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Viral properties, primary structure and phylogenetic analysis of the coat protein of an Olive latent virus 1 isolate from *Olea europaea* L.

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Abstract

An Olive latent virus 1 isolate designated GM6, obtained from a Portuguese olive tree, was characterized and the coat protein gene sequenced and analysed. The purified virus particles showed to be isometric with ca. 30 nm in diameter and contained a single-stranded RNA species with ca. 3.7 kb. The dsRNA profile obtained from infected tissues showed three major species with ca. 3.7, 1.5 and 1.3 kbp. SDS-PAGE analysis revealed a major peptide with an apparent molecular mass of 32 kDa identified as the coat protein. A viral genome region containing the coat protein gene was amplified by RT-PCR and the cDNA was cloned and sequenced. The coat protein gene revealed to be 813 nucleotides long and encode a peptide with 270 amino acid residues and an estimated M_r of 29,851. Alignment of the deduced amino acid sequence with that of other necroviruses showed a higher identity with OLV-1 tulip isolate (97.7%) than with OLV-1 citrus isolate (87.7%). The consensus pattern of the coat protein 'S' domain is conserved in GM6 isolate coat protein sequence, except in amino acid 151, leucine. This is the first report on the coat protein sequence of an OLV-1 olive isolate.
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Keywords: OLV-1; Olive; Necrovirus; Viral properties; Coat protein sequence

Olive latent virus 1 (OLV-1) is a member of genus *Necrovirus*, family *Tombusviridae*. Several isolates have been obtained from symptomless or weakened *Olea europaea* L. trees in Italy, Jordan and Portugal (Gallitelli and Savino, 1985; Martelli et al., 1995; Félix and Clara, 1998), as well as from other quite different diseased hosts, such as citrus trees, affected by chlorotic dwarf disease, in Turkey (Martelli et al., 1996) and tulips, showing necrotic symptoms, in Japan (Kanematsu et al., 2001). In Portugal, OLV-1 was first isolated from symptomatic *Nicotiana benthamiana* plants previously inoculated with extracts of olive fruits and identified by immunoenzimatic assays, using a specific OLV-1 antiserum (Félix and Clara, 1998).

In the present study, we report the molecular characterization of an OLV-1 olive isolate, designated GM6. Its coat protein gene was sequenced, analysed and compared with that of other OLV-1 isolates, as well as of other necroviruses.

The GM6 isolate, originally obtained from fruits of a 'Galega vulgar' cultivar of olive, was propagated in *N. benthamiana* plants. Virus purification was made from 100 g of frozen symptomatic *N. benthamiana* leaves, which were ground in 0.1 M phosphate buffer (pH 6.0). Purified virus preparations, sedimented as single band in sucrose density gradient columns, which revealed to consist in a homogeneous population of isometric particles with ca. 30 nm when observed under the electron microscope (data not shown).

Coat protein subunits were dissociated from virus particles as described by Merciega et al. (1996) and SDS-PAGE analysis (Laemmli, 1970) showed a single major polypeptide with an apparent molecular mass of 32 kDa (data not shown).

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