



UNIVERSITI PUTRA MALAYSIA

**CONSTRUCTION OF A TRANSFORMATION VECTOR CONTAINING
CUCUMBER MOSAIC VIRUS (CMV) COAT PROTEIN (CP) GENE
AND DEVELOPMENT OF AN AGROBACTERIUM TRANSFORMATION
PROCEDURE FOR CHILLI**

LIM JEE HIAN

FSAS 2003 7

**CONSTRUCTION OF A TRANSFORMATION VECTOR CONTAINING
CUCUMBER MOSAIC VIRUS (CMV) COAT PROTEIN (CP) GENE AND
DEVELOPMENT OF AN *AGROBACTERIUM*-TRANSFORMATION
PROCEDURE FOR CHILLI**

By

LIM JEE HIAN

**Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia in Fulfilment of the Requirements for the
Degree of Master of Science
May 2003**



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

CONSTRUCTION OF A TRANSFORMATION VECTOR CONTAINING CUCUMBER MOSAIC VIRUS (CMV) COAT PROTEIN (CP) GENE AND DEVELOPMENT OF AN *AGROBACTERIUM*-TRANSFORMATION PROCEDURE FOR CHILLI

By

LIM JEE HIAN

May 2003

Chairman : Professor Marziah Mahmood, Ph.D.

Faculty : Science and Environmental Studies

Chilli (*Capsicum annuum* L.) is one of the major vegetable and spice crops grown widely, yet it suffers great losses due to infection by various viruses, including Cucumber Mosaic Virus (CMV). The conventional breeding of chilli hybrids for improved disease resistance is a long process with problems of interspecific incompatibility. The objectives of the study were to construct a transforming vector containing Cucumber Mosaic Virus (CMV) Coat Protein (CP) and development of an *Agrobacterium*-transformation procedure which can offer solutions to these problems.

This study was approached primarily from the molecular aspect which the construct of a CMV CP has been successfully cloned. The cloned fragments were 655 bp and exhibited more than 90% similarity to those published CMV CP gene sequences.



SDS-PAGE analysis showed the size of the expressed coat protein to be similar to the predicted size of 30 kD based on the DNA sequences. Western blot analysis was also conducted to confirm the bacterial expressed coat proteins. All these verified that the cloned genes were translatable CMV CP genes.

A protocol for regeneration and genetic transformation was established for chilli. This study showed that GUS reporter gene can be transformed into chilli by using *Agrobacterium*-mediated techniques. Using *Agrobacterium* AGL0 with 24 hours co-cultivation was found to be the best way for chilli transformation as it showed a 100% success rate in the tested explants. However, *Agrobacterium* EHA 105 can also be used for chilli transformation with a shorter period, found to be 3 hours, co-cultivation period of 3 hours to achieve an 87.5% success rate.

Methods that permit normal shoot development have generally been found on regenerated decapitated seedlings. Formation of buds and elongated shoots occurred successfully by injuring the apical region of hypocotyls tissues of seedlings during curved stages. The best responses of regenerative ability showed shoot-like structures induced at the wounded sites on Murashige and Skoog's (MSD) medium supplemented with 2 mg/L Benzyladenine (BAP) and 0.25 mg/L Indole-3-acetic (IAA). Direct shoot elongation appeared after transferring to hormone free MSD medium. These elongated shoots were excised and rooted on MSD medium with 0.05 mg/L IAA and until development into plants.



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

PEMBINAAN VEKTOR TRANSFORMASI YANG MENGANDUNGI GEN CUCUMBER MOSAIC VIRUS (CMV) KOT PROTEIN (CP) DAN KEMAJUAN KAEDAH *AGROBACTERIUM*-TRANSFORMASI UNTUK CILI

By

LIM JEE HIAN

Mei 2003

Pengerusi : Profesor Marziah Mahmood, Ph.D.

Fakulti : Sains dan Pengajian Alam Sekitar

Cili (*Capsicum annum* L.) merupakan salah satu sayuran utama dan tanaman rempah-ratus yang ditanam secara meluas, tetapi ia mengalami kerugian yang besar disebabkan rentan kepada berbagai jenis infeksi virus, termasuk Cucumber Mosaic Virus (CMV). Pembiakbakaan secara konvensional cili untuk memperbaiki rentan terhadap serangan penyakit memakan masa yang lama dengan wujud masalah interspesifik ketidak sepadanan. Objektif kajian ini ialah pembentukkan satu vektor transformasi yang mengandungi gen Cucumber Mosaic Virus (CMV) Kot Protein (CP) serta memajukan kaedah *Agrobacterium*-transformasi untuk penyelesaian masalah tersebut.

Kajian ini dimulakan dengan pembentukkan cassette gen CMV CP yang telah berjaya diklonkan. Fragmen yang diklonkan ialah 655 bp dan mempamerkan lebih daripada 90% persamaan antara rangkaian CMV CP yang telah diterbitkan. Analisis SDS-



PAGE mempamerkan saiz kot protein yang diekspresikan adalah sama dengan saiz ramalan 30 kD berdasarkan rangkaian DNA. Analisis “Western” juga dijalankan untuk mengesahkan pengekspresan kot protein. Kesemua ini membuktikan bahawa gen yang diklon merupakan gen CMV CP yang berupaya diterjemahkan.

Suatu protokol untuk penjanaan dan transformasi genetik telah dibentuk untuk cili. Kajian ini menunjukkan gen penyaringan GUS berjaya tertransformasi ke cili dengan kaedah berperantarakan *Agrobacterium*. Penggunaan *Agrobacterium* AGL0 dengan 24 jam kokultivasi didapati adalah cara terbaik untuk transformasi cili dengan 100% kadar kejayaan dalam eksplan yang diuji. Walau bagaimanapun, *Agrobacterium* EHA 105 juga boleh digunakan dengan 3 jam kokultivasi untuk mencapai 87.5% kadar kejayaan.

Kaedah penjanaan anak benih “decapitated” membolehkan perkembangan pucuk kelihatan normal. Penghasilan tunas dan pemanjangan pucuk terbentuk pada bahagian apical tisu hipokotil, anak benih melengkung yang dilakukan. Keupayaan penjanaan struktur “shoot-like” yang terbaik dijumpai pada bahagian luka anak benih di atas media Murashige and Skoog’s (MSD) dengan tambahan 2 mg/L Benzyladenine (BAP) dan 0.25 mg/L Indole-3-acetic (IAA). Pemanjangan pucuk secara langsung kelihatan selepas pemindahan di atas media MSD tanpa hormon. Anak pucuk yang memanjang akan dipotong dan dipindahkan ke media pengakaran dengan tambahan 0.05 mg/L IAA dan sehingga menjadi tumbuhan.

ACKNOWLEDGEMENTS

First and foremost, the author would like to take this opportunity to express his profound to the supervising committee members, Prof. Dr. Marziah Mahmood and Dr. Mohd Puad Abullah of Biochemistry Department, UPM, Dr. Tan Chon Seng of Biotechnology Department, MARDI for their patience, valuable guidance, exhortation, and encouragement throughout the completion of the research. Without their assistance and valuable contribution, this work would have been impossible. The author grateful to Dr. Tan for his support and encouragement in teaching basic techniques in molecular biology.

Appreciations also go out to everybody in MARDI especially Dr. Lam Peng Fatt and lab-mates who always leads author to complete the work during the course of this project. Appreciation is also extended to members in UPM and all friends for their kind guidance, valuable assistance in helping to complete the research.

Last but not least, the author would like to express his heartiest appreciation and thanks to his beloved family's members. Thanks for all theirs understanding and support throughout the studies. Thanks for all theirs love that sustained author throughout his research.



I certify that an Examination committee met on 8th May 2003 to conduct the final examination of Lim Jee Hian on his Master of Science thesis entitled “Construction of a Transformation Vector Containing Cucumber Mosaic Virus (CMV) Coat Protein (CP) Gene and Development of an *Agrobacterium*-Transformation Procedure for Chilli” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

JANNA ONG ABDULLAH, Ph.D.

Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Chairman)

MARZIAH MAHMOOD, Ph.D.

Professor,
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

MOHD PUAD ABDULLAH, Ph.D.

Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

TAN CHON SENG, Ph.D.

Division of Biotechnology
Malaysian Agricultural Research and Development Institute
(Member)



GULAM RUSUL RAHMAT ALI, Ph.D.

Professor / Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 18 JUL 2003



This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Master of Science. The members of the Supervisory Committee are as follows:

MARZIAH MAHMOOD, Ph.D.

Professor,
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Chairman)

MOHD PUAD ABDULLAH, Ph.D.

Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

TAN CHON SENG, Ph.D.

Division of Biotechnology
Malaysian Agricultural Research and Development Institute
(Member)



AINI IDERIS, Ph.D.

Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 11 JUL 2003



DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



LIM JEE HIAN

Date: 18 JUL 2003

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENTS	vi
APPROVAL SHEETS	vii
DECLARATION FORM	ix
TABLE OF CONTENTS	x
LIST OF TABLES	xiii
LIST OF FIGURES	xiv
LIST OF ABBREVIATIONS	xvi
 CHAPTER	
1 INTRODUCTION	
1.1 Distribution	1
1.2 Economic Importance	1
1.3 Production Related Problem	2
1.4 Aims of This Thesis	4
2 LITERATURE REVIEW	
2.1 Properties of Cucumber Mosaic Virus (CMV)	5
2.1.1 CMV Infection in Chilli	7
2.1.2 Transmission of CMV	7
2.2 Control in Family <i>Solanaceae</i>	9
2.2.1 Conventional Methods	9
2.2.2 Non-Conventional Methods	10
2.2.3 Coat Protein-Mediated Protection	12
2.3 Tissue Culture as a Basis for Plant Biotechnology	15
2.3.1 Tissue Culture and Regeneration	17
2.3.2 Tissue Culture of Chilli	18
2.4 Gene Transfer	20
2.4.1 Goals of Gene Transfer	20
2.4.2 Gene Transfer by Particle Bombardment	21
2.4.3 Gene Transfer by <i>Agrobacterium tumefaciens</i>	22
2.5 <i>Agrobacterium</i> System	23
2.5.1 Ti Plasmids Vector Development	24
2.5.2 Description of Plasmid Used (pCAMBIA 1301)	26
2.6 Plant Transformation	27
2.6.1 Factors Affecting Transformation Frequency	29
2.6.2 Verification of Transformants	29



3	MATERIALS AND METHODS	
3.1	Construction Recombinant Coat Protein (CP) Gene	31
3.1.1	Cucumber Mosaic Virus Isolation and RNA Extraction	31
3.1.2	Primer Design for Coat Protein (CMV CP)	32
3.1.3	RT-PCR Amplification of the CP Gene Fragments from Total RNA of CMV Infected Chilli and Cucumber Leaves	33
3.1.4	Cloning of the RT-PCR Product (CP Gene) into pRSET Vector	34
3.1.5	Transformation of the Ligated Products into <i>E.coli</i> (TOP10F') Competent Cells	37
3.1.6	Protein Expression of CP Gene	39
3.1.7	Cloning of the CP Gene Containing cMyc Tag Gene Fragment into pJIT117 Vector	42
3.1.8	Cloning of the CP Gene Construct into Plant Expression Vector pCAMBIA 2300	43
3.1.9	Introduction of Plasmids (pCAMBIA::CMVCP) into <i>Agrobacterium</i>	43
3.1.10	Determination of Correct Incorporation of PCAMBIA::CMVCP into <i>Agrobacterium</i>	44
3.2	Tissue Culture Studies	44
3.2.1	Direct Regeneration from Explants	44
3.2.2	Explants Materials	46
3.2.3	Media Assessment	48
3.2.4	Rooting Media	48
3.3	Direct Regeneration from Decapitated Seedlings	48
3.3.1	Plant Materials	48
3.3.2	Explants Treatment	49
3.3.3	Explants Culture	49
3.3.4	Elongation Medium	49
3.3.5	Rooting of Shoots	50
3.4	Chilli Transformation	50
3.4.1	Effect of Various Concentrations of Hygromycin on Explants	50
3.4.2	Effect of Various Concentrations of Kanamycin on Explants	50
3.4.3	Mobilisation of pCAMBIA 1301 (GUS) into <i>Agrobacterium</i>	51
3.4.4	Transformation of Chilli	51
3.4.5	Verification of Putative Transgenic Plants	55
4	CLONING OF CMV CP GENE AND EXPRESSION OF CP GENE IN BACTERIA	
4.1	Introduction	56

4.2	Results and Discussions	57
4.2.1	RT-PCR Amplification of the Coat Protein (CP) Gene Fragments from Total RNA of CMV Infected Leaves	57
4.2.2	Cloning of the RT-PCR Products into pRSET Vector	57
4.2.3	Sequence Comparison of the RT-PCR Products with Similar Sequences from Genebank	62
4.2.4	pRSET Vector as Expression Vector	62
4.2.5	Protein Expression and Western Blotting of pRSET::CMVCP	65
4.3	Conclusion	67
5	AGROBACTERIUM-MEDIATED TRANSFORMATION OF CHILLI	
5.1	Introduction	69
5.2	Results and Discussions	71
5.2.1	Cloning of the CP with cMyc Tag Genes into pJIT117 Vector	71
5.2.2	Cloning of the CP Gene Construct in Plant Expression Vector pCAMBIA 2300	73
5.2.3	Introduction of Plasmids Pcambia::CMVCP into <i>Agrobacterium</i>	77
5.2.4	Mobilisation of pCAMBIA 1301 (GUS) into <i>Agrobacterium</i>	77
5.2.5	Effect of Various Concentrations of Hygromycin on Explants	80
5.2.6	Effect of Various Concentrations of Kanamycin on Explants	82
5.2.7	Chilli Transformation	84
5.2.8	Correlation Effect of Co-Cultivation Period and Expression of the GUS Gene in Transformed Tissues	84
5.3	Conclusion	97
6	DIRECT REGENERATION OF CHILLI	
6.1	Introduction	98
6.2	Results and Discussions	101
6.2.1	Direct Regeneration from Explants	101
6.2.2	Direct Regeneration from Decapitated Seedlings	107
6.3	Conclusion	113
7	CONCLUSION	115
	REFERENCES	117
	APPENDICES	131
	VITA	145



LIST OF TABLES

Tables		Page
1	Comparison of the percentage of positive GUS assay in transformed <i>Capsicum</i> explants with different strains of <i>Agrobacterium</i> and co-cultivation period on MS medium supplemented with 2 mg/L BAP and 0.25 mg/L IAA	86
2	Comparison of the percentage of shoots induced on MS medium supplemented with (A) 4 mg/L BAP, (B) 2 mg/L BAP, (C) 2 mg/L BAP and 0.5 mg/L IAA or (D) 2mg/L BAP and 0.25 mg/L IAA	102
3	Percentage of buds initiated in MS medium supplemented with (A) 2 mg/L BAP and 0.25 mg/L IAA, (B) 2 mg/L BAP and (C) hormone free	108



LIST OF FIGURES

Figures		Page
1	Electron microscopy of CMV	6
2	CMV infected chilli plant	8
3	Fruits and seeds of chilli var Kulai	45
4	Explant materials from day 10 seedlings	47
5	Explant materials were dipped in <i>Agrobacterium</i> for 5 min of inoculation time	54
6	RT-PCR of CMV coat protein gene	58
7	Schematic diagram of the CMV coat protein gene construct	60
8	PCR screening of pRSET::CMVCP construct	60
9	Confirmation of pRSET::CMVCP construct by R.E. digestion with <i>Nco I</i> and <i>Xba I</i> resulted in 2 bands	61
10	Alignment of CMV coat protein genes	63
11	SDS-PAGE of bacterial expressed CMV coat protein	66
12	Western Blot analysis of bacterial expressed CMV coat protein	68
13	Confirmation of the CP with cMyc insert was performed by digestion of the pJIT::CP vector with <i>Nco I</i> and <i>EcoR I</i>	74
14	pCAMBIA::CMVCP plasmid isolated from colonies and digested with <i>Kpn I</i>	75
15	Schematic diagram of CMV CP cloned into T-border of pCAMBIA 2300 vector	76
16	Strain of <i>Agrobacterium</i> harbouring pCAMBIA::CMVCP grown on LB plate supplemented with 100 µg/mL kanamycin and 100 µg/mL rifampicin	78
17	Confirmation of pCAMBIA::CMVCP plasmid transformed into <i>Agrobacterium tumefaciens</i>	79



18	Different concentrations of hygromycin to be used for selection of transgenic tissues after transformation with <i>Agrobacterium</i> containing GUS gene	81
19	Different concentrations of kanamycin to be used for selection of transgenic tissues after transformation with <i>Agrobacterium</i> containing GUS gene	83
20	Expression of GUS in cotyledon explants	87
21	Expression of GUS in hypocotyl explants	87
22	Graph showing the different co-cultivation period of strain AGL0	88
23	Graph showing the different co-cultivation period of strain EHA 105	90
24	Graph showing the different co-cultivation period of strain AGL1	91
25	Comparison of GUS expression in control and transformed explants	93
26	(A) Picture showing the shoot-like structures and shoots produced were more vigorous and numerous on 2 mg/L BAP + 0.25 mg/L IAA media (B) Shoots emerged from cotyledon explants (C) Shoots emerged from hypocotyl explants	103
27	Picture showed high amounts of vegetative buds that did not elongate or produced distorted leaves with the appearance of cauliflower leaves without any further elongation	106
28	Picture shown buds formation can be obtained after a week of puncturing by syringe needle on media MSD (BAP + IAA) which produced the highest initiated buds. All seedlings which were able to produce buds managed to elongate further and develop into plants	110
29	Picture shows different stages of shoot elongation within 2 months	111

ABBREVIATION

μL	micro liter
μM	micro molar
$\mu\text{g/mL}$	microgram per milliliter
BAP	benzylaminopurine
bp	base pair
cm	centimeter
CMV	Cucumber Mosaic Virus
DNA	deoxyribonucleic acid
DNTP	deoxynucleoside 5'-triphosphates
EDTA	ethylene diaminetetra acetic acid
g	gram
GUS	β -glucuronidase
HCl	hydrochloric acid
IAA	indole-3-acetic acid
IPTG	Isopropyl β -D-Thiogalactopyronidase
kb	kilo base
kg	kilogram
LB	Luria and Bertani media
M	molar
MgCl_2	magnesium chloride
mg/L	miligram per liter



MgSO ₄	Magnesium sulphate
min	minute
mL	milliliter
mM	millimolar
MS	Murashige and Skoog (1962)
MSD	Murashige and Skoog with Dextrose
MW	molecular weight
NaCl	sodium chloride
NaOH	sodium hydroxide
<i>NptII</i>	Neomycin phosphotransferase II
°C	degree centigrade
%	percentage
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	polymerase chain reaction
PEG	polyethylene glycol
ppm	part per million
RNA	ribonucleic acid
SDS	Sodium dodecyl sulphate
TDZ	thidiazuron
TEMED	tetramethyl-ethylenediamine
Ti	tumor inducing
UV	ultra violet



v/v	volume over volume ratio
var.	variety
w/v	weight over volume ratio
X-Gal	5-Bromo-4-chloro-3-indolyl- β -D-galactoside
YEB	yeast extract broth



CHAPTER 1

INTRODUCTION

1.1 Distribution

Chilli (*Capsicum spp.*) is an important nontuberous vegetable of the family *Solanaceae* besides tomato and potato. It is grown worldwide. Through its important contribution to nutrition and the economy, chilli becomes very closely associated with the Malaysian people and inextricably woven into their lives. Today this important cultivated plant is a source of income for many thousands of farmers. Since cultivation of chilli, people have noted the exceptional flavours, aroma and colourings, as well as its excellent spice quality. As a rich source of vitamins, it has become a popular and widely used spice throughout the world.

1.2 Economic Importance

Chilli is consumed throughout the world. Forty per cent of this crop is produced in Asia, and India is the leading producer in area and production. Greenleaf (1986) has reviewed the economic importance of this crop. In Malaysia, chilli ranks as the most popular fruit vegetable and occupies the highest hectarage among the fruit vegetables in this country (Ong et al., 1979, Inon et al., 1999). *Capsicum annuum* and *Capsicum frutescens* are the two main types of chilli cultivated in Malaysia. Between the two, *C. annuum* is more important in terms of economic and area cultivated. Recent report indicated that over 3,055 ha were planted with this crop, with a production of 21,890 tonnes chilli per year (Inon et al., 1999)



1.3 Production Related Problem

Although chilli is economically important it is highly susceptible to microbial diseases especially viral diseases, resulting in significantly reduction of crop yields and sometimes even complete crop failure. Five types of viruses have been reported in infecting chilli in Malaysia, namely Cucumber Mosaic Virus (CMV), Chilli Venial Mottle Virus (CVMV), Tobacco Mosaic Virus (TMV), Tomato Spotted Wilt Virus (TSMV), and Tobacco Leaf Curl Virus (TLCV) (Ong et al., 1979; Fujisawa et al., 1986; Mohamad Roff and Ong, 1992). Among these, CMV and CVMV were found to be the most common viruses infecting and damaging chilli in Malaysia which results in the losses of yield ranges from 10%-15% if infection came in late and may reach up to 60% if plants were infected at early stage (Ong et al., 1979). As a result, production of chilli is insufficient to meet the local consumption, and approximately RM30 million worth of chilli is imported annually (Ong et al., 1979).

The use of pesticides to control insect vector populations implicated in transmission of the virus represents an important tool to limit the incidence of viral disease outbreaks. However, none of these non-genetic control measures is likely to provide the long term answer to combat viral diseases, because of their expense and their sometimes questionable effectiveness and reliability. Moreover, current concerns about pollution and food safety are forcing hazardous pesticides out of the market.

Since pesticides are progressively abandoned, there is a growing urgency for the development of alternative methods to control viral diseases. Breeding for virus

resistance generally provides the best prospect for virus control in the long term. In the past, the introgression of genetic sources for host plant resistance that are naturally present within the gene pool of the crop involved, has been successfully applied to develop virus resistant crop cultivars for a considerable number of agronomically important crops (Hoisington et al., 1999).

Conventional breeding programs for viral resistance genes in local chilli varieties have been conducted (Ong et al., 1979), only a small group of genotypes were found to be resistant and they do not possess resistance to both CMV and CVMV. The limitations of conventional breeding and routine culture practices urge the need for the development of alternative forms of virus control that can be fully integrated within traditional methods.

This whole concept is an interesting trend. It started with the intention of Takebe in Japan to facilitate the infection of plants cells (tobacco) by plant viruses. A protoplast-to-plant system was achieved (Takebe et al., 1971), and the virus infected the protoplasts and replicated in them. Due to vast progress in molecular methodologies and fruitful investigations in several plant molecular-biology laboratories, especially on the biology and molecular genetics of the plant pathogen *Agrobacterium* spp.- did genetic transformation of plants become a reality.

In recent years, a lot of progress has been achieved on the understanding of plant virus genome organisation and functions (Martin et al., 1997; Parniske et al., 1998; Speulman et al., 1999). This knowledge, together with the virus derived resistance strategies, has been used for developing virus resistant cultivars.



Various DNA sequences (coding for the coat protein, movement protein, the polymerase gene as well as non-coding sequences) have been introduced, through genetic engineering into plant genomes in order to create viral resistance variety. In some instances, high levels of resistance which justify for commercial applications have been successfully obtained (Comai et al., 1985; Powell et al., 1986; Gerlach et al.,1987; Hider et al.,1987). The viral coat protein (CP) gene, however, has thus far been more widely applied than any other viral sequence (Powell et al., 1986; Beachy et al., 1990). Beachy et al. (1990) reported that the transformation of tomato with the CP gene from Cucumber Mosaic Virus was shown to generate high levels of protection to CMV infection in tomato hybrids, not only when challenged by mechanical inoculation, but also when exposed to repeated inoculation by viruliferous aphids in open field.

1.4 Aims of This Thesis

It is foreseen that similar strategies can, therefore, be utilised to give coat protein-mediated protection against CMV diseases in chilli. The objectives of this research are

- (a) to obtain a full-length translatable Coat Protein(CP) gene of a local strain of chilli infected CMV in bacteria and sub-clone it into plant expression cassette
- (b) to develop and optimise an efficient *Agrobacterium* transformation protocols using GUS reporter gene as well as CMV CP gene
- (c) to develop plant regeneration system for chilli.

CHAPTER 2

LITERATURE REVIEW

2.1 Properties of Cucumber Mosaic Virus (CMV)

CMV (Figure 1) is an isometric particle, about 30 nm in diameter and consists of 180 subunits in pentamer-hexamer clusters. The molecular weight of the particle is about $5.8-6.7 \times 10^6$ daltons and has a tripartite genome composed of four RNA species, three genomic and one subgenomic RNA (Peden and Symons, 1973). RNAs 1 (3.3 kb) and RNAs 2 (3.0 kb) encode proteins with molecular weights of 111 kDa and 97 kDa respectively, both of which are essential for virus replication (Nitta et al., 1988; Matthew, 1992). RNA 3 (2.2 kb) encodes for the viral coat protein with a mass of about 24 kDa (Daniels and Campbell, 1992). An additional protein with molecular weight of about 30 kDa, encoded by subgenomic RNA 3, is involved in the cell to cell movement of the virus (Davies and Symons, 1988).

CMV exists as a collection of strains with overlapping host ranges encompassing over 800 plant species (Douine et al., 1979; Kaper and Waterworth, 1981; Palukaitis et al., 1992). CMV exists as a variety of isolates which can be divided into two major serological subgroups defined as DTL and ToRS (Francki et al., 1979). DTL commonly induced more severe symptoms and occurred predominantly in the tropics and subtropics, whereas the ToRs isolates are prevalent in the temperate regions. Most of the approaches for virus detection could be used for characterization and relationship study of a virus. A number of methods and techniques which including biologically, serologically or physically approaches have been used to characterize CMV isolates.

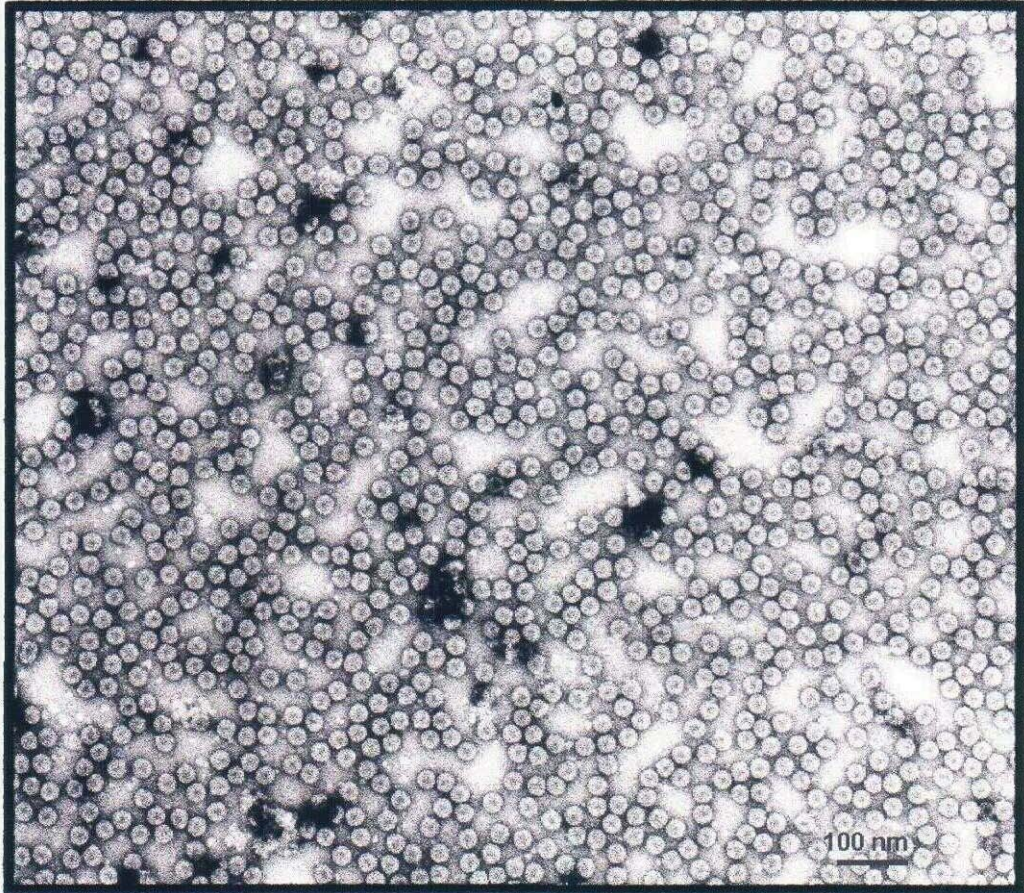


Figure 1: Electron microscopy of Cucumber Mosaic Virus (CMV).