



UNIVERSITI PUTRA MALAYSIA

***CHARACTERISATION OF CUCUMBER MOSAIC VIRUS (CMV) IN
Catharanthus roseus (L.) G. DON AND ITS EFFECTS ON ANTICANCER
METABOLITE PRODUCTION***

MAZIDAH BT. MAT

FP 2012 26

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METABOLITE PRODUCTION**

By
MAZIDAH BT. MAT

**Thesis submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of
Philosophy**

June 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfilment of the requirements for the degree of Doctor of Philosophy

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Chairman: Lau Wei Hong, PhD

Faculty: Agriculture

Natural virus-like disease symptoms such as mosaic and deformation of the leaves and flowers of malformed shape or slight colour-breaking of the petals were observed on *Catharanthus roseus* plants in Serdang, Selangor, Malaysia. Double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) detected cucumber mosaic virus (CMV) in high concentrations in the leaf extract of naturally-infected *C. roseus* as well as in the leaf extract of inoculated *C. roseus*. The purified virions were isometric particles with mean diameter 28.60 ± 0.48 nm and contained a central core. The virus induced systemic leaf mosaic on *N. tabacum* cv. White Burley, *C. sativus*, *N. benthamiana* and *N. glutinosa*. Local lesions and brown necrotic local lesions were produced on the inoculated leaves of *C. amaranticolor* and *V. sesquipedalis*, respectively. A 1000 bp DNA fragment covering the entire coat protein (CP) region of the purified virus was amplified using

the primers CMVF1(5'-TAGACAT/ACTGTGACGCGA-3') and CMVR2 (5'-GTAAGCTGGATGGACAAC-3'). The sequence obtained (GenBank accession number EU726631) revealed a 100% nucleotide and amino acid identities to a CP gene of CMV isolated from *C. roseus* in India (GenBank accession number EU310928) which is a member of subgroup 1B. Cytopathological study showed that CMV infection disrupted the chloroplast ultrastructure in the *C. roseus* leaf cells. The presence of large starch grains in the surrounding necrotic zones caused disintegration of the stromatic lamellae and grana, changing the chloroplasts symmetry. Complex membranous structures in the cells vacuoles and phytoferritin macromolecules in the chloroplast stroma were also observed in the leaf cells of CMV-infected *C. roseus*. Chloroplast was also the most altered organelle following CMV infection in the leaf cells of *N. tabacum* cv. White Burley, *C. sativus* and *C. amaranticolor*. A comparative HPLC analysis on the yields of two anticancer metabolites, vincristine and vinblastine in the *C. roseus* leaf, stem and root tissues at different growing stages indicated that CMV infection modified the metabolism of the metabolites. Following CMV infection, the peak production period of vincristine and vinblastine in leaves was delayed from four months old uninfected *C. roseus* plants to six months old CMV-infected plants. CMV infection also delayed the peak production period of vincristine in roots from five months old uninfected plants to seven months old infected ones. As for vinblastine, the peak production period was delayed from five months old uninfected

plants to eight months old infected plants. Another remarkable alteration after CMV infection is that significant increases in the contents of vincristine in the infected root tissues, particularly at six to nine months old. The concentration of vinblastine which increased about two, four and seven-folds in the roots of six, seven and eight months old CMV-infected plants, respectively, could be another interesting finding of CMV modification, particularly in these anticancer compounds biosynthesis pathway in *C. roseus* plants.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra
Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENCIRIAN CUCUMBER MOSAIC VIRUS DALAM *Catharanthus
roseus* (L.) G. DON DAN KESANNYA TERHADAP PENGHASILAN
METABOLIT ANTIKANSER**

Oleh

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Simptom seakan penyakit virus secara semulajadi seperti mozek dan daun berubah bentuk, bunga berubah bentuk atau mempunyai kelopak yang pecah-warna telah diperhatikan pada tanaman *C. roseus* di Serdang, Selangor, Malaysia. DAS-ELISA telah mengesan CMV dalam kepekatan yang tinggi di dalam ekstrak daun *C. roseus* yang dijangkiti secara semulajadi dan ekstrak daun *C. roseus* terinokulasi. Virus tulen merupakan partikel-partikel isometrik berdiameter purata 28.62 ± 0.48 nm dengan teras tengah. Inokulasi virus secara mekanikal telah menghasilkan mozek daun sistemik pada *N. tabacum* cv. White Burley, *C. sativus*, *N. benthamiana* dan *N. glutinosa*. Lesi-lesi setempat dan lesi-lesi perang nekrotik masing-masing telah terhasil pada daun-daun *C. amaranticolor* dan *V. sesquipedalis* terinokulasi. Suatu fragmen DNA bersaiz 1000 bp yang meliputi keseluruhan kawasan sarung

protein virus tulen telah diamplifikasi menggunakan primer-primer CMVF1(5'-TAGACAT/ACTGTGACGCGA-3') dan CMVR2(5'-GTAAGCTGGATGGACAAC-3'). Data jujukan sarung protein CMV *C. roseus* ini (GenBank nombor aksesii EU726631) mempunyai 100% persamaan identiti nukleotida dan asid amino dengan gen sarung protein isolat CMV daripada *C. roseus* di India (GenBank nombor aksesii EU310928) yang merupakan ahli sub kumpulan 1B. Kajian sitopatologi menunjukkan jangkitan isolat CMV telah memusnahkan ultrastruktur kloroplas dalam sel-sel daun *C. roseus*. Kehadiran butiran kanji yang besar di dalam zon nekrotik telah menyebabkan nyahintegrasi lamella dan grana stroma dan mengakibatkan gangguan kepada simetri kloroplas. Struktur bermembran kompleks di dalam vakol sel dan makromolekul fitoferitin di dalam stroma kloroplas telah diperhatikan dalam sel-sel daun *C. roseus* yang dijangkiti CMV. Kloroplas juga merupakan organel yang paling terubah berikutan jangkitan CMV di dalam sel-sel daun *N. tabacum* cv. White Burley, *C. sativus* dan *C. amaranticolor*. Suatu perbandingan diantara analisis HPLC keatas penghasilan dua sebatian antikanser, vinkristin dan vinblastin di dalam tisu daun, batang dan akar *C. roseus* pada tahap pertumbuhan berbeza menunjukkan jangkitan CMV telah mengubah metabolisme kedua-dua sebatian berkenaan. Berikutan jangkitan CMV, puncak penghasilan vinkristin dan vinblastin di dalam daun telah dilewatkan dari umur empat bulan bagi *C. roseus* yang tidak dijangkiti ke umur enam bulan bagi pokok yang dijangkiti CMV. Jangkitan CMV

juga melewati puncak penghasilan vinkristin di dalam akar dari umur lima bulan bagi pokok yang tidak dijangkiti ke tujuh bulan bagi pokok yang dijangkiti. Untuk vinblastin di dalam akar, puncak penghasilan juga telah dilewatkan dari umur lima bulan bagi pokok tidak dijangkiti ke umur lapan bulan bagi pokok yang dijangkiti CMV. Perubahan lain yang ketara selepas jangkitan CMV ialah penambahan kandungan vinkristin yang jelas dalam tisu akar, terutama pada umur enam hingga sembilan bulan. Kepekatan vinblastin yang telah meningkat secara dua, empat dan tujuh kali ganda dalam akar pada umur enam, tujuh dan lapan bulan masing-masing merupakan suatu penemuan yang menarik berkenaan dengan perubahan akibat jangkitan CMV, terutamanya dalam tapakjalan biosintesis sebatian antikanser untuk tanaman *C. roseus*.

ACKNOWLEDGEMENTS

All praise be to Allah the Almighty, for giving me the strength to write this thesis and complete this study.

I'm so thankful and would like to express my most sincere gratitude and deep appreciation to my supervisor, Dr. Lau Wei Hong for her non-stop support, guidance, encouragement, constructive comments and advices during this study and preparation of this thesis. My most sincere gratitude and appreciation is also dedicated to all my co-supervisors, Professor Datin Paduka Dr. Khatijah Mohd. Yusoff from MOSTI, Associate Professor Dr. Tan Yee How from UPM and Dr. Habibuddin Hashim from MARDI for generous help, guidance and support throughout this study and thesis preparation.

My deepest gratitude and special acknowledgement to MARDI for giving me this opportunity and financial support for my PhD programme.

My special thanks to RIC Reseach Centre, Strategic Research Centre and Biotechnology Research Centre MARDI for kind permission to use research equipments and facilities throughout the study. Technical assistance from Mrs. Siti Maryam in molecular cloning and PCR is greatly appreciated. Not forget Mr. Zaiful and Pak Ya.

To all officers and staffs in the Electron Microscopy Unit, Institute BioScience, UPM especially Mr. Rafiuzaman, Mrs. Aminah, Mr. Ho, Mrs. Farah, Mrs. Yati and Mrs. Faridah, your help and kindness will be always in my mind.

My special thanks to all colleagues and staff members in RIC MARDI especially Samsiah for teaching me HPLC and Mr. Razali for helping me in the thesis preparation. Thanks also to all SR members especially Airin, Dr. Tosiah, Zaimah and Duni. Not forget, Mr. Mahendra and Mr. Malek for helping me in glasshouse works. A very special thanks to my best friend, Mr. Lou of CABI for being very helpful in the statistical analysis. Lastly, special dedication for my beloved family, this is actually our success.

I certify that a Thesis Examination Committee has met on 7th June 2012 to conduct the final examination of Mazidah Bt. Mat on her thesis entitled "Characterisation of Cucumber Mosaic Virus in *Catharanthus roseus* (L.) G. Don and Its Effects on Anticancer Metabolite Production in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, or is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

UPM

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Date: 7 JUNE 2012



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