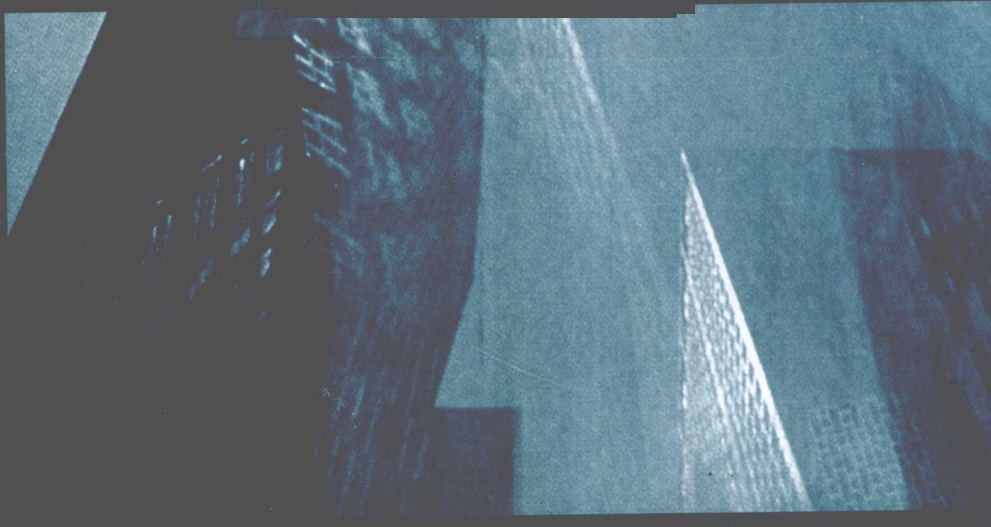
Jishuang Chen

Experimental Plant Virology



ZHEJIANG UNIVERSITY PRESS
浙江大学出版社
Springer

Jishuang Chen

Experimental Plant Virology

With 126 figures

ZHEJIANG UNIVERSITY PRESS
浙江大学出版社

Springer

图书在版编目(CIP)数据

实验植物病毒学 = Experimental Plant Virology：英文 / 陈集双著. —杭州：浙江大学出版社，2010.7
ISBN 978-7-308-07369-1

Ⅰ. ①实… Ⅱ. ①陈… Ⅲ. ①植物病毒－实验－研究－英文 Ⅳ. ①S432.4-33

中国版本图书馆CIP数据核字(2010)第017812号

实验植物病毒学
陈集双 著

责任编辑 王 镨
封面设计 俞亚彤
出版发行 浙江大学出版社
网址：http://www.zjupress.com
Springer-Verlag GmbH
网址：http://www.Springer.com
排 版 杭州中大图文设计有限公司
印 刷 杭州富春印务有限公司
开 本 710mm×1000mm 1/16
印 张 17.75
字 数 492千
版 印 次 2010年7月第1版 2010年7月第1次印刷
书 号 ISBN 978-7-308-07369-1 (浙江大学出版社)
ISBN 978-3-642-14118-8 (Springer-Verlag GmbH)
定 价 120.00元

Preface

Plant Virology, the study of plant viruses and their diseases, is an important branch of science comprised of challenges and opportunities in China and developing countries, where agriculture is of key importance to date. The discovery of principles and the development of new techniques are helpful in feeding the population more safely and in providing more healthy systems of food production, along with many other aspects of social life such as ornamentals and natural medicine.

It is easy to show people fish, no matter how they are obtained, but it is more difficult to tell others how to fish, no matter how simple it becomes after years of practice. Based on the progress of biological studies over the last decades, molecular biology techniques have established a good foundation for realizing the investigation of viral genomes, which is the "stem" for plant virus exploration. Using *Cucumber mosaic virus* (CMV) with its satellite RNA and several other plant viruses with single stranded RNA genome as examples, some newly-obtained principles and the progress of the research are shown in this book, which is composed of six chapters. Chapters 1 to 5 mainly involve topics of genomic characterization, detection and quantitative techniques, especially the infection clone systems of CMV. Host responses to virus infection through plant microRNAs have also been demonstrated as groundwork. In Chapter 6, several plant cryptic viruses with double stranded virus genomes have been described for the first time, treating an understanding of plant viruses as a kind of bio-resource.

I hope this book will provide some practical clues and insight for people who work in this field and in related areas.

I warmly thank all the contributors who have worked with me in the same laboratory, during the year 2000 to 2009, including Ph.D candidates, Junli Feng, Zhiyou Du, Qiansheng Liao, Liqiang Li, Shaoning Chen and Qiulei Lang, with Master Students Liang Cheng, Yanfei chen, Rong Zeng, Jianguang Zhang, Zuodong Qin, Liping Zhu, Qinghua Tian, Hong Guo, Shijie Yan and Susu Shentu.

Jishuang chen
Hangzhou, China
May, 2010

Contents

1

Gene Cloning of *Cucumber Mosaic Virus* and Some Related Viral Agents

1.1 Introduction

Cucumber mosaic virus (CMV) is a typical member of the genus *Cucumovirus*. It has infected more than 1,000 species of monocots and dicots, including many economically important crops (Palukaitis and García-Arenal, 2003; Palukaitis et al., 1992). In China, CMV is commonly detected as the principal virus infecting field crops in the families *Solanaceae* (including tobacco, tomato, potato, pepper, etc.), *Brassicaceae* (including brassicas, radish, turnip, etc.) and *Fabaceae* (including soybean, cowpea, etc.). As shown in Fig. 1.1, CMV strain containing a satellite RNA co-infected with *Tomato mosaic virus* (ToMV), brought fruit necrosis and killed off the whole plant when the temperature was high. CMV infection in early spring used to bring a major lost of radishes and other cruciferous crops.

(a) (b)

Fig. 1.1. Symptoms caused by infection of *Cucumber mosaic virus* in the field (a) Field tomato plant complexly infected by *Cucumber mosaic virus* with a satellite RNA and a strain of *Tomato mosaic virus*; (b) Field radish plant infected by CMV

CMV can easily transmit mechanically to a wide range of plants. This characteristic makes it easier to do more research for virus-host interactions, and also for virus-virus interactions. Typical symptoms induced by CMV are supplied in Fig. 1.2. The rapid replication and high accumulation in leaf tissues of systemic hosts provide another advantage for genomic and quantitative studies. As shown in Fig. 1.3, CMV particles reach a high accumulation condition within four days of inoculation in cells of the inoculated leaf. And the potyvirus infection also shows distinguished characteristics.

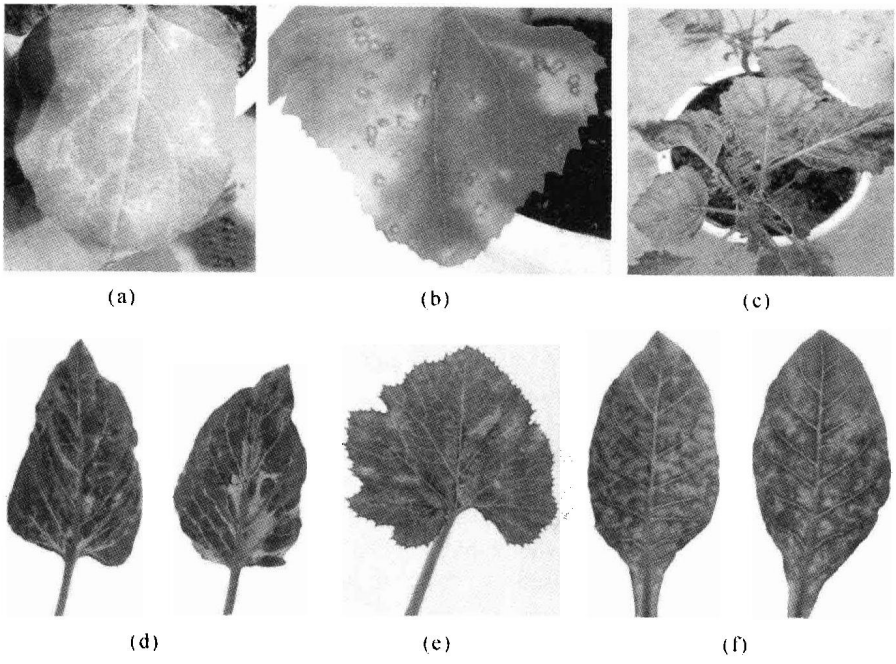
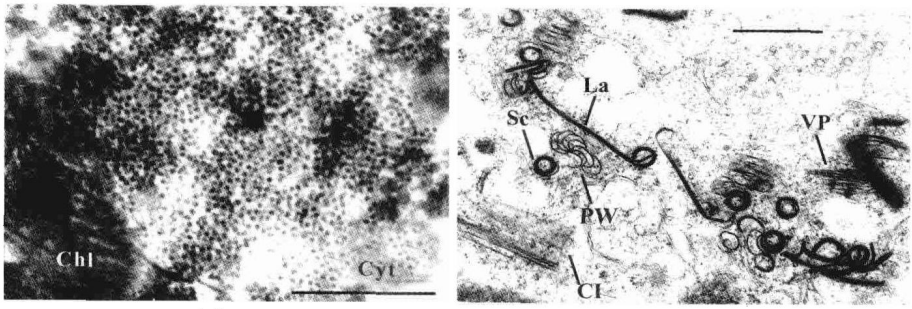


Fig. 1.2. Typical symptoms caused by inoculation of *Cucumber mosaic virus*
(a) *Nicotiana glutinosa*, presenting irregular yellowing spots on the inoculated leaves; (b) *Chenopodiu amaranticolor*, presenting local lesions on the inoculated leaves; (c) *Nicandra physalodes*, presenting systemic mosaic and distortion the lower leaves inoculated dropped; (d) *N. glutinosa*, presenting systemic mosaic; (e) *Lagenaria siceraia*, presenting systemic mosaic; (f) *N. tobacum* (cv. HuangMiaoYu), presenting systemic mosaic

Major differences are also characterized for CMV and the co-infection potyvirus, for CMV is more a hot time virus occurring in seasons with higher temperature, whilst potyvirus such as TuMV is more likely to occur in cool seasons (Table 1.1).

In the same ecological position and transmitted via similar methods (both by aphids and by mechanical transmission), CMV and potyvirus are found to have infected the same crops and express typical mosaic symptoms. It could be considered that the two kinds of viruses can be evaluated together or with similar mechanisms. The morphological characteristics are presented in Fig. 1.4.

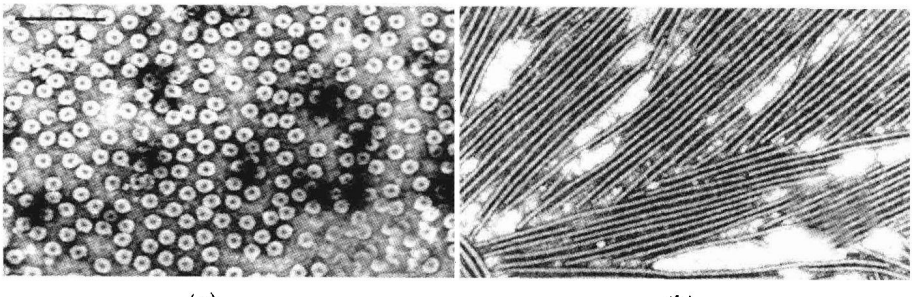


(a) (b)

Fig. 1.3. Cytoplasmic alteration of the host tissues by infection of *Cucumber mosaic virus* and *Turnip mosaic virus* (TuMV)
(a) Cytoplast structure of *N. glutinosa* (inoculated leaf 4 days post inoculation) infected by *Cucumber mosaic virus*, presenting numerous spherical virus particles in the cytoplasm and the remaining chloroplast layers (Chl: chloroplast layer; Cyt: cytoplasm), bar = 750 nm; (b) Cell structure of *Brassica compestris* ssp. chinensis infected with TuMV, presenting different structures of cylindrical inclusion body and aggregated filamentous virus particles (PW: pinwheel structure; CI: cylindrical structure; Sc: scroll-like structure; La: laminated aggregates; VP: virus particle), bar = 600 nm

Table 1.1 Seasonal occurrence in frequencies of principal viruses infecting cruciferous crops

Seasons	CMV	TuMV	CMV +TuMV	CMV (%)	TuMV (%)	CMV/TuMV	Total isolate obtained
Spring (March–May)	12	22	5	44.0	69.2	0.63	39
Summer (June–August)	5	5	0	50.0	50.0	1.00	10
Autumn (September–November)	24	9	5	72.5	35.0	2.10	40
Winter (December–February)	42	51	7	43.3	51.3	0.85	113



(a) (b)

Fig. 1.4. Morphological characteristics of *Cucumber mosaic virus* and potyvirus infecting crucifers
(a) Virus particles of *Cucumber mosaic virus*, bar = 150 nm; (b) Virus particles of Turnip mosaic virus, bar = 350 nm

The survey was done during 1997 and 2001, in Zhejiang Province, eastern China.

In comparison with conventional techniques, viral genome sequence analysis is the most direct and one of the most valid methods to date. As a tripartite RNA virus, CMV contains three capped single-stranded positive-sense genomic RNAs named RNA1, RNA2 and RNA3. RNA1 and RNA2 encode 1a protein and 2a protein respectively, which are involved in virus replication (Hayes and Buck, 1990). RNA3 encodes 3a protein (MP) and coat protein (CP). 3a protein is responsible for virus movement (Ding et al., 1995; Li et al., 2001). CP is translated via a subgenomic RNA4 and involved in virus movement and aphid-mediated transmission (Kaplan et al., 1998; Perry et al., 1994). In addition, 2b protein encoded by subgenomic RNA4A via RNA2 functions in long-distance movement and as a post-transcriptional gene silencing suppressor (Brigneti et al., 1998; Ding et al., 1994; 1995).

A major advantage in using CMV as model ssRNA virus is that it has high copies of double stranded RNAs of the full-length genome. The dsRNA segments are easily extracted and analyzed, and can be regarded as replication forms, as shown in Fig. 1.5.

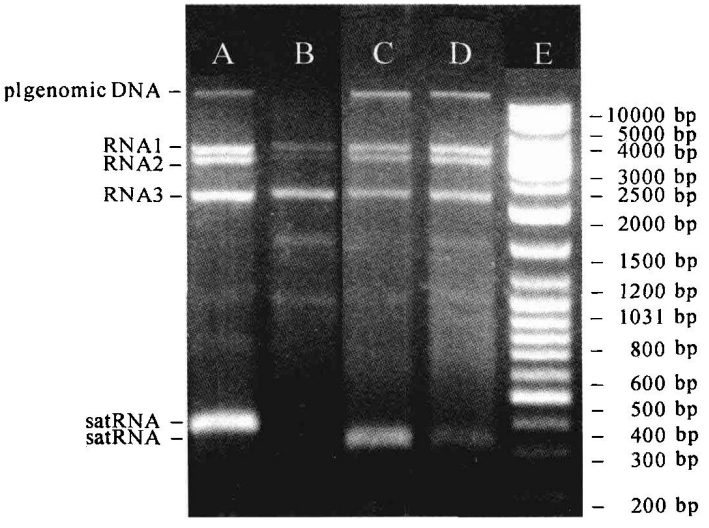


Fig. 1.5. Patterns of dsRNA analysis of several *Cucumber mosaic virus* isolates
The dsRNA pattern shows relative copy numbers of carried satellite RNA and the genome RNAs

When determining the symptom development by host, coat protein is involved in quite a few functions. It is highly expressed via subgenomic RNA4. To outline the fine functional domain and related sequence of CMV RNA3 subgenomic promoter region (SgPr) as a region for regulation between 3a protein and CP sequence, the sequences for SgPr are determined and compared to the reported sequences for CMV RNA3 with different origins. Among the CMV isolates compared, SgPrs are found to consist of 284–323 nt, varying among the isolates.

The SgPr sequence for subgroup I is obviously different from that of subgroup II because of a sequence similarity of <70% between them. This indicates that the SgPr region may have additional biological significance and that the SgPr may have no direct relationship with the presence or absence of a satRNA in CMV (Fig. 1.6).

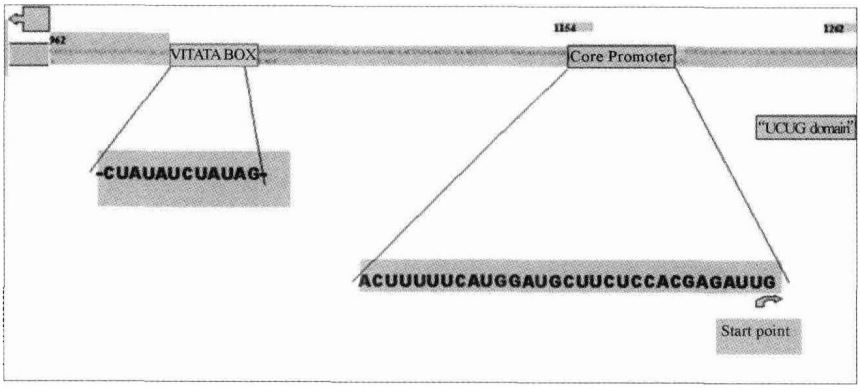


Fig. 1.6. Fine structure and functional motifs of RNA3 subgenomic core promoter region of *Cucumber mosaic virus*

Introduced in this chapter, the evolution mechanism between CMV and the co-infecting potyvirus and the evolution mechanism between CMV and its satellite RNAs (satRNAs) could be different from each other. CMV, as a multipartite ssRNA virus, pseudo-recombination is considered frequently, while potyvirus infecting new host plants seems to utilize a combination among and within gene motifs. For example, the new CMV strain infecting the tomato is discovered as a natural reassortment between the strain of the same virus, but to fit the infection to *Pinellia ternata* from the *Araceae* family, a potyvirus jointly coming from *Soybean mosaic virus* (SMV) and *Watermelon mosaic virus* (WMV). At the same time, an unusual strain of CMV infects *P. ternata* after a period of fitness to vegetative propagation of the host plant displays site mutation and deletion (insertion) mechanisms, with some independent evolution at their UTR terminus (Figs. 1.3 and 1.4). It is believed that the host and geographical environment had an impact on evolutionary types of this virus.

As for satRNA of CMV, the secondary structures are essential for replication and stability. Changes at a single base could influence survival or interaction.

1.2 A Tomato Strain of *Cucumber Mosaic Virus*, a Natural Reassortant Between Subgroups IA and II

According to serological relationships and nucleic acid identities, CMV isolates have been classified into two main subgroups, namely subgroup I and subgroup II (Palukaitis et al., 1992). The analysis of a larger numbers of CP genes and 5′

non-translated regions of CMV isolates' RNA3 has led to a further division of subgroup I into subgroups IA and IB (Roossinck et al., 1999). The nucleotide sequence identity between CMV subgroups I and II strains ranges from 69% to 77%, while it is above 90% within a subgroup (Palukaitis et al., 1992). CMV strains of subgroups IA and II have been reported from most parts of the world, while subgroup IB strains are considered to be mainly restricted to Asia (Roossinck, 2002). Considering the tripartite nature of the CMV genome, reassortment is one of the mechanisms for genetic variation and new strain generation of multipartite RNA viruses (Chao, 1997). Reassortment of multipartite RNA viruses has been displayed for many animal viruses and plant viruses, such as the influenza virus (McCullers et al., 1999) and tobravirus (Robinson et al., 1987). Among cucumoviruses, an interspecific reassortant, composed of CMV RNA3 and *Peanut stunt virus* (PSV) RNAs 1 and 2, and an intraspecific reassortant of PSV, have been discovered (Hu and Ghabrial, 1998; White et al., 1995). Studies of natural CMV populations have showed that mixed infection by different CMV strains is frequent and genetic exchange by reassortment occurred (Bonnet et al., 2005; Fraile et al., 1997).

However, natural reassortants between CMV subgroups and strains should survive against selection and could not become established as dominating populations before a favorite condition appears. Furthermore, reassortment does not occur randomly. The fraction of reassortants between CMV subgroups IA and IB is found to be larger than that of reassortants between subgroups I and II. Before, only one naturally occurring reassortant between CMV subgroups I and II strains was found by Bonnet et al. (2005).

A CMV strain, represented as an isolate, namely CMV-Tsh has been detected for its wide distribution in a tomato field in Shanghai, emerging in spring 2005. This isolate is found to be a natural reassortant between subgroups I and II based on sequence analysis.

Based on biological inoculation, virus isolation, serological identification and, especially, double stranded RNA analysis, the existence of CMV with a satRNA is found to co-exist with ToMV to cause severe systemic mosaic and necrosis synergy. After gene cloning with full-length cDNA amplified with primer pairs against all the subgroups I and II strains, the genomic sequences of this CMV are obtained. The full length RNA1 is obtained by cloning two RT-PCR products respectively. The primers used for amplification CMV genomic RNAs are listed in Table 1.2. The full length sequences of CMV-Tsh RNA1, RNA2 and RNA3 have been submitted to GenBank under the accession number EF202595, EF202596 and EF202597, respectively. CMV-Tsh RNA1 is found to consist of 3,394 nucleotides (nt), encoding 1a protein of 994 amino acids from 96 to 3,077 nt. RNA2 is consisted of 3,047 nt, containing two partially overlapped ORFs 2a and 2b. The 2a ORF encoding 2a protein of 858 amino acids extends from 86 to 2659 nt, and the 2b ORF is positioned at the sequence from 2,418 to 2,750 nt, encoding 2b protein of 111 amino acids. RNA3 contains 2,206 nt, encoding 3a protein of 280 amino acids and CP of 219 amino acids, corresponding to the sequences from 97 to 936 nt, and 1,229 to 1,885 nt respectively.