

Natural Infection of Banana by a Satellite-Containing Strain of Cucumber Mosaic Virus: Nucleotide Sequence of the Coat Protein Gene and the Satellite RNA

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Banana plants expressing mosaic symptoms, from the Jordan Valley in Israel, were shown to be infected by a satellite-RNA-containing strain of cucumber mosaic virus (CMV). Double-stranded RNA isolated from field-infected banana, without passage through another host, was used as a template for synthesis of cDNA. The cDNAs corresponding to the coat protein (CP) gene and to the satellite RNA were cloned after polymerase chain reaction amplification. The nucleotide sequences of the CP and the satellite cDNAs were determined. The CP gene and its 3' flanking sequence had 98% similarity to the CMV Fny nucleotide sequence and the two strains differed in only one amino acid of the CP. The associated satellite had a sequence similarity ranging from 95% to 85.6% with other CMV satellites. Analysis of banana suckers differing in symptoms' severity indicated a correlation between the presence of satellite and attenuation of symptoms.

KEY WORDS: Double-stranded RNA; satellite RNA; polymerase chain reaction, PCR; cucumber mosaic virus; banana.

INTRODUCTION

The small icosahedral virions of cucumber mosaic virus (CMV) encapsidate three single-stranded plus-sense genomic RNAs and a fourth subgenomic RNA which is the messenger RNA for the coat protein (CP) (20). Some strains of CMV contain a fifth RNA often designated CARNAS (CMV associated RNA 5) (14,21). CARNAS is a linear satellite RNA which is dependent on the helper virus for replication and encapsidation (16). The presence of satellite RNA can affect CMV symptoms development following infection. In tomatoes infected by CMV, satellite RNA can co-determine various symptoms, such as necrosis (14), severe white leaf symptoms (9), stunting of the plant (10), or attenuate disease symptoms (13). The effect of CMV satellite RNA on symptom expression is the result of interaction of the satellite, its helper virus and the host plant genome. The same satellite RNA can ameliorate or exacerbate the disease symptoms, depending on the particular combination of helper virus, plant host and growing conditions (13,21).

CMV was identified as an agent of mosaic disease of banana (*Musa* sp.) (18,26). The disease is present in all banana-growing areas (1). In this paper we report the detection of a satellite-containing strain of CMV in naturally infected banana plants in Israel. The

Contribution from the Agricultural Research Organization. No. 1463-E, 1994 series. Received July 30, 1995; received in final form Oct. 19, 1995.

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CP gene, its 3' flanking sequence and the associated satellite RNAs were cloned and their nucleotide sequences were determined.

MATERIALS AND METHODS

Extraction of double-stranded RNA from infected banana plants

Infected banana plants were individually analyzed for the presence of CMV satellite RNA by dsRNA analysis. Double-stranded RNA was isolated by CF11 (Whatman) chromatography followed by CC41 (Whatman) chromatography, as described by Dodds (4). Polyacrylamide gel electrophoresis (PAGE) and silver staining of dsRNA were done as described by Dodds (4) and Igloi (11).

cDNA synthesis

cDNA was synthesized on dsRNA template isolated from CMV-infected banana by the method of Wexler *et al.* (25). Initial nucleotide sequence information was derived from plasmid pCBTw9, a previously described partial cDNA clone of RNA-3 (25). Comparison of nucleotide sequence with the CMV sequences in the GenBank and EMBL database revealed that the cDNA contained the 3' 778 nucleotides of RNA-3. The most similar sequence in the database was that of the Fny strain of CMV (19). For cloning of the intact CP gene, two oligonucleotides were synthesized. Oligonucleotide I was used for cDNA synthesis of RNA-3: 5'-CGCGGATCCGAATTCTCCTCTCCTTTTGGAGGC-3' (underlined sequence represents BamH I and EcoR I restriction sites). Oligonucleotide II was designed according to the published Fny sequence (19) and used for second strand synthesis: 5'-GCGAGCTCTAGAAGCCATGGACAAATCTGA-3' (underlined sequence represents Sac I and Xba I restriction sites).

cDNA corresponding to the associated satellite RNA was synthesized using two degenerative oligonucleotides. They were designed according to sequence variations commonly found in published CMV satellite sequences (21). Oligonucleotide III was used for first strand cDNA synthesis: 5'-GACAAGCTTGGGTCTGXAGAGGAAT-3' (X=T or C, underlined sequence represents Hind III restriction site). Oligonucleotide IV was used for second strand synthesis: 5'-GACGAATTCGTTTTGTTTGTG ATY TZGAGAATT-3' (Y=A or T; Z=A or G; underlined sequence represents EcoR I restriction site).

Polymerase chain reaction amplification and cloning

Oligonucleotides I and II were used for PCR amplification of the cDNA corresponding to the CP gene and the 3' non-translated sequence of RNA-3. PCR was performed as described previously (25). Cycling conditions were: one cycle of 94°C for 3 min, 45°C for 1 min and 72°C for 2 min; one cycle of 94°C for 1 min, 45°C for 1 min and 72°C for 2 min; 35 cycles of 94°C for 1 min, 65°C for 1 min and 72°C for 2 min. PCR amplification was performed using a Hybaid Thermo-Cycler.

Oligonucleotides III and IV were used for PCR amplification of satellite-derived cDNA. PCR cycling conditions for the satellite cDNA were: one cycle of 94°C for 3 min, 45°C for 1 min and 72°C for 2 min; two cycles of 94°C for 5 sec, 40°C for 1 sec and 72°C for 5 sec; 30 cycles of 94°C for 1 sec, 50°C for 1 sec and 72°C for 5 sec. PCR amplification of the satellite cDNA was performed on an Idaho Air Thermo-Cycler.

The amplification products were isolated after agarose gel electrophoresis using

Jet Sorb Gel extraction kit (Genomed, Research Triangle Park, NC, USA). The DNA was restricted by the appropriate enzymes (depending on restriction sites incorporated into the specific primers) and cloned by conventional protocols (23) in pBLUESCRIPT (Stratagene, La Jolla, CA, USA).

Nucleotide sequence analysis

DNA sequencing was performed by the Sequenase version 2 kit (USB, Cleveland, OH, USA) according to the manufacturer's instructions. Nucleotide sequences were compared with the GenBank and EMBL database using the UWGCG program (3).

RESULTS AND DISCUSSION

Banana plants showing mosaic symptoms were collected from a commercial plot in the Jordan Valley, Israel. Samples were collected from ten individual plants displaying symptoms varying from mild mosaic to severe mosaic and stunting. dsRNA was isolated directly from infected banana plants without passage in other hosts. In Figure 1 are shown the PAGE profiles of dsRNA extracted from several naturally infected banana plants. A dsRNA preparation from a previously characterized satellite containing CMV from *Nicotiana glauca* (22) was run as a marker with the banana preparation (not shown). dsRNA species corresponding in size to RNAs 1 through 4 of CMV were detected in

Fig. 1. Polyacrylamide gel electrophoresis of double-stranded RNA extracted from: 1, healthy banana plant; 2–7, field-collected samples of cucumber mosaic virus-infected banana plants. A dsRNA preparation from a previously characterized satellite containing CMV from *Nicotiana glauca* (22) was run as a marker (not shown), the bands corresponding to the five CMV dsRNA are indicated.

all symptomatic plants. All preparations also contained a dsRNA species corresponding in size to CMV satellite RNA (CARNA5). Additional low-molecular-weight dsRNA molecules of unknown origin were also present in all samples (Fig. 1).

dsRNA was used as a template for reverse-transcription and PCR amplification of the CP gene as described in the Materials and Methods section. The amplified DNA fragment was cloned and its nucleotide sequence was determined (Fig. 2). Comparison with the EMBL and GenBank nucleotide sequence data revealed that CMV strain Fny (19) is the most similar in sequence to the banana isolate. The CP gene and its 3' flanking sequence showed a 98% similarity to strain Fny at the nucleotide level. The CP of the banana strain differed from the Fny CP only in one out of 218 amino acids, phenylalanine in the banana isolate replacing leucine in Fny at position 166.

The satellite RNA associated with the banana strain of CMV was cloned using dsRNA isolated from a banana plant showing mild symptoms and the nucleotide sequence was determined (Fig. 3). The 339 nucleotide sequence was compared with the CMV satellite sequences found in the EMBL and GenBank data bases. Sequence similarity ranged from 95% with the B1 satellite (8) to 85.6% with the S19 satellite (17).

Suckers from field-infected bananas were maintained in an insect-proof screen house. Some of the plants developed symptoms of severe mosaic and stunting while others showed only mild mosaic. PAGE analysis of dsRNA extracts showed the presence of the satellite RNA in plants exhibiting mild symptoms. No satellite dsRNA was detected in plants with severe mosaic and stunting (Fig. 4).

While satellite RNA is often associated with CMV infection in the greenhouse, it is only infrequently isolated from field plants (20). CMV satellite was detected in tomatoes (6,9,12,14) and tobacco (24). It was also found in samples of eggplant, squash, watermelon and tomato in Spain (6) and in naturally infected *N. glauca* and the ornamental plant *Pachystachys coccinea* in Israel (22), but was not detected in a field survey in Australia (7). In surveys of field-infected plants, satellite RNA was found in two of 106 CMV-positive samples in New York (15) and in 13 of 97 CMV-positive weeds collected in Italy (2). It was not detected in 28 CMV-positive samples in Bermuda (15).

Satellite was detected by hybridization in a single CMV-infected banana sample from Bermuda (5). In the present study, satellite RNA was detected in all field-infected plants analyzed and in some of the plants grown from infected suckers. In suckers, mild mosaic symptoms were associated with the presence of satellite. It will be important to examine carefully the possibility that satellite RNA affects the concentration of viral antigens in banana plants and consequently interferes with virus detection by serological methods.

M D K S E S T S A G R N R R R
ATG GAC AAA TCT GAA TCA ACC AGT GCT GGT CGT AAC CGT CGA CGT 45

R P R R G S R S A P S S A D A
CGT CCG CGT CGT GGT TCC CGC TCC GCC CCC TCC TCC GCG GAT GCT 90

N F R V L S Q Q L S R L N K T
AAC TTT AGA GTC TTG TCG CAG CAG CTT TCG CGA CTT AAT AAG ACG 135

L A A G R P T I N H P T F V G
TTA GCA GCT GGT CGT CCA ACT ATT AAC CAC CCA ACC TTT GTA GGG 180

S E R C R P G Y T F T S I T L
AGT GAA CGC TGT AGA CCT GGG TAC ACG TTC ACA TCT ATT ACC CTA 225

K P P K I D R G S Y Y G K R L
AAG CCA CCA AAA ATA GAC CGT GGG TCT TAT TAT GGT AAA AGG TTG 270
C

L L P D S V T E Y D K K L V S
TTA CTA CCT GAT TCA GTC ACG GAA TAT GAT AAG AAG CTT GTT TCG 315

R I Q I R V N P L P K F D S T
CGC ATT CAA ATT CGA GTT AAT CCT TTG CCG AAA TTT GAT TCT ACC 360

V W V T V R K V P A S S D L S
GTG TGG GTG ACA GTC CGT AAA GTT CCT GCC TCC TCG GAC TTA TCC 405

V A A I S A M F A D G A S P V
GTT GCC GCC ATC TCT GCT ATG TTC GCG GAC GGA GCC TCA CCG GTA 450

L V Y Q Y A A S G V Q A N N K
CTG GTT TAT CAG TAT GCC GCA TCT GGA GTC CAA GCC AAC AAC AAA 495

L
F L Y D L S A M R A D I G D M
TTT TTG TAT GAT CTT TCG GCG ATG CGC GCT GAT ATA GGT GAC ATG 540
C G

R K Y A V L V Y S K D D A L E
AGA AAG TAC GCC GTC CTC GTG TAT TCA AAA GAC GAT GCG CTA GAG 585
C

T D E L V L H V D I E H Q R I
ACG GAC GAG CTA GTA CTT CAT GTT GAC ATC GAG CAC CAA CGC ATT 630

P T S G V L P V
CCC ACG TCT GGA GTG CTC CCA GTC TGATTCCGTGTTCCAGAACCCCTCCCTC 682
A T

CGATTCTGTGGCGGGCAGTGAAGTTGGCAGTTCTGCTATAAACTGTCTGAAGTCACTAAA 742
C AGC

CGC--TTTACGGTGAACGGGTTGTCCATCCAGCTTACGGCTAAAATGGTCAGTCGTGGAG 800
TT

AAATCCACGCCAGTAGATTTACAAATCTCTGAGGCGCCTTTGAAACCATCTCCTAGGTTT 860
C

CTTCGGAAGGACTTCGGTCCGTGTACCTTCCAG--CAACGTGCTAGTTTCAGGGTACGGG 918
- T CA

TGACCCCCACTTTCGTGGGGGCTCCAAAAGGAGACCA 957
C

Fig. 2. The nucleotide sequence and deduced amino acid sequence of cDNA corresponding to the CP gene and its 3' flanking non-translated region of the banana strain of cucumber mosaic virus. Nucleotides and amino acids different in the CMV Fny strain are indicated below and above the sequence, respectively.

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      10          20          30          40          50
GTTTGTGTTG ATGGAGAATT GCGTAGAGGG GTTGTATCTA CGTGAGGATC
      60          70          80          90         100
TATCACTCGG CGGTGTGGGT TACCTCCCTG CTACGGCGGG TTGAGTGACG
      110         120         130         140         150
CACCTCGGAC TGGGGACCGC TGGCTTGCGA GCTATGTCCG CTACTCTCAG
      160         170         180         190         200
CACTACGCAC TCATTTGAGC CCCCCTCAG TTTGCTAACA AAACCCGGCC
      210         220         230         240         250
CGTGGTTTGC CGTTACCGCG GAAATTCGA AAGAAACACT CTGTTAGGTG
      260         270         280         290         300
GTATCAGATG ACGACGCACG CAGGGAGAGG CTAAACCTA TAAGGTCATG
      310         320         330
CTGATCTCCG TGAATGTATA CCATTCCTCT ACAGGACCC

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Fig. 3. The nucleotide sequence of cDNA corresponding to the satellite RNA associated with the banana strain of cucumber mosaic virus.

Fig. 4. Polyacrylamide gel electrophoresis of double-stranded RNA extracted from six cucumber mosaic virus-infected banana plants grown from suckers. Plants 1 and 2 showed symptoms of severe mosaic and stunting, plants 4 and 5, very mild mosaic; plants 3 and 6, symptoms of intermediate severity. The bands corresponding to the five CMV dsRNA are indicated.

ACKNOWLEDGMENTS

The authors thank Ms. Rose Gofman for technical help. This study was supported by grants from the Chief Scientist of the Ministry of Agriculture, Israel, and The Banana Growers Association of Israel. The nucleotide sequences reported in this paper have been submitted to the EMBL and GenBank and assigned accession no. U43888 for the co-protein gene sequence and accession no. U43889 for the satellite RNA sequence.

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