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INCIDENCE AND DIFFERENTIATION OF Cucumber mosaic virus (CMV) ISOLATES IN PENINSULAR MALAYSIA

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INCIDENCE AND DIFFERENTIATION OF *Cucumber mosaic virus* (CMV) ISOLATES IN PENINSULAR MALAYSIA



By

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

INCIDENCE AND DIFFERENTIATION OF Cucumber mosaic virus (CMV) ISOLATES IN PENINSULAR MALAYSIA

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Chair: Associate Professor Kamaruzaman Sijam, PhD

Faculty: Agriculture

A survey on the occurrence and incidence of *Cucumber mosaic virus* (CMV) was carried out on selected horticultural crops in Peninsular Malaysia. A total of 1293 leaf samples showing virus-like symptoms comprising of chilli (527 samples), tomato (63 samples), eggplant (183 samples), cucumber (209 samples), pineapple (161 samples), long bean (49 samples), and loofah (101 samples) were collected from the field at 35 different locations in 7 states (Selangor, Pahang, Melaka, Johor, Kelantan, Perak and Pulau Pinang) to detect the presence of CMV. The detection of CMV was done using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Based on the DAS-ELISA results, CMV was detected in 9% of the entire surveyed crops. The highest percentage of CMV incidences was found in Selangor with 48% infection, followed by Kelantan and Melaka (15%), Pahang (8%), Johor (7%), Pulau Pinang (4%), and Perak (1%). Amongst the

samples collected, CMV was discovered to be the most prevalent on eggplant and chilli with 12% disease incidence each, followed by tomato (10%), cucumber (5%), long beans (4%) and pineapple and loofah (3% each).

CMV-infected samples detected by DAS-ELISA were used in reversetranscription polymerase chain reaction (RT-PCR) analysis. Gene-specific primers were designed and a primer was then selected to amplify the region of *coat protein* (*CP*) gene, producing a specific band sized of approximately 600 base pair (bp). Samples used for RT-PCR included crops collected from Selangor, Pahang, Melaka, Johor, Kelantan, Perak, Pulau Pinang and with an addition of samples from Terengganu. Only crops collected from Johor (long beans, eggplant, cucumber, chilli and pineapple), Pahang (chilli, tomato, cucumber and eggplant), Terengganu (chilli, loofah and eggplant), Perak (cucumber), Pulau Pinang (chilli) and Selangor (chilli, tomato and eggplant) produced DNA bands of approximately 600 bp in size.

Nucleotide sequences of the *CP* gene of 20 CMV isolates from tomato, eggplant, loofah, chilli and tobacco collected in Terengganu, Pahang, Penang, Perak and Selangor were confirmed as CMV. Phylogenetic analysis of the partial *CP* genes suggest that the local CMV isolates belong to subgroup I (S-I) and are grouped together with the subgroup IB (S-IB) strains. The results further proposed that the isolates can be arranged according to the type of crops and geographical location.

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

INSIDEN DAN PEMBEZAAN ISOLAT Cucumber mosaic virus (CMV) DI SEMENANJUNG MALAYSIA

Oleh

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Satu kajian ke atas kejadian dan insiden *virus cucumber mosaic* (CMV) telah dijalankan pada tanaman hortikultur terpilih di Semenanjung Malaysia. Sejumlah 1293 sampel daun yang menunjukkan simptom serangan virus yang terdiri daripada cili (527 samples), tomato (63 samples), terung (183 samples), timun (209 samples), nenas (161 samples), kacang panjang (49 samples), dan petola (101 samples) telah dikutip daripada lapangan dalam 35 lokasi berbeza di 7 buah negeri (Selangor, Pahang, Melaka, Johor, Kelantan, Perak dan Pulau Pinang) untuk mengesan kehadiran CMV. Pengesanan CMV telah dibuat menggunakan "double antibody sandwich enzyme-linked immunosorbent assay" (DAS-ELISA). Berdasarkan kepada keputusan DAS-ELISA, CMV telah dikesan di dalam 9 % daripada keseluruhan sampel. Peratusan tertinggi insiden CMV ditemui di Selangor dengan 48 % jangkitan, diikuti dengan Kelantan dan Melaka (15%), Pahang (8%), Johor (7%), Pulau Pinang (4%), dan Perak (1%). Di kalangan sampel

yang dikutip, CMV didapati lebih tersebar luas pada terung dan cili dengan 12 % insiden penyakit setiap satu, diikuti dengan tomato (10%), timun (5%), kacang panjang (4%), serta nenas dan petola (3% setiap satu).

Sampel yang dikesan dijangkiti CMV oleh DAS-ELISA telah digunakan dalam analisis "reverse-transcription polymerase chain reaction" (RT-PCR). Primer yang khusus terhadap gen telah direka dan dipilih untuk menggandakan gen "coat-protein" (*CP*) dan menghasilkan satu jalur spesifik berukuran dalam lingkungan 600 pasangan bes (bp). Sampel yang digunakan untuk RT-PCR merangkumi tanaman yang dikutip dari Selangor, Pahang, Melaka, Johor, Kelantan, Perak, Pulau Pinang dan dengan tambahan sampel dari Terengganu. Hanya tanaman yang dikutip dari Johor (kacang panjang, terung, timun, cili dan nenas), Pahang (cili, tomato, timun dan terung), Terengganu (cili, petola dan terung), Perak (timun), Pulau Pinang (cili) dan Selangor (cili, tomato dan terung) menghasilkan jalur DNA bersaiz dalam lingkungan 600 bp.

Urutan nukleotida gen *CP* 20 isolat daripada tomato, terung, petola, cili dan tembakau yang dikutip di Terengganu, Pahang, Penang, Perak and Selangor telah disahkan sebagai CMV. Analisis filogenetik gen *CP* mencadangkan bahawa isolat CMV tempatan tergolong dalam subkumpulan I (S-I) dan dikumpulkan bersama-sama strain subkumpulan IB (S-IB). Keputusan kajian seterusnya mencadangkan bahawa isolat tersebut boleh disusun mengikut jenis tanaman dan lokasi geografi.

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I certify that an Examination Committee has met on to conduct the final examination of Norsazilawati binti Saad on her degree thesis entitled "Incidence and Differentiation of *Cucumber Mosaic Virus* (CMV) Isolates in Peninsular Malaysia" in accordance with the Universities and University Colleges Act 1971 and Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science

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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, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



NORSAZILAWATI BINTI SAAD

Date: 25 April 2012

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LIST OF ABBREVIATIONS

%	Percent
μg	Microgram
µg/mL	Microgram per millilitre
μl	Microlitre
bp	Base pair
, cDNA	Complementary deoxyribonucleic acid
CP	Coat protein
DAS-ELISA	Double antibody sandwich ELISA
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate mixture
dsDNA	Double-stranded deoxyribonucleic acid
FDTA	Ethylenediamine tetra acetic acid
EtBr	Ethidium bromide
0	Gram
GA	Glutaraldehyde
laG	
M	Molar
ma/ml	Milligram per millilitre
min	Minute
ml	Millilitre
mM	Milimolar
MP	Maximum Parsimony
N.I	Neighbor-ioining
nm	Nanometer
°C	Degree Celsius
	Ontical density
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
PNP	P-nitrophenyl phosphate
RNA	Ribonucleic acid
rom	Rotation per minute
RT	Reverse Transcription
S	Second
TAF	Tris-acetate EDTA
U	Unit
UPGMA	Unweighted Pair-Group Method
UV	Ultraviolet light
V	Volt
v/v	volume per volume
w/v	weight per volume

CHAPTER 1

INTRODUCTION

Cucumber mosaic virus (CMV) is the type member of the *genus Cucumovirus*, which consists of a positive-sense RNA with a tripartite genome. CMV is distributed worldwide and is detrimental to crops due to its non-persistent manner of transmission by more than 80 species of aphids, infecting over 1200 plant species belonging to more than 500 genera of over than 100 families including horticultural crops, fruits, legumes, ornamentals and weeds (Palukaitis & García-Arenal, 2003; Aramburu et al., 2007; Palukaitis et al., 1992; Edwardson & Christie, 1991; Kaper & Waterworth. 1981).

Tomlinson (1987) has listed CMV as the most important virus of some annual crops mainly in the temperate countries, imposing serious yield losses of up to 100% (Galitelli, 2000). In Malaysia, CMV was identified as one of the virus that causes destruction in chilli and cucurbit growing areas (Fujisawa et al., 1986 and 1990). The presence of CMV in Malaysia was also detected in weeds growing around vegetable plots (Sidek et al., 1993). Due to the virus dominance in causing great financial losses on some horticultural crops and the unavailable data recorded on the occurrence and distribution of CMV, a survey was therefore needed to update the current status of CMV in Peninsular Malaysia.

To date, current management practices employed by most of the local farmers to prevent the spread of CMV are through the use of pesticides (Yew et al., 1992; Mohamad Roff & Salleh, 1992). Insecticide sprays has been one of the most popular treatments to effectively control aphid populations. However, this approach does not work rapidly to interrupt CMV transmission by the aphids (Galitelli, 2000) especially during the probing stage on plant. Besides the possibility of causing the vector to develop resistance against insecticides, the use of chemical substances in an excessive amount can pose a great danger to humans and the environment (Akhtar et al., 2010).

Former strategies of managing CMV by cultural practices and pesticides usage seemed to be ineffective due to the stated reasons. Therefore, development of CMV-resistant varieties provides an alternative in dealing with this virus because of its efficiency and environmentally safe feature. Recent studies using pathogen-derived gene sequences to confer resistance against plant viruses in potential host have been well reported (Baulcombe, 1996; Lomonossoff, 1995). Cuozzo et al (1988) has demonstrated the use of coat protein (CP) gene of CMV in transgenic plant or known as CP-mediated protection. The CP-mediated protection of a virus is strain specific in which the transformed plants only provide resistance to the protecting strain (Quemada et al., 1991). However, transgenic plant expressing CP gene of CMV against a strain from either subgroup I or II are protected from other strains within the same subgroup (Cuozzo et al., 1988). Hence, it is important to identify the CP gene sequence and classify the subgroup of CMV isolates in Peninsular Malaysia.

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The congregated information in this research may perhaps provide a sound corroboration for developing a successful control strategy to diminish the spread of CMV, particularly in breeding program. This study was therefore conducted with the following objectives:

- 1. To survey for CMV incidences on selected horticultural crops in Peninsular Malaysia.
- 2. To identify and characterize the molecular properties of *CP* gene of CMV isolates in selected crops in Peninsular Malaysia.

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