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Molecular characterization of *Cucumber mosaic virus* isolates isolated in Korea

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Cucumber mosaic virus (CMV) belongs to genus *Cucumovirus*. The *Cucumovirus* group contains three distinct members: CMV, *Tomato aspermy virus* (TAV), and *Peanut stunt virus* (PSV). The type member, CMV is the most widespread and most studied. CMV is isometric particles about 30 nm in diameter. The genome of CMV is divided into three RNAs. In addition, RNA extracted from virus particles contains a fourth RNA that is a subgenomic RNA generated from RNA3. RNA1 and RNA2 are each encapsidated in separate particles, whereas RNAs3 and 4 are coencapsidated in a third particle. Hence, inoculation by three particles, transmitted either mechanically or by the aphid vector, is required to infect plants.

CMV has the largest host range throughout the temperate regions of the world. It infects cereals, forages, woody and herbaceous ornamentals, vegetables, and fruit crops. The number of plant species identified as hosts for CMV has increased steadily year after year. In the most recently published literature survey of the CMV host range, many weed hosts of CMV were listed, bringing the number to 775 species in 365 genera from 85 families. It is now considered that the host range of CMV is in excess of 1,000 species. There are many strains of CMV, i.e., isolates that show differences in symptoms and/or in host range. In the past year, this has given rise to numerous false identification of new viruses that have turned out to be strains of CMV. On the basis of serological relationships, peptide mapping of the viral coat protein, and nucleic acid hybridization analysis, all but one strain of CMV fall into two major subgroups, which are equivalent to subgroups I and II. Furthermore, sequence analysis of a representative strain from each subgroup verified the designations, and recent analysis of the coat protein (CP) gene and 5′ non-translated region (5′NTR) of RNA3 of several subgroup I CMV strains suggested that they can be further divided into two groups, IA and IB. The relationships of virus strains within each subgroup are very close, and less so between subgroups, as determined by each of the above properties. The CMV has been investigated extensively at the molecular level to understand the mechanism of viral gene expression and replication, and to study the molecular basis of symptom expression. These informations have facilitated the development of specific oligo- nucleotides for use as nucleic acid probes, and as primers of reverse transcription polymerase chain reaction (RT-PCR) that enable accurate identification of CMV.

Last some ten years, research about identification and classification of CMV isolates in Korea was also recorded from much crops or plants. However, as case of other virus, the systematic and

scientific approach of identification of CMV is rare extremely, and mostly remain by simple record only. Because it is real condition that sources of virus identified previously has not been preserved, the viruses were not been utilizing from various plant virus research and were not been achieving in the current to scientific basis extremely. CMV is very important with one of the pathogenic virus in crops been cultivating in Korea. Specially, the virus isolates have been isolated from much crops and plants because CMV has a serious pathogenic specialization, and isolated viruses show variously different characteristics. Therefore, correct classification and identification about CMV isolates which distribute in our country can decide as important work serving diagnosis or control of CMV disease. In the meantime, recently, along with development of molecular biological techniques, plant viruses are turning many attentions in practical use attribute as genetic resources with concept that is control object as pathogens of plant.

Identification and differentiation of *Cucumber mosaic virus* isolates in Korea

Eight isolates of *Cucumber mosaic virus* (CMV) from Korea were identified and differentiated by dsRNA analysis, reverse transcription-polymerase chain reaction (RT -PCR) assay, *Msp* I restriction mapping, single-stranded conformational polymorphism (SSCP) analysis, serological property, and biological reaction in several hosts. All isolates revealed four major dsRNA species with estimated molecular size of 3.4, 3.2, 2.1 and 1.0 kbp. Among them, however, isolate Gs showed a slightly smaller RNA 1 and larger RNA 2 compared to those of other isolates. An RT-PCR assay with primers designed in a conserved region of the 3′ half of the CMV coat protein gene amplified approximately 490 bp DNA fragment from all isolates. Restriction *Msp*I analysis of the RT-PCR products produced distinct digestion patterns that assigned the CMV isolates into one of two subgroups. As a detailed indicator of heterogeneity, the CMV isolates were examined by SSCP analysis to detect sequence variation in the cDNAs produced by RT-PCR. In this way, the CMV isolates could be classified in either of four subgroups. Isolates Pa, Mf, Sa, Ga, and Rs were placed in one subgroup, while isolates Ph, Lc, and Gs were different from one another. In immunodiffusion tests, the isolates Gs only was classified into serotype Ⅱ, and the other isolates which were serologically indistinguishable were classified as serotype Ⅰ. All isolates caused systemic symptoms to upper leaves of *Nicotina glutinosa*, *Melandryum firmum* and *Gentiana axillariflora*. The systemic symptoms were also induced by six isolates in *Cucumis sativus*. However, isolates Ga and Gs were asymptomatic in the cucmber plants. In soybean, only isolate Pa caused systemic infection. The similar result was obtained in radish by inoculation of isolate Rs.

Construction of full-length cDNA of *Cucumber mosaic virus* genomic RNAs, complete nucleotide sequence and generation of infectious RNA transcripts

Full-length cDNAs of *Cucumber mosaic virus* (CMV) RNAs 1, 2 and 3 of the Mf strain were constructed from cDNA copies derived using a method based on the polymerase chain reaction (PCR) with primers that include the T7 promoter and were cloned. The complete nucleotide sequences of all three genomic RNAs of Mf-CMV were determined using the cloned cDNAs. The three genomic RNAs, RNA 1, RNA 2 and RNA 3, are 3,357, 3053 and 2,215 nucleotides long, respectively. RNA 1 and RNA 2 contain a single open reading frame (ORF) of 2,979 and 2,572

bases encoding proteins 1a and 2a, respectively, and the RNA 2 encodes an additional ORF, 2b, that overlaps the 3′ end of ORF 2a. RNA 3 is involved two ORFs of 837 and 654 bases encoding proteins 3a and coat protein, respectively. There was almost complete sequence homology between Mf-CMV and Fny-CMV genomic RNAs (95.0% at the nucleotide level) and lower degree of homology between Mf-CMV and Q-CMV genomic RNAs (65.0% at the nucleotide level). The full-length cDNA clones of all three genomic RNAs of Mf-CMV have been cloned downstream from a bacteriophage T7 RNA polymerase promoter. The RNAs synthesized by *in vitro* run-off transcription in the presence of the 5′cap analog m^7GpppG were infectious in *Nicotiana benthamiana*. Inoculations of the local lesion host *Vigna unguiculata* indicated that the infectivity of the synthetic transcripts was about 1% of that of the native viral RNAs.

A novel strain of *Cucumber mosaic virus* Isolated from *Lilium longiflorum*

A new strain of *Cucumber mosaic virus* (CMV) from easter lily (*Lilium longiflorum*), Ly2-CMV, was identified and compared to the well-characterized Mf-CMV (subgroup I) and LS-CMV (subgroup II) by host reaction in several indicator plants, dsRNA analysis, serological property, RT-PCR analysis, restriction enzyme profile of the PCR products and nucleotide sequence of coat protein (CP) gene. Remarkable differences in symptoms of Ly2-CMV were found between Mf-CMV or LS-CMV in tobacco plants and *Datura straminium*. Ly2-CMV induced small necrotic ringspots on the inoculated leaves of *Nicotiana tabacum* cvs. Xanthi nc and Burley 21 and *D. stramonium*, and failed to infect these species systemically. Of the indicator plants tested, *N. benthamiana* only reacted with systemic infection by inoculation of Ly2-CMV. In experiments of dsRNA analysis, serology and RT-PCR of coat protein (CP) gene, Ly2-CMV was come within subgroup I CMV. However, restriction enzyme analysis of the PCR products using *Msp*I showed that Ly2-CMV was distinct to Mf-CMV. The CP gene of Ly2-CMV contains 657 nucleotides, and the nucleotide sequence is similar to that of Mf-CMV. There is also a high degree of conservation between their putative gene products in Ly2-CMV and Mf-CMV, with five amino acid changes in the 218 amino acids of the CPs.

RT-PCR detection and identification of three species of *Cucumoviruses* with a genus-specific single pair of primers

Reverse transcription and polymerase chain reaction (RT-PCR) was used for detection and identification of three *Cucumoviruses* (*Cucumber mosaic virus*, CMV; *Peanut stunt virus*, PSV; *Tomato aspermy virus*, TAV) in various plants sources with a single pair of primers, designed as CPTALL-3 and CPTALL-5. The pair of *Cucumovirus* genus specific primers that flank the coat protein gene were designed and used to amplify a DNA fragment of approximately ranging from 938 to 966 bp. The RT-PCR with the set of primers specifically amplified the target size of DNA fragment in all the tested *Cucumoviruses* (CMV S-IA, S-IB and S-II, PSV and TAV). No DNA product of any length was produced when brome mosaic virus or tobacco mosaic virus RNA was used as templates. The *Cucumoviruses* examined were differentiated by PCR-restriction fragment length polymorphism with different enzymes. This indicates that the designed primers are only specific for the *Cucumoviruses* and useful for reliable information of identification of members of the

Cucumovirus genus.

Cross-protection effectiveness of *Cucumber mosaic virus* (CMV) isolates associated with satellite RNA for prevention of CMV disease in pepper plants

Two attenuated *Cucumber mosaic virus* (CMV) isolates, Paf-CMV and Rs2-CMV, that have been selected from CMV isolates associated with satellite RNA (satRNA) were tested for cross-protection effect in pepper plants. The viruses selected as attenuated strains appeared to be identical serologically and physically to the challenge virus (Mf-CMV), but they were lower in the dilution end-point of infectivity of crude sap than Mf-CMV. When symptoms were observed in several indicator plants after inoculation, Paf-CMV and Rs2-CMV were symptomless or showed mild mosaic symptoms on the indicator plants, while Ap-CMV, another satRNA isolate, developed severe mosaic symptoms on the leaves as the symptoms induced by Mf-CMV. The nucleotide sequences of the satRNAs were determined by sequencing full-length cDNA clones. Paf-, Rs2- and Ap-satRNAs were 386, 335, and 347 nucleotides long, respectively, and showed nucleotide sequence homologies, varying from 61.7 to 89.3%. The sequences were then compared with the other known Y-satRNA, revealing that nucleotide sequences of the satRNAs consisted of 5′- and 3′-terminal conserved regions. However, variations occurred on the middle regions of the sequences, especially those related to symptom interference showing significant differences between Paf-satRNA and other isolates. Infectious transcripts of Paf-satRNA and Rs2-satRNA induced mild mosaic symptoms in pepper plants when supported by genomic RNAs of Mf-CMV. Under greenhouse conditions, Paf-CMV and Rs2-CMV were tested for cross-protection effect in pepper and tobacco (*Nicotiana tabacum* cv. Xanthi nc) plants against Mf-CMV. No symptoms were developed on the plants vaccinated with Paf-CMV until 3 weeks after inoculation with the virulent strain; however, another attenuated isolate, Rs2-CMV, showed less effectiveness in cross-protection. Depending on the concentration of the challenged virus, symptoms sometimes appeared later in the upper leaves. However, in plants challenged with low concentrations (below 0.2 mg/ml) of the challenge inoculum, symptoms caused by the virulent strain did not develop on the plants vaccinated with Paf-CMV. In the field experiments, the number of pepper plants with severe mosaic symptoms in the control plots was progressively increased after transplanting and reached approximately 50% after 50 days. On the other hand, the incidence of mosaic disease appeared very low plants that had received the protective inoculation with Paf-CMV.

Transgenic tobacco plants introduced with cDNA of *Cucumber mosaic virus* satellite RNA

The cDNA of *Cucumber mosaic virus* (As-CMV) satellite RNA was introduced into tobacco plants (*Nicotiana tabacum* cv. Samaun NN) using a binary Ti plasmid vector system of *Agrobacterium tumefaciens*. The cDNA of satellite RNA introduced into tobacco plants was detected by polymerase chain reaction (PCR) and molecular hybri- dization analyses. Symptom development was distinctly suppressed in the transgenic tobacco plants when inoculated with Co-CMV. CMV concentration in the transgenic tobacco plants was decreased to 1/40 of non-transgenic tobacco plants. The kanamycin resistance gene of the transgenic tobacco plants was also detected in the progeny.

Generation of a murine single chain Fv (scFv) antibody specific for *Cucumber mosaic virus* using a phage display library

With the long-term goal of generating *Cucumber mosaic virus* (CMV)-resistant transgenic plants using antibody genes, a single-chain variable fragment (scFv) antibody that binds to the CMV was isolated from a scFv phage display library by four rounds of affinity selection with Mf-CMV as an antigen. The scFv has the identical binding specificity to CMV as a monoclonal antibody that is generated by the hybridoma fusion technique, and recognized purified preparations of CMV isolates belonging to either subgroup I or II in immunoblotting. The nucleotide sequences of the recombinant antibody showed that a heavy chain variable region (V_H) gene belonged to the V_H3 subgroup and the kappa light chain variable region (V kappa) came from the V_K4 subgroup. Our results demonstrate that the scFv phage display library, an alternative approach to the traditional hybridoma fusion technique, has a potential applicability in the study of plant virus and plant pathology.

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