telah digunakan untuk mengesan CbVd melalui penggunaan tiga pasang CbVd primer (universal CbVd primer, spesifik CbVd1K dan spesifik CbVd 5 primer). Hasil pengesanan molekul oleh RT-PCR menunjukkan bahawa 47 daripada 49 sampel adalah positif dengan CbVd-1 (CbVd1K primer) dan 41 sampel positif dengan CbVd-5 (CbVd 5 primer). Manakala, 40 daripada 49 sampel positif dengan kedua-dua varian CbVd-1 dan CbVd-5. Kesemua sampel yang positif dengan CbVd-1 mempunyai saiz diantara 249 nt hingga 250 nt dan CbVd-5 dengan 274 nt. Analisis jujukan kedua-dua klon CbVd-1 (MF176948-MF176951) dan CbVd-5 (MF176952-MF176955) menunjukan kadar persamaan 99% hingga 100% kepada CbVd-1 klon 1, genom lengkap yang dilaporkan di China (GenBank Accession No. DQ178399.1) dan Korea (GenBank Accession No. EU410620.1) dan CbVd-5 klon 1, genom lengkap (GenBank Accession No. FJ151370.1) yang dilaporkan di China. Hasil daripada analisis filogenik, isolat CbVd-1 Malaysia dengan isolat dari Korea, India, China,

Brazil dan German menunjukkan isolat Malaysia membentuk klad yang sama dengan Korea (EU410620), India (AB740017 dan AB740018), China (DQ178397-DQ178399) dan Brazil (X69293) manakala isolat CbVd-5 dengan isolat dari China (FJ151370-FJ151372 dan NC012127) dan Jepun (LC068970). Kajian patogenisiti menunjukkan anak benih *C. blumei* kultivar 'Dipt in wine' yang diinokulasi dengan sap daun dan plasmid yang mengandungi selitan CbVd-1 (CbVd-1_J1MY) dan CbVd-5 (CbVd-5_R3MY) hanya menunjukan gejala pudar warna daun. Manakala, bagi anak benih yang diinokulasikan dengan gabungan varian CbVd-1 dan CbVd-5 (CbVd-1_J1MY and CbVd-5_R3MY) menunjukkan gejala pudar warna daun dan pertumbuhan terbantut sepanjang 12 minggu pemerhatian. CbVd-1, CbVd-5 dan gabungan kedua-dua varian telah dikesan dalam anak benih *C. blumei* cultivar 'Dipt in wine' yang diinokulasi di antara 3 hingga 9 minggu per-inokulasi oleh RT-PCR. Penjujukan amplikon mengesahkan kehadiran CbVd-1, CbVd-5 dan campuran jangkitan CbVd-1 dan CbVd-5 dengan persamaan lebih 95% pada varian CbVd-1 (CbVd-1_J1MY) dan CbVd-5 (CbVd-5_R3MY) yang digunakan untuk inokulasi. Secara ringkas, CbVd-1 dan CbVd-5 dikesan pada *C. blumei* di Semenanjung Malaysia dan mereka mampu berreplikasi dalam *C. blumei* cultivar 'Dipt in wine'. Laporan ini merupakan laporan pertama varian CbVd-1 dan CbVd-5 di Malaysia.

ACKNOWLEDGEMENTS

"Allah thank you for giving me the strength and encouragement especially during all the challenging moments in completing this thesis. I am truly grateful for your exceptional love and grace during this entire journey"

Firstly, I would like to express my sincere gratitude to my supervisor, Assoc. Prof. Dr. Ganesan Vadamalai for continuous support, guidance, encouragement, patient, motivation and critical discussion throughout my study at Universiti Putra Malaysia (UPM). His fantastic mentorship and immense knowledge have help me in all the time of research and writing of this thesis. I feel very fortunate to have advisor and mentor like him for my master study. I extend my gratitude to my Co-supervisor, Dr. Kong Lih Ling for her insightful comments, encouragement, and suggestion which incented me to widen my research from various perspectives. My sincere gratitude to Dr. Sathis Sri Thanarajoo for her encouragement, moral support, long discussion and expert suggestion.

I would like to thanks my best friends, Khoo Ying Wei and Nurulatika Binti Minhad for support, crying and all the fun we had in the last three years. Thanks also to my fellow lab mate, Muhammad Bukhari for helping me during conducting pathogenicity study. My gratitude to MyBrain 15 scholarship scheme for funding me to complete my studies. I would like to extend my gratitude to all staffs at Faculty of Agriculture, Institute Tropical of Agriculture (ITA) and Institute of Plantation Study (IKP) for giving me the access to use the laboratory and research facilities to carry out the experiments for this study.

I would like to dedicate this dissertation to my beloved parents, parent in law and my lovely siblings for their endless love, support and prayers. My special thanks goes to my husband, Wan Muhammad Hanif Bin Wan Aziz for your endless love, financial support and motivating me spiritually throughout completing this study and my life in general. Special thank also goes to my son for always accompany me during sampling and being my stress relieves pills.

Finally, a huge thank you to everyone who has been a part of my life directly or indirectly supported and prayed for me along the way.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follow:

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LIST OF ABBREVIATIONS

%	Percentage
2D-PAGE	Two-dimensional polyacrylamide gel electrophoresis
AMV-RT	Avian myeloblastosis virus reverse transcriptase
ANOVA	Analysis of variant
ASBVd	*Avocado sunblotch viroid*
Blast	Basic local alignment search tool
bp	Base pair
CA	Chlorophorm: isoAmyl alcohol
CbVd	*Coleus blumei viroid*
CbVd-1	*Coleus blumei viroid-1*
CbVd-2	*Coleus blumei viroid-2*
CbVd-3	*Coleus blumei viroid-3*
CbVd-4	*Coleus blumei viroid-4*
CbVd-5	*Coleus blumei viroid-5*
CbVd-6	*Coleus blumei viroid-6*
CCCVd	*Coconut cadang-cadang viroid*
CChMVd	*Chrysanthemum chlorotic mottle viroid*
CCR	Conserved central region
cDNA	Complementary deoxyribonucleic acid
CEVd-A	*Citrus exocortis viroid* –A
cm	Centimeter
CRD	Completely randomized design
CTAB	Cetyl trimethylammonium bromide
CTiVd	*Coconut tinangaja viroid* (CTiVd)
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dGTP	Deoxyguanosine triphosphate
DNA	Deoxyribonucleic acid
dTTP	Deoxythymidine triphosphate
EDTA	Ethylenediaminetetraacetic acid
ELVd	*Eggplant latent viroid*
EtBr	Ethidium bromide
EtOH	Ethanol
g	Gram
g	Relative centrifugal force
HLVd	*Hop latent viroid*
HSVd	*Hop Stunt Viroid*
M	Moles/ liter (Molarity)
Mg	Milligram
MgCl2	Magnesium chloride
min	Minute
mL	Milliliter
mM	Millimolar
Multiplex RT-PCR	Multiplex reverse transcriptase polymerase chain reaction
NaCl	Sodium chloride

ng	Nanogram
nt	Nucleotide
P	Pathogenicity
PLMVd	*Peach latent mosaic viroid*
pmole	Picomole
PSTVd	*Potato tuber spindle tuber viroid*
RNA	Ribonucleic acid
RNAsin	RNase inhibitor
R-PAGE	Return-polyacrylamide gel electrophoresis
rpm	Revolutions per minute
RT-PCR	Reverse transcription polymerase chain reaction
sec	second
TASVd	*Tomato apical stunt viroid*
TBE	Tris-borate EDTA
TL	Terminal left
TPMVd	*Tomato planta macho viroid*
TR	Terminal right
Tris-HCL	*Tris*(hydroxymethyl) aminomethane *hydrochloride*
UV	Ultraviolet
V	Variable
V	Voltage
X-gal	5-bromo-4-chloro-3-Indolyl –D-galactopyranoside
µL	Microliter
µM	Micromolar

CHAPTER 1

INTRODUCTION

Plectranthus scutellarioides or namely known as *Coleus blumei* is one of the flowering plant from family *Lamiaceae.* This plant was originated from Africa and Indonesia but it is now distributed throughout the world including Malaysia. *C. blumei* is widely grown in many countries as bedding and garden plant. This ornamental plant can be raised through seed and stem cutting (Toogood, 1999; Rogers, 2008; Manoj *et al.,* 2015).

C. blumei is susceptible to various pests and diseases including viroid diseases. *Coleus blumei viroid* (CbVd) from the genus *Coleviroid* is reported worldwide to cause infection in *C. blumei.* The infection of this viroid on *C. blumei* resulted in symptomatic or asymptomatic infection depending on the cultivars (Fonseca *et al.,* 1989; Spieker, 1996a). The symptoms induced by CbVd includes stunting, chlorosis or purple pigmentation on leaf margin, leaf color fading and reduction in leaf size (Fonseca *et al.,* 1989; Spieker, 1996a; Singh and Boucher, 1991; Chung and Choi, 2008). CbVd can be transmitted among coleus through seeds, mechanical and grafting inoculation.

C. blumei has been reported worldwide infected with six main CbVd variants which are CbVd-1 to CbVd-6. However, there is no report on CbVd infecting *C. blumei* in Malaysia. Previous studies has proven the imported seed from Japan and United State available in commercial market in China, Korea, and Canada have been infected with CbVd (Singh and Boucher, 1990; Chung and Choi, 2008, Hou *et al.,* 2009a). Since CbVd are transmitted through seed, there is a probability that CbVd are widely present in Malaysia as most of the *C. blumei* planting materials in Malaysia are imported. In addition, most nurseries in Malaysia propagate this species through stem cutting.

There are many molecular diagnostic tools that can be used for viroids detection in plants as well as CbVd in *C. blumei*. Previous studies showed detection of CbVd mainly conducted using Polyacrylamide gel electrophoresis (PAGE) variations such as two dimensional polyacrylamide gel electrophoresis (2D-PAGE) and return polyacrylamide gel electrophoresis (R-PAGE) (Singh *et al.,* 1991; Hou *et al.,* 2009b; Jiang *et al.,* 2013), molecular hybridization such as northern blot hybridization and dot-blot hybridization (Li *et al.,* 2006), RT-PCR (Jiang *et al.,* 2011) microarray (Zhang *et al.,* 2013) and next generation sequencing (Zhang *et al.,* 2014). However, in this research, RT-PCR will be used for detection of CbVd in *C. blumei* in Peninsular Malaysia.

This study was conducted to confirm the present of CbVd in *C. blumei* in Peninsular Malaysia. In addition, this study will prove the Koch postulate of CbVd. The

outcomes of the present study will enable the development of disease management strategies in order to avoid a possible outbreak of CbVd in Malaysia. In view of this, the objectives of this study are:

1. To detect and characterize CbVd from *C. blumei* Benth. in Peninsular Malaysia using RT-PCR, cloning and sequencing.

2. To test the pathogenicity of CbVd variants on *C. blumei* cultivar 'Dipt in wine'

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PUBLICATION

Journal Paper

Roslan, N. N. C., Thanarajoo, S. S., Kadir, J., Kong, L. L., & Vadamalai, G. (2017). FIRST REPORT OF COLEUS BLUMEI VIROID IN MALAYSIA. *Journal of Plant Pathology*, *99*(3), 800.

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