Complete nucleotide sequence of Oat necrotic mottle virus: A distinct Tritimovirus species (family Potyviridae) most closely related to Wheat streak mosaic virus

Brief Report

D. C. Stenger and R. French
United States Department of Agriculture, Agricultural Research Service and Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska, U.S.A.

Received July 22, 2003; accepted September 22, 2003
Published online November 26, 2003 © Springer-Verlag 2003

Summary. The RNA genome (9346 nucleotides) of Oat necrotic mottle virus (ONMV) was cloned and sequenced. Complete genome comparisons indicated that ONMV, currently classified as a rymovirus, was most closely related (\(\sim 73\%\) nucleotide, \(\sim 79\%\) amino acid identity) to the tritimovirus Wheat streak mosaic virus (WSMV). ONMV encoded a single polyprotein, with protease cleavage sites very similar to those of WSMV. Pairwise comparison of ONMV and WSMV cistrons revealed that P3 was most conserved (\(\sim 79\%\) nucleotide, \(\sim 86\%\) amino acid), whereas HC-Pro was most divergent (\(\sim 67\%\) nucleotide, \(\sim 69\%\) amino acid). In contrast, the ONMV sequence was distantly related (\(\sim 40\%\) nt, \(\sim 26\%\) amino acid identity) to that of the rymovirus Ryegrass mosaic virus, with highest sequence conservation noted within the NIb cistron (\(\sim 47\%\) nucleotide, \(\sim 41\%\) amino acid identity). These results firmly establish that ONMV is not a rymovirus but is instead a distinct species of the genus Tritimovirus.

* Oat necrotic mottle virus (ONMV) is a poorly characterized virus of the family Potyviridae only known to occur in Manitoba, where it causes a minor disease of oat [2]. ONMV was assigned to the genus Rymovirus [20], based on a weak serological relationship with Wheat streak mosaic virus (WSMV) [3]. At that time the genus Rymovirus included all monocot-infecting potyviruses known, or suspected, to be transmitted by eriophyid mites. It since has been demonstrated that several eriophyid mite transmitted viruses (e.g. WSMV and Brome streak mosaic virus [BrSMV]) share an evolutionary history distinct from that of the rymoviruses...
Ryegrass mosaic virus (RGMV), Agropyron mosaic virus, and Hordeum mosaic virus [6, 12, 15]. As a result, WSMV and BrSMV were removed from the genus Rymovirus and placed within the newly erected genus Tritimovirus [18]. More recently, comparison of 3′-terminal nucleotide (nt) sequences coding for CP and part of Nib [10] suggested that ONMV also should be removed from the genus Rymovirus and reclassified as a Tritimovirus species. However, as taxonomic placement may be most accurately accomplished through analysis of complete genome sequences, the generic affiliation of ONMV remains uncertain. To resolve the taxonomic status of ONMV, we report here the cloning and analysis of the complete ONMV nucleotide sequence.

A culture of the Type isolate of ONMV (ONMV-Type, ATCC PV-107) maintained at the University of Nebraska since ca. 1988 was used in this study, and propagated in mechanically inoculated oat (Avena sativa L.) cv. ‘Shaw’. This Nebraska culture, designated here as ONMV-Type_{NE}, was authenticated by host range and symptom expression: systemic infection of oat with mild mosaic, symptomless systemic infection of Kentucky blue grass (Poa pratensis L.), and inability to infect wheat (Triticum aestivum L.). The Nebraska culture was further authenticated by comparing sequence of the 3′-terminal 1728 nucleotides, derived from a reverse transcription-polymerase chain reaction (RT-PCR) product, with the corresponding sequence of two isolates of ONMV (ONMV-Type maintained in Aschersleben, Germany [ONMV-Type_{A}] and ONMV-Poa pratensis [ONMV-Pp]) for which this same region has been sequenced [10]. The RT-PCR product of ONMV-Type_{NE} was generated and cloned using the same conditions as reported [10], with the nt sequence used for comparison being a consensus derived from three independent clones. These results indicated that the 1728 nt 3′-terminal sequence of ONMV-Type_{NE} shared 99.3% identity with ONMV-Type_{A} [AF454460] and 99.1% identity with ONMV-Pp [AF454461].

ONMV-Type_{NE} virions were purified from systemically infected oat using a protocol modified from Schubert and Rabenstein [13]. Briefly, frozen infected tissue was ground in 1.5 volumes of extraction buffer (0.1 M sodium citrate, pH 7.0) and filtered through Miracloth. The supernatant recovered after centrifugation (20 min., 16,000 × g, 4 °C) was adjusted to 2% triton X-100 and mixed for 5 min. Virions were concentrated by centrifugation (2 hr, 101,000 × g, 4 °C) through a 20% sucrose pad and resuspended in extraction buffer. Virions were further purified by density gradient centrifugation in which resuspended virions were layered onto 28% CsCl and centrifuged in a Beckman SW 55.1 rotor (19 hrs, 35,000 rpm, 10 °C). Virions recovered from gradients were diluted with extraction buffer, concentrated by centrifugation (2 hr, 107,000 × g, 4 °C), and resuspended in a minimum volume of extraction buffer.

ONMV-Type_{NE} RNA (~2 µg) extracted from virions was used as template for reverse transcription with oligo dT or random hexamers as primers. Both first strand and second strand cDNA were synthesized using the Universal Ribo Clone cDNA Synthesis System (Promega, Madison, WI, U.S.A.). Double stranded cDNA was made blunt-ended using T4 DNA polymerase and size fractionated on a 1.2% agarose gel. Gel-purified DNA (≥2 kbp) was A-tailed using Taq polymerase