Emergence of a new satellite RNA from cucumber mosaic virus isolate P1*

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Abstract: The cucumber mosaic virus (CMV) isolate P1 caused very mild symptoms on many plant species. After serial passages by mechanical inoculation over five years, CMV P1 caused severe symptoms on several tobacco cultivars and tomato. A specific band of approximately 0.3 kb in length was amplified by RT-PCR with primers synthesized based on reported CMV satellite RNA (satRNA) sequences. Sequence analysis showed there were two satRNAs (Sat-P1-1 and Sat-P1-2). Sat-P1-1 contained 335 nucleotides, and Sat-P1-2 contained 394 nucleotides. These two satRNAs shared 64% overall nucleotide sequence homology, and differences between the two satRNAs included mutations as well as deletions. Sat-P1-1 was identical to a satRNA (Z96099) reported in 1995 in CMV P1. Based on differences in the sequence and secondary structure between these two satRNAs, we conclude that Sat-P1-2 represents the emergence of a new satellite (necrotic satellite) from attenuated satRNA populations. The possible effect of the emergence of this new satRNA is discussed.

Key words: Cucumber mosaic virus, Satellite RNA, Sequence
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INTRODUCTION

Cucumber mosaic virus (CMV), the type species of the genus *Cucumovirus* of family *Bromoviridae*, is an isometric virus with a tripartite single-stranded RNA genome of positive polarity. CMV has a particularly wide host range, infecting more than 800 species in over 70 families of mono- and dicotyledonous plants, and is an economically important pathogen of vegetables, ornamentals, legumes and other important crops in many areas of the world (Grieco *et al.*, 1997). The virus is transmitted effectively by more than 60 aphid species.

The satellite RNA (satRNA) of CMV is a small 332-405-nucleotide linear molecule. The presence of this satRNA results in a decreased accumulation of CMV in the tissues of infected plants and modifies the symptoms induced by CMV, according to a complex interaction that is dependent on the strain of CMV, the variant of satRNA and the species of host plant (Garcia-Arenal *et al.*, 2000). A number of variants of CMV satRNA intensify symptoms induced by CMV in different plant species, whereas others attenuate them.

We identified a CMV isolate from pea (CMV P1), which caused very mild symptoms on many plant species due to a satRNA that attenuated viral symptoms (Zhou *et al.*, 1994; 1995a). After serial passages mechanical inoculation over 5 years, CMV P1 was found to cause more severe symptoms on several tobacco cultivars and tomato. In order to determine the reason for the symptom changes, sequences of satRNA were determined, and a new satRNA was discovered in CMV P1.

MATERIALS AND METHODS

1. Virus strain and inoculation

CMV P1 was isolated from pea (*Pisum sativum*) (Zhou *et al.*, 1995a), and propagated

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in tobacco (*Nicotiana tabacum*). Tobacco and tomato plants were inoculated with CMV P1 by mechanical inoculation and the plants were maintained in an insect-proof greenhouse.

2. Double-stranded RNA (dsRNA) purification

The dsRNAs were purified from CMV P1 infected tobacco leaves by the method of Zhou *et al*. (1995b).

3. RT-PCR and Cloning

dsRNA was denatured by boiling for 3 min and then immediately placed on ice. The first-strand cDNA was synthesized by Expand™ reverse transcriptase (Bohringer Banhhein) using primer I with denatured dsRNA as template. PCR and PCR product cloning were carried out as previously described (Zhou *et al*., 2000). The primers were designed based on reported satRNA sequences (Zhou *et al*., 1995a). The primer sequences were 5'-CACTGCAGGTTTTGTTTGATGGAG-3' (I) and 5'-GACCCGGGTCCTGTAGAGGAA-3' (II).

4. Nucleotide sequencing and sequence analysis

Plasmids were purified using a QIAprep spin plasmid kit (Qiagen). The clones were sequenced using an ALFexpress DNA Sequencer (Pharmacia). Universal M13 primer and reversal primer were used for sequencing. Sequencing was performed in both directions. Sequences were assembled and analyzed with the aid of GCG Program of the Wiscosin Package Version 8.1.

RESULTS

1. Symptomology of CMV P1

The original CMV P1 isolate caused very mild symptoms on many plant species. After more than 50 passages by mechanical inoculation over five years, CMV P1 caused more severe symptoms (Fig.1).

2. cDNA synthesis and PCR

cDNA was synthesized and used as a template for PCR. A specific band of approximately 0.3 kb was amplified (Fig.2). The PCR products were purified and cloned into pGEMT-Easy vector.

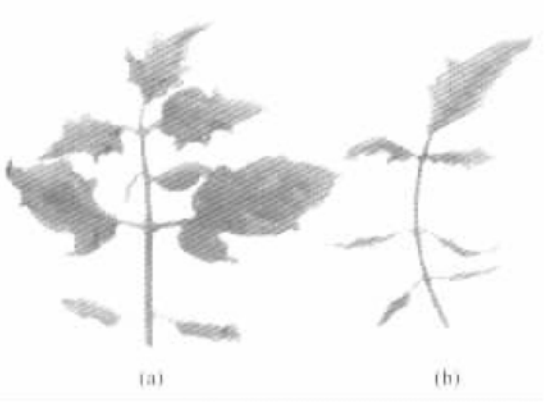

Fig.1 Symptoms produced by CMV P1 on tomato
(a) original; (b) passage

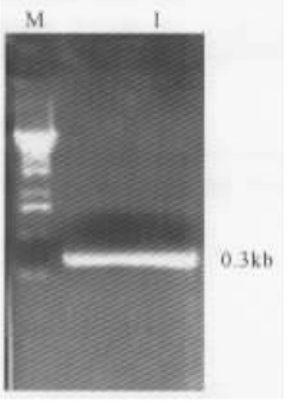

Fig.2 Agarose gel electrophoresis of PCR product
Lane M: 1kb DNA ladder; lane 1: PCR product

3. Sequence analysis

Two recombinant clones were selected. The plasmids were purified and sequenced. Two satRNAs (Sat-P1-1 and Sat-P1-2) were obtained. Sat-P1-1 contained 335 nucleotides (EMBL accession number AJ457163), while Sat-P1-2 contained 394 nucleotides (EMBL accession number AJ457164). Comparison showed Sat-P1-1 had 100% nucleotide sequence homology with a satRNA (Z96099) reported in 1995 from CMV P1 (Zhou *et al*., 1995a), while Sat-P1-2 had very high nucleotide sequence homology (97%) with a reported Chinese satRNA tn (D84389) (Fig. 3), which was associated with CMV and induced tomato necrosis (Cheng *et al*., 1997). Sat-P1-2 also had high nucleotide sequence homology with satRNA d27 (85%, U31661) and satRNA t43 (88%, D10039). Both satRNA d27 and t43 had necrogenic properties that could intensify symptoms in tomato. CMV P1 Sat-1 and Sat-2 shared only 64% over-

all nucleotide sequence homology, and differences between the two satRNAs included mutations as well as deletions (Fig.3).

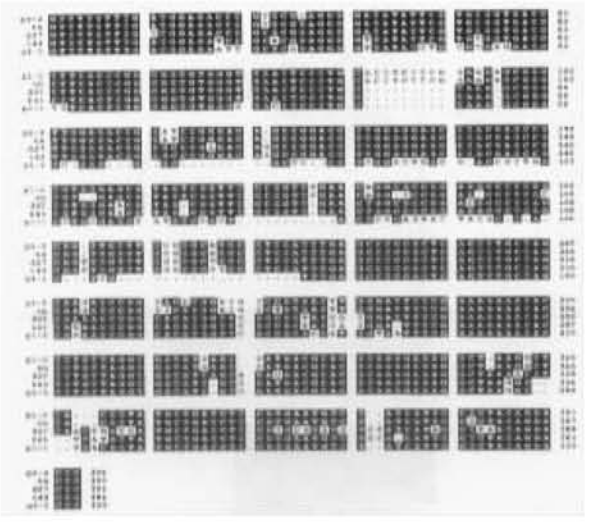

Fig.3 Sequence comparison of two satRNAs with the reported necrotic satRNAs (tn, d27 and t43).

In order to examine if there were more types of satRNA, 21 recombinant clones were selected, and the purified recombinant plasmids were digested with *Nco* I, *Sca* I and *Kpn* I, electrophoresis showed that 13 clones had restriction pattern identical to that of Sat-P1-1, while the pattern of another 8 clones resembled that of Sat-P1-2, suggesting that there were two main types of satRNA.

The secondary structures of the two satRNAs were predicted by computer analysis using the GCG Program of the Wiscosin Package Version 8.1 (Fig.4). Both satRNA sequences had very high base pairing, but the secondary structure of the two satRNAs was distinct. The structure proposed for Sat-P1-2 (free energy of -129.8 kJ/mol) was more stable than that of Sat-P1-1 (free energy of -102.7 kJ/mol).

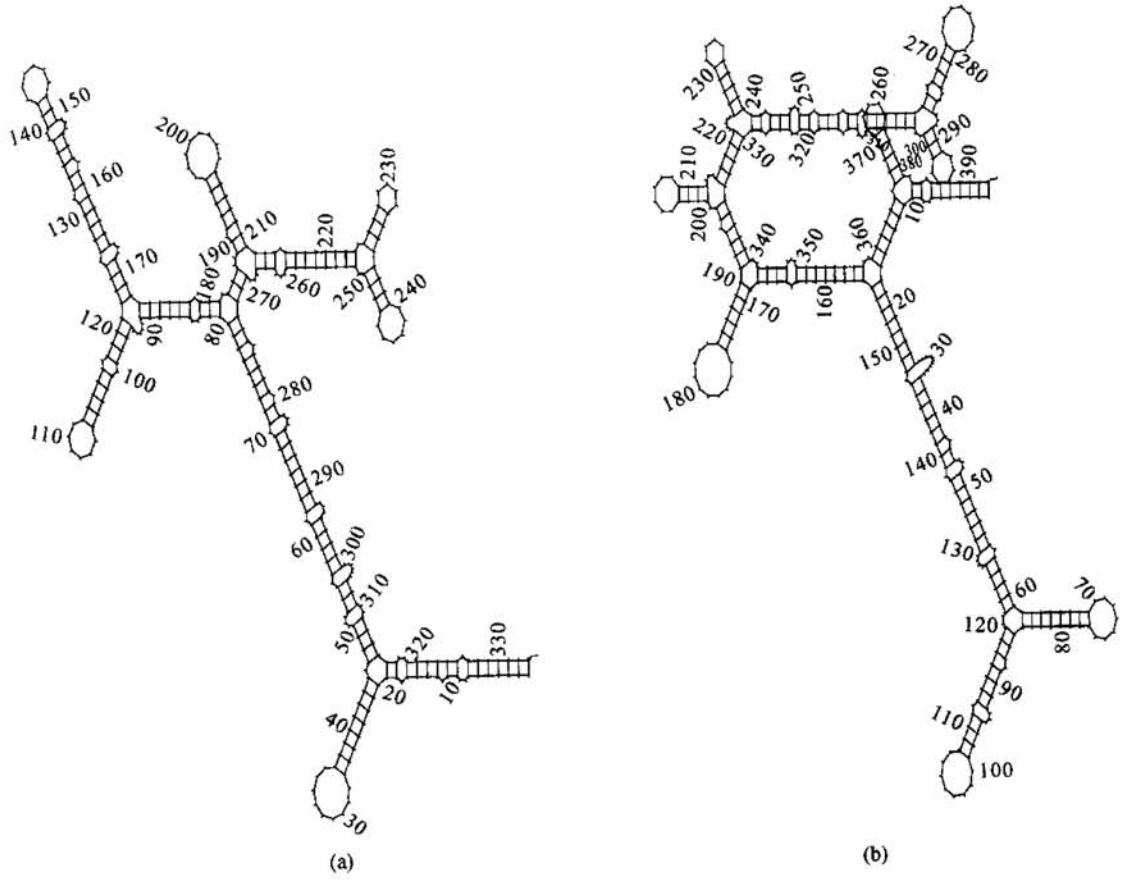

Fig.4 Predicted secondary structures of the two CMV satRNAs. (a) Sat-P1-1; (b) Sat-P1-2. The secondary structures were predicted using the GCG Program of the Wiscosin Package Version 8.1.

DISCUSSION

Two satRNAs were recovered from a CMV P1 isolate (Zhou *et al*., 1994; 1995a) that had become more pathogenic with extensive passages. The satRNA sequences were determined and the results revealed that one satRNA (Sat-P1-1) had remained the same while a second satRNA had emerged. Based on the difference of the sequence length, large sequence deletions and the resulting secondary structure differences between these two satRNAs, we concluded that sat-P1-2 represented the emergence of a new satellite (necrotic satellite) from attenuated satRNA populations. The emergence of a new satRNA led to more severe symptoms in tomato.

A number of variants of CMV-satRNA attenuate symptoms were induced by CMV in different plant species, whereas others intensified them. Variants with attenuating effects, therefore, had been extensively studied as potential or actual biocontrol agents for control of CMV-induced diseases (Tien and Wu, 1991). Our results demonstrated that satRNA populations have genetic divergence and necrogenic satRNA can evolve from attenuated satRNA populations. This is a warning on the wide-spread use of attenuated CMV satRNA in biocontrol programs.

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