Brief Report

Genome sequence of a Japanese isolate of Radish mosaic virus:
the first complete nucleotide sequence of a crucifer-infecting comovirus

K. Komatsu¹, M. Hashimoto¹, K. Maejima¹, J. Ozeki¹, S. Kagiwada², S. Takahashi²,
Y. Yamaji¹, S. Namba¹,²

¹ Laboratory of Plant Pathology, Department of Agricultural and Environmental Biology,
Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
² Laboratory of Clinical Plant Science, Department of Agricultural and Environmental Biology,
Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan

Received 25 March 2007; Accepted 26 April 2007; Published online 29 May 2007
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Summary

The complete nucleotide sequences of RNA1 and RNA2 of a Japanese isolate of Radish mosaic virus (RaMV-J), a crucifer-infecting comovirus, were determined. RNA1 is 6064 nucleotides long and encodes a 210-kDa polyprotein containing conserved motifs that are required for replication. RNA2 is 4020 nucleotides long and encodes a 123-kDa polyprotein containing the putative movement protein and two coat proteins. Comparisons of the encoded proteins confirmed that RaMV-J and a Czech RaMV isolate are isolates of the same species in the genus Comovirus. A phylogenetic analysis of RaMV-J and other comoviruses revealed that legume-infecting comoviruses constitute a single branch to which RaMV is distantly related.

Radish mosaic virus (RaMV) is a member of the genus Comovirus in the family Comoviridae. RaMV is a beetle-transmitted virus that infects most cruciferous plants, causing mosaic, ringspots, and leaf crinkling. It was first reported in California by Tompkins [27] and was rediscovered later in the same region by Campbell [1]. This crucifer-infecting virus has also been reported in Europe, Iran, Japan, and Morocco [7, 13, 19, 26]. In Japan, Tochihara [26] described a virus from radish with many characteristics similar to RaMV but initially referred to it as radish enation mosaic virus (REMV). After a subsequent study showing that REMV and RaMV are serologically indistinguishable, REMV was considered an isolate of RaMV [2].

Comoviruses have positive-sense single-stranded bipartite RNA genomes designated RNA1 and RNA2. These genomes contain a poly(A) tract at
the 3' terminus and a genome-linked protein (VPg) covalently linked to the 5' end. Numerous studies of cowpea mosaic virus (CPMV), the type member of the genus Comovirus, have revealed that each RNA has a single long open reading frame (ORF). The ORF of RNA1 encodes proteins that are involved in replication, whereas that of RNA2 encodes structural proteins and proteins required for cell-to-cell movement. The functional viral proteins are produced by cleavages of both the RNA1 and RNA2 polyproteins by a viral-encoded protease at conserved processing sites [20].

In addition to the nucleotide sequences of CPMV (GenBank accession numbers X00206 and X00729), the genomic RNAs of several comoviruses have been sequenced. Complete sequences of both RNA1 and RNA2 are available for isolates of Bean pod mottle virus (BPMV; U70866 and M62738) [6, 17], Cowpea severe mosaic virus (CPSMV; M83830 and M83309) [3, 4], Squash mosaic virus (SqMV; AB054688 and AF059533) [9, 10], and Red clover mottle virus (RCMV; X64886 and M14913) [22, 23]. Moreover, the complete nucleotide sequence of RNA2 of an Andean potato mottle virus isolate (APMoV; L16239) has been determined [24]. Similar to CPMV, a single long ORF is present in all of these comoviral genomic RNAs. A sequence alignment analysis of the encoded polyproteins revealed putative functional motifs similar to those reported in CPMV [3, 4, 6].

The host ranges of comoviruses are narrow: 11 of the 15 members of the genus infect only plant species of the family Leguminosae. Four of the five comoviruses for which the complete nucleotide sequences of both RNAs have been determined have a narrow host range limited to the Leguminosae. However, several other comoviruses, including APMoV, RaMV, and SqMV, naturally infect non-leguminous host plants.

RaMV is the only comovirus that is known to infect plant species in the family Cruciferae, but its genomic sequences have not yet been determined except for that of the putative RdRp coding region of a Czech isolate (AY965345) [19]. In this study, we determined the complete nucleotide sequences of the genomic RNAs of a Japanese isolate of RaMV, termed RaMV-J, and compared them to those of other sequenced comoviruses. This is the first report of the genomic sequence of a crucifer-infesting comovirus.

RaMV-J was originally isolated from infected leaves of radish, Raphanus sativus, in Omagari, Akita Prefecture, Japan, and was supplied by the National Institute of Agrobiological Sciences Genebank. Raphanus sativus was used for virus propagation. Virus purification and extraction of viral RNA were performed using standard methods for comoviruses [28]. Viral cDNA was synthesised with an oligo(dT) primer using a SMART PCR cDNA Synthesis Kit (Clontech). Cloning and sequencing of cDNA fragments were performed as described [12].

The complete genomic sequence of RaMV-J RNA1 is 6064 nucleotides (nt) long, excluding the 3' terminus poly(A) tail. The sequence begins with UAUUAAAAU, which is the consensus sequence found at the 5' termini of many comoviruses [3, 4, 6]. Analysis of the nucleotide sequence revealed a single ORF that begins at an AUG codon (nt 340–342) and terminates with a UAA codon (nt 5899–5901). The 5' untranslated region (UTR) consists of 339 nt, and the 3' UTR is 163 nt long. The predicted translation product of the ORF is 1853 amino acids (aa) in length with a calculated Mr of 210,469 Da (a 210-kDa protein). The 210-kDa protein encoded by RaMV-J RNA1 showed 42 to 44% amino acid identity to RNA1 polyproteins of other comoviruses.

A comparison with other comovirus sequences suggests that the 210-kDa polyprotein could be cleaved to yield five final protein products: from the amino (N) to the carboxy (C) terminus: the protease cofactor (Co-pro), the putative helicase (Hel), the VPg, the protease (Pro), and the putative RNA-dependent RNA polymerase (RdRp) (Fig. 1) [20]. Four putative cleavage sites were predicted from an amino acid sequence alignment of the comoviral RNA1-encoded polyproteins using CLUSTAL W (Table 1a). The putative cleavage sites all contain a glutamine residue at position -1 and particularly conserved alanines at positions -2 and -4. Although the RaMV-J putative cleavage dipeptide at the Co-pro/Hel (Q/G) site is identical to that of RCMV and the dipeptide at the Hel/VPg (Q/S) site is identical in all comoviruses, the other dipeptides