UNIVERSITI PUTRA MALAYSIA

DIFFERENTIATION AND GENETIC STUDIES OF SEVERAL ISOLATES OF CUCUMBER MOSAIC VIRUS

OMAR MUSSA EL-SANOUSI

FSAS 1997 17

DIFFERENTIATION AND GENETIC STUDIES OF SEVERAL ISOLATES OF CUCUMBER MOSAIC VIRUS

BY

OMAR MUSSA EL-SANOUSI

Dissertation Submitted in Fulfilment of the Requirements
for the Degree of Doctor of Philosophy in the
Faculty of Science and Environmental Studies,
Universiti Putra Malaysia.

June 1997

ACKNOWLEDGEMENT

In the name of ALLAH, Most Gracious, Most Merciful.

Praise be to ALLAH, the Cherisher and Sustainer of the worlds, May He bless and grant peace to our Lord Muhammad who was sent by Him to save these worlds. My deepest gratitude and love to my father, my mother, my wife and other family members who constantly pray for my success.

I express sincere appreciation and deep gratitude to Assoc. Prof Dr. Norani Abdul-Samad, chairman of my supervisory committee, for her wise counselling, guidance, support and encouragement throughout the entire graduate programme. Grateful appreciation is extended to Dr. Ong Ching Ang a member of the supervisory committee, for providing the CMV isolates, constructive suggestions and comments at various stages of this study.

I owe my sincere gratitude and appreciation to Assoc. Prof. Dr. Khatijah Mohd. Yusoff and Dr. Suhaimi Napis , members of the supervisory committee for their constructive comments in the preparation of the final manuscript.

I am very much grateful to all the staff of Department of Biochemistry and Microbiology for their support, cooperation and friendship.

The financial support from the University of Omar Al-Mukhtar El-Bida Libya and the National Council for Research and Development, Malaysia (IRPA) is gratefully acknowledged.

The companionship of friends is highly appreciated for their motivation throughout the study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

bp	=	base pair
CARNA5	=	CMV Associated Ribonucleic Acid 5
CMV	=	Cucumber Mosaic Virus
cv	=	Cultivar
DAS-ELISA	=	Double Antibody Sandwich Enzyme Linked Immunosorbent Assay
DEPC	=	Diethyl Pyrocarbonate
DNA	=	Deoxyribonucleic Acid
cDNA	=	Complementary deoxyribonucleic acid
ds	=	Double Stranded
EDTA	=	Ethylenediaminetetra Acetic Acid
g	=	Gravity
Kb	=	Kilo bases
dNTP	=	Deoxynucleotides Triphosphate
PBS	=	Phosphate Buffer Saline
PCR	=	Polymerase Chain Reaction
PSV	=	Peanut Stunt Virus
PVP	=	Polyvinylpyrrolidone
RNA	=	Ribonucleic Acid

RT-PCR	=	Reverse Transcription Polymerase Chain Reaction
SDI	=	Serological Differentiation Indices
SDS	=	Sodium Dodecyl Sulphate
ss	=	Single Stranded
TAV	=	Tomato Aspermy Virus
v/v	=	Volume/Volume
w/w	=	Weight/Weight

Abstract of dissertation submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

DIFFERENTIATION AND GENETIC STUDIES OF SEVERAL ISOLATES OF CUCUMBER MOSAIC VIRUS

By

OMAR MUSSA EL-SANOUSI

June 1997

Chairman: Associate Prof. Dr. Norani Abdul Samad

Faculty : Science and Environmental Studies

Four cucumber mosaic virus (CMV) isolates from different host and localities were differentiated on the basis of biological and serological properties and polymerase chain reaction (PCR). The first two isolates (CMV-3 and CMV-7) were isolated from tobacco in Telong, Kelantan; the third (CMV-4) was from chilli in MARDI Jalan Kebun, Klang; and the fourth isolate (CMV-6) was from purple cleome in Sri Kembangan, Selangor. These isolates could be distinguished from each other by the symptoms produced in several plant species. CMV-3 and CMV-7 were more similar to each other than to the other isolates. Aphid transmission test revealed that all the isolates could be transmitted by *A. gossypii* with higher efficiency than *A. craccivora*.

Immunodiffusion tests revealed that all the isolates were closely related since all the heterologous titres were within the same two-fold dilutions of each other. In the DAS-ELISA tests, there were variations observed between the homologous and the heterologous antigens which revealed that the isolates CMV-3, -6, -7 had a closer relationship to each other than to CMV-4; and the isolate CMV-7 had a more distant relationship to isolate CMV-4 than to the others. All the isolates showed a closer relationship to D strain (DTL serogroup) but could not be detected by Q strain (ToRS serogroup) when the DAS-ELISA was used. By indirect-ELISA, all the isolates could be detected by D and Q strains.

A single band of about 487 bp were successfully amplified from the coat protein gene of the CMV isolates . The PCR product could be digested by *Msp* I to produce two bands of approximately 337 and 151 bp but could not be digested by *Eco*R I. Result of analysis of the biological and serological properties as well as PCR confirmed that the isolates belonged to subgroup I of CMV.

To determine the gene or genes location of symptoms determinants in the RNA segments, six pseudorecombinants were constructed *in vitro* between the RNAs of CMV-4 and CMV-3; and two pseudorecombinants by exchanging RNA3 between CMV-4 and CMV-7. The observations on chilli cv. MC4, tomato cv. Eggtomato, *N. glutinosa* and *N. tabacum* cv. White Burley infected with these

pseudorecombinants indicated that RNA 2 or 3 or both were involved in symptoms production.

Two different types of sat-RNA, named sat-RNA6 and sat-RNA7 were found to be naturally associated with CMV-6 and CMV-7, respectively. The association of these sat- RNAs with the CMV isolates reduced the virus concentration up to ten times. These sat-RNAs induced systemic necrosis in tomato and attenuated the symptoms produced by the genomic RNAs in MC4 chilli and White Burley tobacco. Sat-RNA6 showed a higher virulence by inducing systemic necrosis in tomato and produced local necrosis in inoculated White Burley tobacco leaves.

Abstrak disertasi yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi syarat keperluan untuk Ijazah Doktor Falsafah

PEMENCILAN DAN KAJIAN GENETIK BEBERAPA PENCILAN CUCUMBER MOSAIC VIRUS

Oleh

OMAR MUSSA EL-SANOUSI

June 1997

Pengerusi: Prof. Madya Dr. Norani Abdul Samad

Fakulti : Sains dan Pengajian Alam Sekitar

Empat pencilan cucumber mosaic virus (CMV) dari beberapa tumbuhan perumah dan keadaan persekitaran telah diperbeza berasaskan ciri-ciri biologi dan serologi serta kaedah tindakbalas berangkai polimerase (PCR). Dua pencilan pertama (CMV-3 dan CMV-7), telah diperolehi dari pokok tembakau di Telong, Kelantan; pencilan ketiga (CMV-4) telah diperolehi dari pokok cili di MARDI. Jalan Kebun, Klang; dan pencilan keempat (CMV-6) telah diperolehi dari pokok maman liar di Sri Kembangan, Selangor. Pencilan-pencilan ini boleh dibezakan di antara satu dengan yang lain melalui simptom yang dihasilkan di beberapa jenis tumbuhan. Pencilan- pencilan CMV-3 dan CMV-7 adalah hampir serupa berbanding dengan pencilan yang lain. Ujian transmisi afid menunjukkan

kesemua pencilan boleh ditransmisikan oleh *A. gossypii* dengan keupayaan yang lebih tinggi jika dibandingkan dengan *A. craccivora*.

Ujian-ujian imunoserapan dua hala menunjukkan bahawa ke semua pencilan mempunyai pertalian yang rapat antara satu sama lain berdasarkan kepada persamaan titer heterolog dalam lingkungan pencairan dua kali. Dalam ujian DAS-ELISA, terdapat beberapa perbezaan di antara antigen homolog dan antigen heterolog yang menunjukkan bahawa CMV-3, -6, -7 mempunyai pertalian yang lebih rapat di antara satu sama lain daripada pertalian dengan CMV-4; sementara pencilan CMV-7 pula mempunyai pertalian yang paling jauh dengan pencilan CMV-4 berbanding dengan yang lain. Dengan menggunakan DAS-ELISA, kesemua pencilan menunjukkan pertalian yang rapat dengan strain D (kumpulan serum DTL) tetapi tidak dapat dikesan oleh strain Q (kumpulan serum ToRS). Akan tetapi dengan mclakukan ujian "indirect-ELISA", kesemua pencilan dapat dikesan oleh strain D dan Q.

Kesemua pencilan-pencilan CMV telah berjaya menghasilkan satu jalur hasilan PCR bersaiz 487 bp dari cDNA yang mengkod protein virus dengan menggunakan primer-primer yang direka untuk gen protein kot. Hasil PCR boleh dihadamkan dengan enzim pembatas *Msp* I dengan menghasilkan dua jalur bersaiz

sekitar 337 bp dan 151 bp , tetapi tidak boleh dihadamkan oleh enzim *Eco*R I. Pencirian biologi dan serologi dan teknik PCR membuktikan bahawa pencilan-pencilan tersebut tergolong dalam CMV sub-kumpulan I.

Untuk menentukan gen atau lokasi gen bagi penentu simptom dalam RNA, enam `pseudorecombinant' telah dibentuk *in vitro* di antara segmen-segmen RNA dari CMV-4 dan CMV-3; dan dua pseudorecombinant melalui pertukaran RNA3 di antara CMV-4 dan CMV-7. Pemerhatian-pemerhatian pada cili cv. MC4, tomato cv. Eggtomato, *N. glutinosa* dan *N. tabacum* cv White Burley yang disuntik dengan pseudorecombinant tersebut menunjukkan bahawa RNA2 atau 3, atau kedua-duanya sekali terlibat di dalam penghasilan simptom.

Dua jenis sat-RNA yang berbeza, sat-RNA6 dan sat-RNA7 telah ditemui yang mempunyai hubungan semulajadi dengan CMV-6 dan CMV-7, masing-masing. Hubungan di antara sat-RNA tersebut dengan pencilan-pencilan CMV menurunkan kepekatan virus pada daun hingga sepuluh kali ganda. Sat-RNA ini mengaruhkan nekrosis sistemik pada tomato dan melemahkan kesan simptom genom RNA pada cili MC4 dan tembakau White Burley. Sat-RNA6 menunjukkan daya kevirulenan yang tinggi dengan mengaruhkan nekrosis sistemik pada tomato dan menghasilkan nekrosis setempat dalam daun tembakau White Burley.

CHAPTER I

INTRODUCTION

Cucumber mosaic virus (CMV) is the type species of the genus Cucumovirus of Bromoviridae Family (Murphy *et al.,* 1995). CMV was first reported as the causal agent of cucumber mosaic disease in U.S.A. (Doolittle, 1916; Jagger, 1916). Since then CMV has been found in most countries of the world. CMV is often the most prevalent virus in a number of surveys on plant virus infections in different parts of the world including Hungary (Gaborjanyi and Nagy, 1972), Iran (Rahimian and Izadpanah, 1978), Germany (Schimanski *et al.*, 1976), France (Quiot *et al.*, 1979a), Korea (Choi and Park, 1982), Spain (Garcia-Luque *et al.*, 1983; Luis-Arteaga *et al.*, 1988; Blas *et al.*, 1993), New Zealand (Burgman *et al.*, 1986) , Morocco (Bouhid and Lockhart, 1990), U.S.A. (Provvidenti *et al.*, 1984; Rist and Lorbeer, 1989, 1991), Malaysia (Mohamad Roff and Ong, 1992) Indonesia, Malaysia, Sri Lanka, Taiwan and Thailand (Green, 1992). In the last two decades, CMV has been identified as the causal agent of several disease epidemics, for instance, necrosis of tomato in France (Quiot *et al.*, 1979b), Italy (Gallitelli *et al.,* 1990) and Spain (Jorda *et al.,* 1992); severe stunting and leaf epinasty of lupine in Australia (Alberts *et al.,* 1985), mosaic in sweet potato in

Israel (Cohen *et al.*, 1988) and in banana in Morocco (Bouhida and Lockhart, 1990). In China, severe losses caused by CMV were reported in vegetable crops (Tien and Wu, 1991).

The CMV has a very broad natural host range throughout the temperate, tropical and subtropical areas. It is pathogenic to cereals, forages, woody and herbaceous ornamental, vegetables and fruit crops (Kaper and Waterworth, 1981).

In Malaysia, CMV was reported as a major virus disease on cucurbitts (Ong and Ting, 1977) and one of the main virus diseases on chilli (Mohamad Roff and Anang, 1989; Mohamad Roff and Ho, 1989; Fujisawa *et al.*, 1990; Mohamad Roff and Ong, 1992; Green 1992).

Based on symptomology, the CMV was first reported in Malaya by Milsun and Grist earlier in 1941. Later in 1968 Miller reported the infection of squash and pumpkin with CMV (Ong, 1972). Ong (1972) studied CMV isolated from infected cucumber in more detail by using physical properties, transmission, extensive host range, serology and cross protection. Since then several studies had been conducted to differentiate CMV isolates using selected hosts and electrophoretic patterns of double stranded RNA (dsRNA) (Ahmad, 1991 ; Abu-Baker, 1990; Mohd Nadzir 1992; Mustapha, 1992). Since information are still lacking on the existence of CMV

subgroup, and the association of satellite RNA, the main objectives of the study were to:

1. differentiate several CMV isolates from different hosts and locality and classify them into subgroup by using, i) biological properties; ii) serological properties and iii) molecular techniques.
2. study the transmission efficiency of these isolates by the two main aphid species in Malaysia.
3. study the presence of the satellite RNA with the CMV isolates and their interaction with the virus genome.
4. study the distribution of genetic determinants for symptom production and host range.

CHAPTER II

LITERATURE REVIEW

Structure and Composition of CMV

Particle Structure

CMV has isometric particles about 28-30 nm in diameter. The capsid is composed of 180 protein subunits in pentamer-hexamer cluster with T=3 surface lattic symmetry (Francki *et al*., 1979). The stability of CMV particles depends largely on protein-RNA interaction which occur between the positively charged basic residues of capsid polypeptide and the negatively charged phosphate groups of RNA (Kaper, 1975 ; 1976). This stability can readily be disrupted in low concentration of ionic detergents such as sodium dodecyl sulphate or high concentration of neutral chloride salts (Francki *et al*., 1966; Kaper and Geelen, 1971; Boatman and Kaper, 1976) and its infectivity is sensitive to ribonuclease (Francki, 1968; Kaper and Geelen, 1971; Habili and Francki, 1974a). The particles sediment as a single component with S = 98.6 - 1.04 C where C is the virus concentration in milligram/ millilitre. The extinction coefficient at 260 nm is 5 (Francki *et al*., 1966).