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Nucleotide Sequence Analysis for RNA 3 of pepo-, SO- and MY17-Strains of *Cucumber Mosaic Virus* and its Comparison with Y-Strain*

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Abstract

RNA 3 sequence of pepo-, SO- and MY17-*Cucumber Mosaic Virus* (CMV), which infect *Cucumis figarei* at 36° C, was determined and compared with that of Y-CMV. The sequences indicated that both SO- and MY17-CMV were classified in subgroup I as pepo- and Y-CMV, which further supported that there were no relationships between the subgroups and the ability to infect systemically at 36° C. There were minor differences in the nucleotide sequence among pepo-, SO- and MY17-CMV RNA 3, however, no considerabledifferences in the nucleotide sequence homology were detected between Y- and the three other strains.

Key Words: *Cucumber Mosaic Virus*, strains, RNA 3, nucleotide sequence, systemic resistance.

Cucumis figarei Des. et Naud. is an African wild species relative to *Cucumis melo* L.(melon) and highly resistant to several cucurbit-infecting viruses, including *Cucumber mosaic virus* (CMV). Comparative analysis for replication and movement of CMV in *C. figarei* and *C. melo* indicated that the resistance of *C. figarei* to CMV might be caused primarily by the inhibition of virus movement via both cell-to-cell and long-distance pathways (Saiga *et al.*, 1998). In the previous study, we demonstrated that the systemic resistance in *C. figarei* to Y- and two other CMV strains was sustained at 36℃, but the resistance to pepo-, SO- and MY17-CMV was breakable at the temperature (Saitoh *et al.*, 1998). *C. figarei*-CMV is, therefore, an useful model system for analyzing the virus movement in systemic infection as well as the resistance mechanism.

CMV is a tripartite single-stranded, positive sense RNA virus in *Cucumovirus* of *Bromoviridae*. RNA 1 and RNA 2 encode the 1a and the 2a proteins, respectively, both of which are components for the viral RNA-dependent RNA polymerase (Nitta *et al.*, 1988b; Hayes and Buck, 1990). RNA 3 encodes the 3a movement protein (3a MP) and the coat protein (CP) that is expressed from subgenomic RNA 4 (Palukaitis *et al.*, 1992). Both 3a MP and CP are reported to be implicated in the long-distance movement of CMV (Taliansky and García-Arenal, 1995; Blackman *et al.*, 1998). Hence, we determined the RNA 3 sequence of pepo-, SO- and MY 17-CMV and compared them with that of Y-CMV.

cDNA clones for pepo-, SO- and MY17-CMV RNA 3

Three CMV strains, pepo-CMV (Osaki and Shohara, 1973; Saitoh *et al.*, 1998), SO-CMV (Saitoh *et al.*, 1998) and MY17-CMV (Saitoh *et al.*, 1998) were used.

Full-length cDNA clones of pepo-CMV RNA 3 was constructed as follows. The sequence of 3' terminus was determined after polyadenylation (Drummond *et al.*, 1985) by dideoxynucleotide sequencing (Meshi *et al.*, 1983; DeBore

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et al., 1986) that was primed using an oligonucleotide (5'-TTTTTTTTTTTTG-3'). The 5' terminus was determined by dideoxynucleotide sequencing using an oligonucleotide (5'-ACCTTGGAAAGCCAT-3'). First-strand cDNA was primed using an oligonucleotide (5'-CCGGATCCTGGTCTCCTTTTGGAGGCCC-3') complementary to 3' proximal 20 nucleotides of CMV RNAs (Nitta *et al.*, 1988a; Owen *et al.*, 1990) with reverse transcriptase (Nippongene). Second-strand cDNA synthesis of pepo- CMV RNA 3 was primed using an oligonucleotide (5'-CGCCTAATACGACTCACTATA GTAATCTTACCACTGTGT-3', underlined shows nucleotides for T7 RNA polymerase promoter). The primer contains 17 nucleotides that constitute most of T7 RNA polymerase promoter sequence followed by 18 nucleotides that correspond to the viral 5' terminal sequence of pepo-CMV RNA 3. Full-length cDNA of pepo-CMV RNA 3 was amplified by RT-PCR and cloned into the plasmid vector pCRII following the protocol and materials provided in the TA Cloning Kit (Invitrogen). *Bam*HI and *Eco*RI fragment from the clone was further cloned into the plasmid pUC18, which had been digested with the same enzymes. The resultant cDNA clone of pepo-CMV RNA 3, which infectivity was confirmed by the bioassay, was designated pCP 3TP2 (P3).

Full-length cDNA clones for SO-CMV RNA 3 and MY17-CMV RNA 3 were constructed as follows. The sequences of the 3' termini were determined as described (Symons, 1979) after polyadenylation (Sanger *et al.*, 1977) and those of the 5' termini were determined by primer extension method (Meshi *et al.*, 1983) using an oligonucleotide (5'-ACCTTGGAAAGCCAT-3') complementary to the representative Y-CMV (Nitta *et al.*, 1988a) and Fny- (Owen *et al.*, 1990) with RAV-2 reverse transcriptase (TaKaRa). To facilitate the cloning of cDNAs, the 5' and 3' primers used for RT-PCR were designed based on the nucleotide sequences of RNA3s to contain *Sna*BI and *Eco*RI sites respectively. The amplified cDNA products were cloned into the *Nsi*I/*Eco*RI sites of plasmid pUCT19, a modified pUCT7 (Mori *et al.*, 1991) after blunting the *Nsi*I site with T4 DNA polymerase with a correct fusion of the viral 5' terminus to a T7 RNA polymerase promoter. The resultant infectious cDNA clones of SO- and MY17-CMV RNA 3 were designated pCS3TP1 (S3) and pCM3TP2 (M3), respectively.

P3, S3 and M3 were then subjected to nucleotide sequencing by dideoxy chain termination method (Sanger *et al.*, 1977) with AutoRead Sequencing Kit (Amersham Pharmacia). Comparative studies on the RNA 3 sequences including Y-CMV RNA 3 (Nitta *et al.*, 1988a) were assisted by computer software program GENETYX (Software Development).

RNA 3 sequences of Y-, pepo-, SO- and MY 17-CMV

The nucleotide sequences of the four CMV RNA 3 are presented in Fig. 1. The accession numbers in GenBank database for pepo-, SO- and MY17-CMV RNA 3 are AF103991, AF10 3992 and AF103993, respectively. The total length of the RNA 3s ranged between 2212 (MY17) to 2217 (Y), but the two open reading frames (ORFs) for 3a MP and CP were composed of the same numbers of amino acid residues, 840 and 657, respectively, among the four strains. Although the pepo-strain reported in Chaumpluk *et al.* (Chaumpluk *et al.*, 1996) has the same origin with our pepo-CMV, there are considerable differences between their sequence data (D28488) and ours. These changes are possibly due to the variation during continuous propagation on plant hosts at individual laboratories.

The entire nucleotide sequence homologies were over 96% among the four strains. Especially the nucleotide sequences in the ORFs for 3 a MP and CP showed higher homologies as

Table 1. Comparative homology (percentage) of nucleotide sequences of the two ORFs among Y-, pepo-, SO- and MY 17-cucumber mosaic virus (CMV)

		3a Protein ORF			
	Strain	Y	pepo	SO	MY17
Coat Protein ORF	Y	100	98.3	98.2	97.5
	pepo	96.3	100	98.9	97.3
	SO	97.0	97.6	100	97.4
	MY17	97.0	95.3	96.3	100

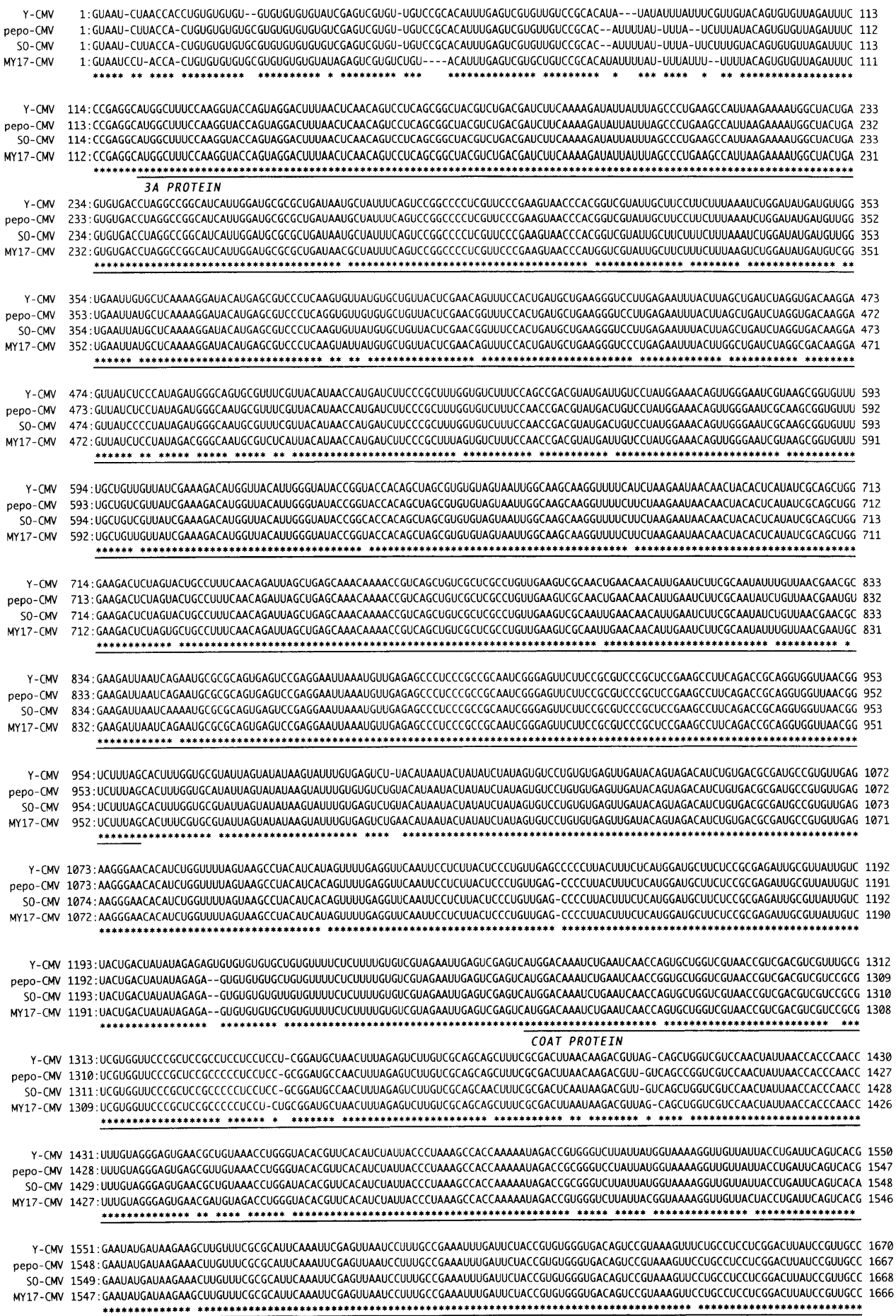
3A PROTEIN
COAT PROTEIN

Fig. 1. Nucleotide sequence of RNA 3 of Y- (Nitta *et al.*, 1988), popo- (AF103991), SO- (AF103992) and MY 17 -CMV (AF 103993).

The asterisk indicates an identical sequence. The open reading frames of the 3 a and the coat protein genes are underlined.

shown in Table 1. Nucleotide sequence homology for the 5' noncoding region, the intercistronic region, and the 3' noncoding region among the four strains were over 89%, 98%, and 65%, respectively (data not shown). Since nucleotide sequence homology of CMV RNA 3 was over 88% within the subgroups but under 67% between them (Chaumpluk *et al.*, 1996), these results verified that both SO- and MY 17-CMV are classified in subgroup I. This further supported that there were no relationships between the subgroups and the ability to infect systemically at 36℃ (Saitoh *et al.*, 1998).

There are minor differences in the nucleotide sequence among pepo-, SO- and MY17-CMV RNA 3, though all three strains can systemically infect *C. figarei* at high temperature. No considerable differences in the nucleotide sequence homology were detected between Y- and the three other strains. On the contrary, critical differences were ascertained in the predicted amino acid sequences, as we reported in a separate paper (Saitoh *et al.*, 1999).

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