Nucleotide sequence and infectious transcripts from a full-length cDNA clone of the carmovirus Melon necrotic spot virus

Brief Report

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Summary. We have studied the biological and molecular characteristics of a MNSV isolate collected in Spain (MNSV-Mα5) and generated a full-length cDNA clone from which infectious RNA transcripts can be produced. The host range of MNSV-Mα5 appeared to be limited to cucurbits and did not differ from that of MNSV-Dutch [4, 21]. However, differences were observed in the type of symptoms that both isolates could induce. A full-length cDNA of MNSV-Mα5 was directly amplified by reverse-transcription polymerase chain reaction (RT-PCR) using a 5′-end primer anchoring a T7 RNA promoter sequence and a 3′-end primer, and cloned. Uncapped RNAs transcribed from this cDNA clone were infectious and caused symptoms indistinguishable from those caused by viral RNA when mechanically inoculated onto melon, cucumber or watermelon plants. The complete genome sequence of MNSV-Mα5 was deduced from the full length cDNA clone. It is 4271 nt long and, similarly to MNSV-Dutch, consists of 5′ and 3′ untranslated regions (UTRs) and five open reading frames (ORFs) coding for 29, 89, 42 and two small 7 kDa proteins. One notable difference between MNSV-Mα5 and other sequenced MNSV isolates was found, as for MNSV-Mα5 the first of the two small ORFs, which are contiguous in the genome, terminates with a genuine stop codon, whereas for MNSV-Dutch and other sequenced MNSV isolates it terminates with an amber codon. This suggested that the putative p14 readthrough

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The protein that could be expressed from the MNSV-Dutch and other MNSV genomes could not be expressed from the MNSV-Mα5 genome. Also, the nucleotide and amino acid sequences comparisons showed a distant relationship of MNSV-Mα5 with other known MNSV isolates.

*Melon necrotic spot virus* (MNSV) is an isometric monopartite plant virus which measures about 30 nm in diameter [13]. In nature, MNSV is transmitted by the chytrid fungus *Olpidium bornovanus* [5, 6, 13] and through the seed [1, 12]. Experimentally, MNSV can be readily transmitted by mechanical inoculation. MNSV host range is limited almost exclusively to species in the family *Cucurbitaceae*, in which the symptoms are necrotic spots or large necrotic lesions of the leaves, and necrosis of the stems [13, 16]. The virus is endemic in cucurbit crops worldwide and epidemic outbreaks of economic importance have been reported in several occasions [3, 4, 16, 25]. The characterization of several MNSV isolates revealed that there were differences in the symptoms they could induce and in their host ranges [2, 3, 4, 10, 13, 16, 18]. However, the identification of genetic determinants for the differences observed has not been carried out and even information on genomic nucleotide sequences is rather limited. In fact, to our knowledge, there is only one complete nucleotide sequence of a MNSV isolate (MNSV-Dutch [21]). An analysis of the MNSV-Dutch genome sequence indicated that this virus belongs to the genus *Carmovirus* (family *Tombusviridae*) and that it has a genome organization essentially similar to that of *Carnation mottle virus* (CarMV), the type member of the genus. Thus, MNSV-Dutch has a single stranded and positive sense RNA genome of 4.3 kb long which contains at least five open reading frames (ORFs). The most 5′ ORF terminates in an amber codon. The translation of this ORF would yield a 29 kDa protein and a 89 kDa readthrough product. Two small centrally located ORFs encode each a 7 kDa protein. The first of these two ORFs terminates in an amber codon whose readthrough could result in the production of a 14 kDa fusion protein. The 3′ proximal ORF encodes the 42 kDa coat protein [21]. The functions of the 29/89 kDa and the 7 kDa proteins have been proposed to be replication and movement respectively, by analogy with the more studied homologue proteins of *Turnip crinkle virus* (TCV), another member of the genus *Carmovirus* [7, 20]. The MNSV coat protein might be involved in the fungal transmission process, as has been demonstrated for *Cucumber necrosis virus* [14, 17], a member of the family *Tombusviridae* [15] also transmitted by *O. bornovanus*. However, the scanty information in genome sequences and the lack of infectious cDNA clones has hampered the establishment of correlations between genomic information and biological properties in the case of MNSV. In this paper we describe the complete sequence of the genome of the MNSV-Mα5 isolate and the obtention of infectious transcripts from a full-length cDNA clone. This may provide useful information and tools for a more complete understanding of the MNSV biology.

MNSV-Mα5 was collected from a naturally infected melon plant grown in Almeria (southern Spain) and isolated by three consecutive serial single-lesion