Virus Genes 15:1, 73–77, 1997

Nucleotide Sequences of the 3′ Terminal Region of Onion Yellow Dwarf Virus Isolates from Allium Plants in Japan

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Received December 15, 1996; Accepted February 26, 1997

Abstract. The 2032 nucleotide sequence of the 3′ terminal region of onion yellow dwarf virus (OYDV) isolated from *Allium wakegi*, bearing the genes for viral coat protein (CP) and a truncated RNA-dependent RNA polymerase, has been determined. Respecive homologies of the nucleotide sequence in the corresponding region and the deduced amino acid sequence of CP with the equivalents of leek yellow stripe virus (LYSV) from garlic were 68.0 and 59.3%. Variation in the nucleotide sequence is concentrated in the boundary region between the putative RNA-dependent RNA polymerase gene and the CP gene as well as in the 3′ noncoding region. These sequence divergencies, including the deletion of 79 nucleotides, resulted both in alterations to the amino acid sequence and the absence of 28 amino acid residues in the amino terminal region of OYDV CP in comparison with LYSV CP. In addition, the lenght of the 3′ noncoding sequence of OYDV was one-third that of LYSV. Comparison of the 3′ terminal 1197 nucleotides sequence of OYDV with sequences of the respective cDNAs cloned by RT-PCR directly from the total RNA of infected Allium plants that included two varieties of *A. fistulosum*, ''Wakenegi' and ''Shimonita-negi'', and *A. chinense*, showed 90.7% overall identities, even though they have long been cultivated in locally restricted area in Japan. These findings appear to suggest that a single strain of OYDV invaded Japanese Allium plants long ago and spread throughout them.

Key words: *A. wakegi, A. fistulosum, A. chinense,* garlic, leek yellow stripe virus, RT-PCR

Virus diseases are widespread in Allium plants throughout the world. A number of reports on viruses that infect Allium species have been published, but the findings are somewhat confusing, especially as to viruses which infect vegetatively propagated plants, including garlic and shallot (1–9). Infection by a complex of two or more viruses and difficulties in separating these viruses because of their restricted host ranges are the main reason for the confusion. Van Dijk (5) recently differentiated four potyviruses; leek yellow stripe virus (LYSV), onion yellow dwarf virus (OYDV), shallot yellow stripe virus (SYSV), and Welsh onion yellow stripe virus (WoYSV), based on their differences in host range and serology. The detailed relationship of these potyviruses, based on the nucleotide sequence of genomic RNA or the amino acid sequence of viral CP, however, have yet to be shown.

Recent advances in molecular biology provide new tools for the identification and classification of viruses (10–12). Such molecular characteristics as the viral genome sequence, its organization, and the deduced amino acid sequence of CP from the cloned viral cDNA sequence are useful for distinguishing viruses from strains and for determining the relationships between genera, species, and subspecies of distinct viruses. Moreover, the usefulness of molecular biology methods has been shown both for the characterization of viruses that are difficult to isolate and finely characterize by traditional methods and for the development of novel methods for detecting viruses (13).

To clarify the interrelations among the Allium potyviruses at the nucleotide sequence level, we cloned the cDNA of OYDV genomic RNA. We described this cDNA cloning of the genome of the

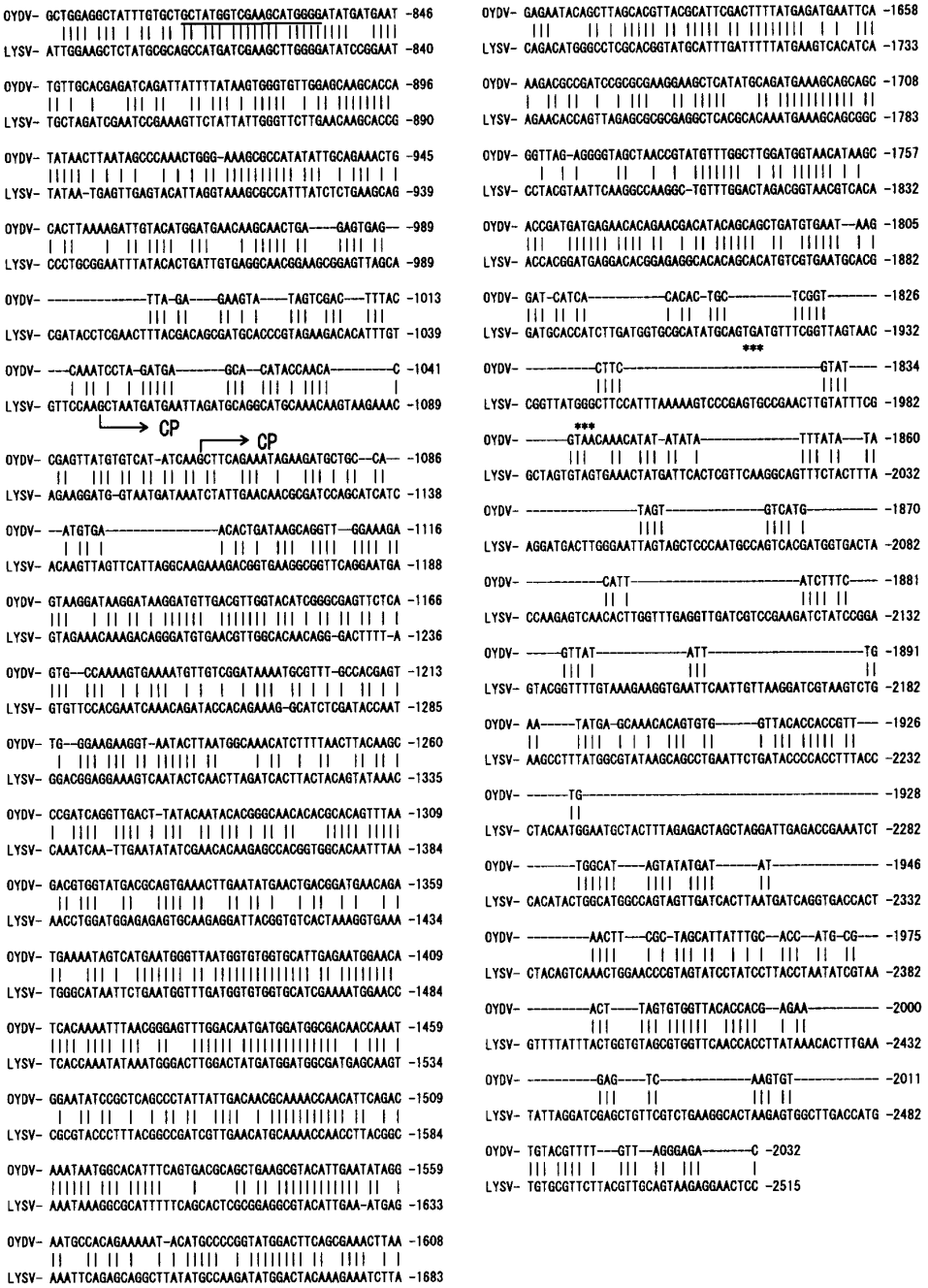

Fig. 1. Comparison of the 3′ terminal nucleotide sequence of OYDV genome with the equivalent sequence of LYSV. The 3′ terminal 1236 nucleotide sequence in cDNA clone OYDV-M15 is aligned with the corresponding region of garlic LYSV cDNA, GV-7 (13). The brackets mark the beginning of the viral CP genes. The termination codon is indicated by asterisks. The nucleotide sequence underlined is the 5′ primer sequence used for cDNA cloning of OYDV strains from Allium plants by RT-PCR. The number corresponds to the position of the OYDV cDNA sequence of 2032 nucleotides, deposited in DDBJ database.

OYDV isolated from *Allium wakegi* and show the similarities in the 3′ terminal sequence, including viral CP gene, among the OYDV genomes of virus isolates from Allium plants cultivated in locally restricted areas in Japan (which included *A. wakegi, A. fistulosum* cultivars ''Shimonita-negi'' and ''Wakenegi'', and *A. chinense*) as well as the difference in the cDNA sequences of OYDV and LYSV.

Onion yellow dwarf virus was partially purified from *A. wakegi* leaves infected solely with OYDV as described previously (14). Infection only with OYDV was confirmed by the direct tissue blotting immunoassay (DTBIA), immunoelectron microscopy and bioassays of several plants (data not shown). Three hundred and sixty micrograms of virus particles was obtained from 43.1 g of fresh leaves as estimated from the A_{260} of the purified preparations, assuming $E_{0.1\%} = 2.5$. Nucleic acids were extracted from the partially purified virus with ISOGEN (Nippon Gene, Toyama, Japan). Eleven micrograms of RNA was obtained from 240 μg of the purified virus.

Double stranded cDNA was synthesized with a Time Saver cDNA Synthesis Kit (Pharmacia Biotech, Uppsala, Sweden) and an *Eco*RI adapter was ligated to both ends of the cDNA according to the manufacturer's instructions. The cDNA was ligated to the pT3T7 plasmid vector (Pharmacia Biotech, Uppsala, Sweden) after double digestion with *Eco*RI and *Not*I, and the resultant chimeric plasmid was used to transform *E. coli* JM109. Two representative cDNA clones, designated OYDV-M15 and OYDV-N24, which both had approximately 2 kb cDNA inserts were selected. The nucleotide sequences of the respective 2032 and 1766 bases (excluding the poly(A) tail) of the 3′ terminal regions of their viral genomic RNAs, cloned respectively in OYDV-M15 and -N24, were determined. The 3′ terminal 1766 nucleotide sequence coincided with the sequence between M15 and N-24. We therefore used the cDNA clone M-15 for subsequent characterization. The sequence of M-15 has been deposited in the DDBJ (DNA Data Bank of Japan) database under accession number D73378.

The cDNA clone M-15 contains only a single potential open reading frame (ORF) which encodes a truncated RNA-dependent RNA polymerase and CP. We compared the nucleotide sequence of M15 to the corresponding region of LYSV cDNA, previously reported as GV-7 (14) (Fig. 1). Although 68.0% of the

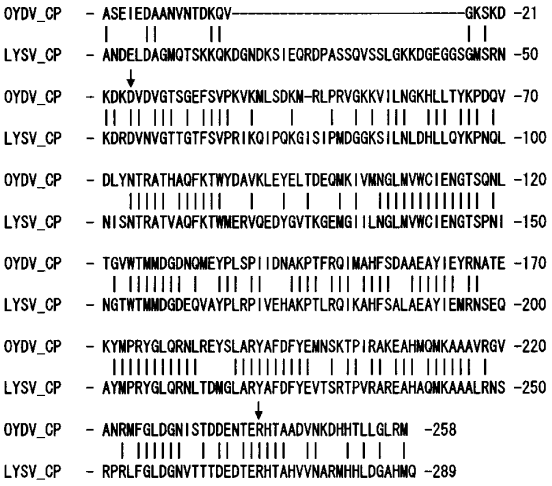

Fig. 2. Comparison of the amino acid sequences of putative viral CPs between OYDV and LYSV. The core region of CP marks with arrow and gaps are introduced for maximum alignment.

sequence was identical, divergence was concentrated in the boundary area between the genes for RNA polymerase and viral CP as well as in the 3′ noncoding region (Fig. 1). Sequence variation, including the deletion of 79 nucleotides, in the boundary region resulted in alterations to both the N-terminal amino acid sequence and the molecular mass of CP between OYDV and LYSV. The overall homology of the deduced amino acid sequence of CP in them was 59.3%, and in the core region corresponding to the region from D^{33} to R^{248} in potato virus Y (15) it was 63.6% (Fig. 2). Both the similarity and length of the 3′ noncoding region of the viral genome are reported to be useful markers for virus taxonomy (16). In addition, Shukla and Ward (17,18) showed that similarity in the core regions of viral CP is one of the most useful criteria for distinguishing viruses, in particular from strains, and establishing evolutionary relationships between groups of distinct potyviruses. Homologies of the CP core regions which range from approximately 20 to 30%, and from 55 to 75%, 74 to 88%, and from 90 to 99% indicate that the respective viruses probably belong to different genera, species, subspecies, and strains (19). The sequence data obtained in this experiment therefore supports a classification based on differences in host range and serology that places OYDV and LYSV in distinct species of the *Potyviridae*.

We directly cloned the 3′ terminal region of the viral genome that carries the CP gene from the total

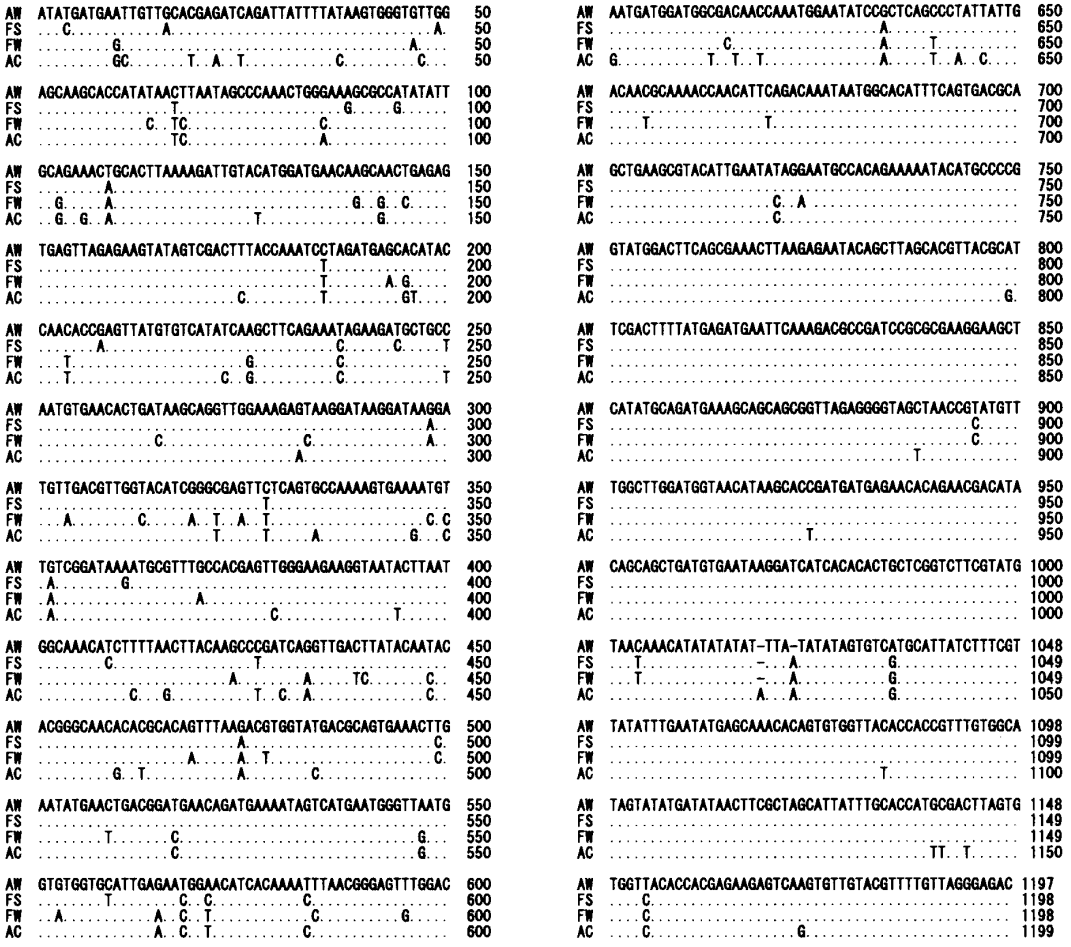

Fig. 3. Alignment of the nucleotide sequences of the 3′ terminal 1197 nucleotides among OYDV strains. AW, FS, FW, and AC indicate the strains of OYDV from *A. wakegi, A. fistulosum* cultivars ''Shimonita-negi'' and ''Wakenegi'', and *A. chinense,* respectively. The only nucleotides that differ from *A. wakegi* OYDV sequence are indicated, and gaps are introduced for maximum alignment.

RNA of infected Allium plants cultivated in Japan using the RT-PCR procedure and determined the nucleotide sequence in order to investigate alterations in the sequence of each isolate. We chose the *A. fistulosum* cultivars, ''Wakenegi'' and ''Shimonita-negi'', as well as *A. chinense* as the OYDV-infected plant materials because these plants have been shown to be infected with viruses identified serologically, biologically, or both, as OYDV (20,21, unpublished data). All these plants have long been cultivated in locally restricted areas in Japan as special agricultural products and are vegetatively propagated except for ''Shimonita-negi'' which is propagated by seed. The *A. wakegi*, the *A. fistulosum* cultivars ''Wakenegi'' and Shimonita-negi'', and the *A. chinense* respectively were collected from Hiroshima, Chiba, Gunma, and Tottori prefecture. Infection of the individual plant materials with OYDV was confirmed by DTBIA (13,22).

Total RNA was extracted from 200 mg fresh leaves from the infected Allium plants using ISOGEN (Nippon Gene, Toyama, Japan). Approximately 40 μg of the total RNA was obtained. Reverse transcription (RT) was performed on about 1 μg of the RNA with 0.2 μg of *Not*I-d$(T)_{18}$ as the primer in a 15-μl reaction volume with a First-Strand cDNA Synthesis Kit (Pharmacia Biotech, Uppsala, Sweden). For the PCR, 1 μl of the RT mix was the template, 30 reaction cycles being performed that included an annealing period of 30 s, at 55°C, synthesis for 60 s, at 72°C, and melting for 30 s, at 94°C. The 5′ primer was 5′-GCTATGGTCGAAGCATGGGG-3′, and the 3′ primer 5′-

ACCGATTCAACTGGAAGAATTCGCGG-3′, corresponding to a part of the *Not*I-d$(T)_{18}$ primer sequence. In the RT-PCR of the total RNAs from all the Allium plants, 1197 to 1199 base DNA fragments, excluding poly(A) tracts were amplified. The respective nucleotide sequences were determined and deposited in the DDBJ database under accession numbers AB000472, AB000473 and AB000474. Alignment of the nucleotide sequence of each amplified fragment showed very high homology of 90.7% (Fig. 3). The amino acid sequence homologies of the CP core regions were more than 98%, and the sequences of the isolates from *A. wakegi* and ''Shimonita-negi'' were identical. The facts that these plants have long been cultivated in locally restricted areas in Japan and that OYDV is transmitted by aphids in a non-persistent manner appear to suggest that a single strain of OYDV long ago invaded and spread through Japanese Allium plants, but more sequence data are required to be definitive.

Quite recently, Kobayashi et al. reported the nucleotide sequences of OYDV garlic and onion strains (23). The similarities between the deduced amino acid sequences of these isolates and the corresponding sequences of the OYDV isolates from the Japanese Allium species were less than 76.7%. The degree of similarity between them suggests that the Japanese OYDV isolates represent different viral species or subspecies from the OYDV isolates reported by Kobayashi et al. (23), by the taxonomic criterion based on CP sequence similarity (17,18). We presume that the Japanese OYDV isolates may closely relate to WoYSV or SYSV differentiated from typical OYDV, based on the differences in host range and serology (5) and we are currently examining this hypothesis by cDNA cloning of potyvirus isolates from Allium species throughout the world.

Acknowledgments

We thank Dr. I. Sako (Tottori Horticultural Experiment Station), Messrs. H. Ikeda (Gunma Agricultural Experiment Station) and M. Fukami (Chiba Prefectural Agricultural Experiment Station) for collecting Allium plants.

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