

Contents

1	Gene Cloning of <i>Cucumber Mosaic Virus</i> and Some Related Viral Agents.....	1
1.1	Introduction.....	1
1.2	A Tomato Strain of <i>Cucumber Mosaic Virus</i> , a Natural Reassortant Between Subgroups IA and II.....	5
1.3	The <i>Araceae</i> Strain of <i>Cucumber Mosaic Virus</i> Infecting <i>Pinellia ternata</i> Suggested to be a Novel Class Unit Under Subgroup I.....	10
1.3.1	Phylogenetic and Sequence Divergence Analysis of 3a and CP ORFs.....	15
1.3.2	Phylogenetic and Sequence Divergence Analysis of 5' UTR and 3' UTR, 2a and 2b ORFs of RNA3.....	15
1.4	The Potyvirus Infecting <i>Pinellia ternata</i> is a Recombinant Contributed by <i>Soybean Mosaic Virus</i> and <i>Lettuce Mosaic Virus</i>	17
1.4.1	DAS-ELISA Analysis of Field Samples for Detecting the Potyvirus.....	18
1.4.2	Sequencing and Nucleotide Sequence Analysis of the Potyvirus Infecting <i>Pinellia</i>	20
1.4.3	Amino Acid Sequence Analysis for CP of the Potyvirus Infecting <i>Pinellia</i>	22
1.4.4	Nucleotide Sequence Analysis for CP N-terminal of the Potyvirus Infecting <i>Pinellia</i>	24
1.4.5	Amino Acid Sequences for N-terminal and for the Conserved Region of the Potyvirus Infecting <i>Pinellia</i>	25
1.4.6	Nucleotide Sequences for 3' UTR of the Potyvirus Infecting <i>Pinellia</i>	27
1.4.7	The General Character and Possible Origin of the Potyvirus Infecting <i>Pinellia</i>	27
1.5	The 5' Terminal and a Single Nucleotide Determine the Accumulation of <i>Cucumber Mosaic Virus</i> Satellite RNA.....	31
1.5.1	GUUU- in 5' Terminal is Necessary to Initiate Replication of 2msatRNA.....	32

3.2.3	ToMV-N5-initiated Necrosis on Tomato Cannot be Protected by Previous Inoculation with Potato Virus X.....	103
3.2.4	CMV-initiated Protection against ToMV-N5 is Related to the Replication and Accumulation of Challenging Virus.....	104
3.3	Pseudo-recombination between Subgroups of <i>Cucumber Mosaic Virus</i> Demonstrates Different Pathotypes and Satellite RNA Support Characters.....	105
3.3.1	Wildtype and Pseudo-recombinants and with or without satRNA Induce Different Symptoms on <i>N. glutinosa</i>	105
3.3.2	Wildtype and Pseudo-recombinants with or without satRNA Induce Different Symptoms on <i>N. benthamiana</i>	107
3.3.3	Wildtype and Pseudo-recombinants with or without satRNA Induce Different Symptoms on Tomato Varieties.....	108
3.3.4	The Pathogenicity of Wildtype and Pseudorecombinants with or without satRNA-Tsh are Related to Viral Accumulation....	110
3.4	Synergy via <i>Cucumber Mosaic Virus</i> and <i>Zucchini Yellow Mosaic Virus</i> on Cucurbitaceae Hosts.....	111
3.4.1	Assessment of Symptom and Synergic Interaction by <i>Cucumber Mosaic Virus</i> and <i>Zucchini Yellow Mosaic Virus</i> ...	112
3.4.2	Accumulation Kinetics for CMV ORFs in Single or Complex Infection.....	113
3.4.3	Accumulation Kinetics of ZYMV CP ORF in Single or Complex Infection.....	116
3.5	Methodology.....	117
3.5.1	The Interaction Study on CMV and ToMV Interaction.....	118
3.5.2	Pseudo-recombination of CMV Subgroups.....	119
3.5.3	Synergy between CMV and ZYMV on <i>Cucurbitaceae</i>	121
	References.....	122
4	Gene Function of <i>Cucumber Mosaic Virus</i> and its Satellite RNA Regarding Viral-host Interactions.....	125
4.1	Introduction.....	125
4.2	The 2b Protein of <i>Cucumber Mosaic Virus</i> is a Determinant of Pathogenicity and Controls Symptom Expression.....	127
4.2.1	Infectivity and Stability of Fny-CMV Derived Mutants.....	128
4.2.2	Replacement of the 2b ORF Affected Capsidation of Viral RNA 2.....	130
4.2.3	Intraspecies Hybrid Viruses by Changing 2b Gene Induce Different Virulence.....	132

4.2.4	Divertive Virulence is Mediated by the 2b Protein Rather than by the C-terminal Overlapping Parts of the 2a Protein.....	132
4.2.5	Virulence is Associated with the Accumulation of Viral Progeny RNAs Affected by 2b Protein.....	134
4.3	Function of CMV 2b Protein and the C-terminus of 2a Protein in Determining Viral RNA Accumulation and Symptom Development...	137
4.3.1	The Systemic Necrosis-inducing Domain is Related to a 125-nucleotide Region of RNA 2.....	138
4.3.2	Effect of 2b Protein Amino Acid 55 on Viral Accumulation and Symptom Development.....	140
4.3.3	Sequence Analyses of the 2b Proteins and the C-top of the 2a Proteins.....	141
4.3.4	Effect of the C-terminus of 2a Protein on Symptom Expression and Virus Accumulation.....	143
4.4	Satellite RNA-mediated Reduction in Accumulation of CMV Genomic RNAs in Tobacco Related to 2b Gene of the <i>Helper Virus</i>	146
4.4.1	Symptom Expression on <i>N. Tabacum</i> Inoculated with CMV-Fsat.....	146
4.4.2	Effect of satRs on the Accumulation of CMV-Fny Genomic RNAs.....	148
4.4.3	Symptom Expression on the Host Plants Inoculated with CMV-FnyΔ2b.....	148
4.4.4	Accumulation of CMV-FnyΔ2b Genomic RNAs and the Effect of satRNAs.....	149
4.4.5	Accumulation of CMV-Fny Genomic RNAs in the Inoculated Leaves and the Effect of satRNAs.....	151
4.4.6	The Effect of satRNAs on Long-distance Movement of CMV-Fny Genomic RNAs.....	152
4.5	Methodology.....	153
4.5.1	Plants, Viruses and Plasmid Constructs.....	153
4.5.2	Plant Inoculation and Viral Progeny RNA Analysis.....	158
4.5.3	Quantifying the Accumulation of Viral RNAs in Leaf Tissue...	159
	References.....	159
5	Plant MicroRNAs and Their Response to Infection of Plant Viruses ...	163
5.1	Introduction.....	163
5.2	Methodology.....	165
5.2.1	Computational Prediction of miRNAs and Their Target Genes	

for Plant Species with Known Genome Sequences.....	165
5.2.2 Use Plant miRNA Microwarrays to Identify Conservative miRNAs from New Host Plants.....	167
5.2.3 Use Plant miRNA Microarrays to Identify Conservative miRNAs Response to Virus Infection.....	169
5.2.4 Quantitative Determination of miRNAs by Stem-loop Real-time RT-PCR.....	170
5.2.5 Design of Plant miRNA-array and Data Analysis.....	173
5.2.6 Confirmation of miRNAs by Northern Blotting and Target mRNA by 3'-RACE.....	174
5.3 Tomato miRNAs Predicted from Known Genomic Sequences and Discovered by miRNA Microarray.....	174
5.3.1 Potential Tomato miRNAs Predicted Computationally According to Known Genomic Sequences.....	175
5.3.2 Potential Targets of Newly Predicted miRNAs and Their Function.....	178
5.3.3 Confirmation of Tomato miRNAs Expression and Survey by Microarray.....	180
5.4 Mechanisms Involved in Plant miRNA Expression with Regard to Infection of ssRNA Viruses.....	185
5.4.1 Phenotype in Tomato Under Infection with CMV/satRNA Combinations and ToMV.....	186
5.4.2 Response of Tomato miRNA Expression to Virus Infection.....	187
5.4.3 MiRNA Expression Profiles between CMV-Fny and CMV-Fny Δ 2b Infections.....	193
5.4.4 MiRNAs Expression Profiles Altered with Addition of satRNAs.....	194
5.4.5 A Comparison of miRNAs Expression Profiles between CMV and ToMV Infections.....	195
5.5 Tomato miRNA Response to Virus Infection Quantified by Real-time RT-PCR.....	197
5.5.1 Identification of Tomato ARF8- and AGO1-like Genes.....	199
5.5.2 Analytical Validation of Real-time RT-PCR for Amplification of miRNAs.....	200
5.5.3 Quantification of Tomato miRNAs Expression by Stem-loop Real-time RT-PCR.....	202
5.5.4 Quantification of miRNAs Targets in Tomato under <i>Cucumovirus</i> Infection.....	204
References.....	206

6 Genomic Characterization of New Viruses with Double Stranded RNA Genomes.....	211
6.1 Introduction.....	211
6.2 Novel dsRNA Viruses Infecting <i>Raphanus sativus</i>	212
6.2.1 Yellow Edge Symptoms and dsRNA Patterns in the Radish.....	213
6.2.2 Genome Characterization of <i>Raphanus sativus Cryptic Virus 1</i>	217
6.2.3 Genome Characterization of <i>Raphanus sativus Cryptic Virus 2</i>	222
6.2.4 Correlation of <i>Raphanus sativus Cryptic Virus 2</i> with <i>Raphanus sativus Cryptic Virus 1</i>	224
6.2.5 Genome Characterization of Suggested <i>Raphanus sativus Cryptic Virus 3</i>	226
6.2.6 The Possible Existence of More dsRNA Viruses in Radish.....	229
6.3 Double Stranded Viruses in <i>Vicia faba</i>	229
6.3.1 Two dsRNA Viruses Infecting <i>V. faba</i>	230
6.3.2 A Partitiviruss Infecting <i>Aspergillus</i> sp. Associated with Leaf Tissue of <i>Vicia faba</i>	237
6.4 A Novel dsRNA Virus Infecting <i>Primula malacoides</i> Franch.....	243
6.5 Derivation and Evolutionary Relationship of dsRNA Viruses Infecting plants.....	249
6.6 Conclusion.....	257
6.7 Methodology.....	258
6.7.1 Plant Material and dsRNA Extraction.....	258
6.7.2 Purification of Virus Particles.....	260
6.7.3 Amplification of Unknown dsRNA Sequence by Modified Single-primer Amplification Technique (SPAT).....	260
6.7.4 Sequence Analysis.....	261
6.7.5 Dot-Blot Hybridization.....	262
References.....	262
Index.....	267