

## SHORT COMMUNICATION

AN ISOLATE OF *OLIVE LATENT VIRUS 2* INFECTING CASTOR BEAN IN GREECE

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## ABSTRACT

During 2001, in the Aegean Greek islands of Rodi and Kastellorizo, wild castor bean plants (*Ricinus communis* L.), showing yellowish vein netting and mottling of the leaves were observed. A virus was isolated by mechanical inoculation from such plants to herbaceous hosts, which based on biological, serological and molecular assays was identified as an isolate of *Olive latent virus 2* (OLV-2/CB), virtually indistinguishable from the type strain. The field syndrome was experimentally reproduced in artificially inoculated castor bean seedlings and the virus reisolated, thus fulfilling Koch's postulates. This is the first report of OLV-2 outside Italy in a natural host other than olive.

*Key words:* castor bean, oleavirus, OLV-2, Greece, molecular characterization.

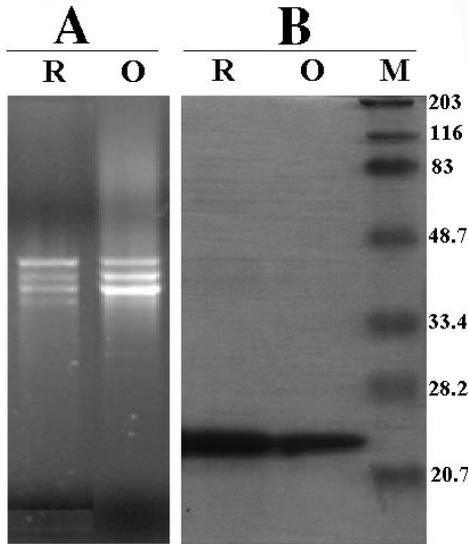
*Olive latent virus 2* (OLV-2) (genus *Oleavirus*, family *Bromoviridae*) has quasi spherical to bacilliform particles and a non-polyadenylated, positive sense ssRNA genome, made up of four major RNA species (Martelli and Grieco, 1997). RNA1 (3126 nt) and RNA2 (2734 nt) are both monocistronic molecules coding for replication-related proteins, with conserved helicase, methyltransferase (RNA1) and RNA polymerase (RNA2) motifs of Tobamo lineage (Koonin and Dolja, 1993). RNA3 (2438 nt) codes for a 36.5 kDa movement protein (MP), and the 20 kDa coat protein (CP). RNA4 (2078 nt) is co-terminal with RNA3 and has an undetermined function. A subgenomic RNA (*ca* 1042 nt), encoding the CP, is formed in infected plants but it may not be encapsidated (Martelli and Grieco, 1997).

During a surveys carried out in the Aegean Greek islands of Rodi and Kastellorizo, a number of castor bean plants (*Ricinus communis* L.), showing yellowish vein netting and mottling of the leaves were observed.

A virus was isolated by mechanical inoculation from these plants to herbaceous hosts and recognised as OLV-2 (Vovlas *et al.*, 2002). Since there are no reports of OLV-2 infections in hosts other than olive and outside Italy, the biological and physico-chemical characteristics of this virus (OLV-2/CB) and the molecular organisation of its RNA3 were investigated.

The isolate was mechanically transmitted to *Gomphrena globosa*, which was used as inoculum source for determining host range reactions. The virus was then propagated in *Nicotiana occidentalis*, from which it was purified as described by Grieco *et al.* (1992). Nucleic acids were extracted from virus preparations fractionated in a sucrose gradient (Gonsalves and Shepherd, 1972). RNA preparations were analysed by electrophoresis in 1.2% low melting point agarose and stained with ethidium bromide. Protein for polyacrylamide gel electrophoresis (PAGE) were resuspended in SDS-buffer, boiled and electrophoresed in PAGE as described by Laemmli (1970). Immunodiffusion was used for investigating serological relationships of OLV-2/CB with *Alfalfa mosaic virus* (AMV). RNA extracted from purified virus preparations was subjected to RT-PCR using specific primers F = 5'-ATGGTG-GTTTGTGGCGTTCTAACTCC-3' (homologous to nt 360-386 of OLV-2 RNA3) and primer R = 5'-CAA-GACGAACTCTCAAGTAACGC-3 (complementary to nt 2033-2055 of OLV-2 RNA3). RT-PCR assay RT was carried out according to Grieco *et al.* (1996) and amplification products were analysed in 1.2% agarose gel electrophoresis (Sambrook *et al.*, 1989). Amplicons were gel-purified, ligated into pGEM-T plasmid (Promega, USA) and cloned in *Escherichia coli* strain DH5 $\alpha$  as previously described (Grieco *et al.*, 1996). Plasmid DNA was prepared using a commercial kit (JetQuick, Genomed, Germany) and sequence analysis done by custom automatic sequencing (MWG, Germany). The nucleotide sequence was analysed with the assistance of the University of Wisconsin Genetics Computer Group programs (Anonymous, 1994) and the ClustalW program (Higgins and Sharp, 1988). Generation of DIG-RNA probes was done using a DIG RNA Labeling kit (Boehringer Mannheim, GmbH),

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**Fig. 1.** **A.** Comparative electropherogram of RNA preparations from unfractionated OLV2/CB (R) and OLV-2 (O) isolate. **B.** Electropherogram of CPs of OLV2/CB (R) and OLV-2 (O). Protein markers in lane M with sizes in kDa.

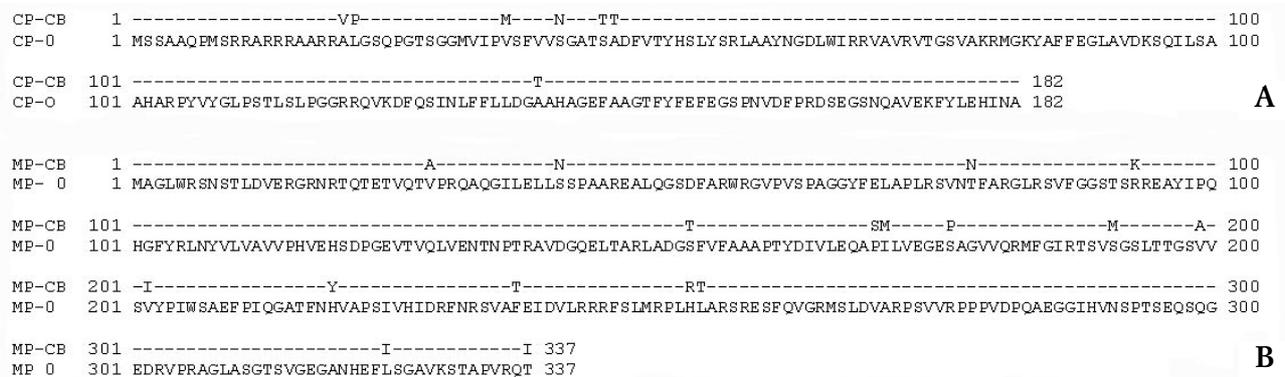
using *EcoRI*-linearised pSPOLV2 plasmid (Grieco *et al.*, 2000). Non-radioactive dot-blot hybridization assay was done as previously described (Saldarelli *et al.*, 1996).

The following herbaceous plants showed symptoms after mechanical inoculation: *Nicotiana benthamiana*: systemic chlorotic ringspots; *N. clevelandii*, *N. occidentalis*, *N. glutinosa* and *N. megalosiphon*: chlorotic/necrotic local lesions, followed by systemic mottling; *N. rustica* and *Nicotiana tabacum* cv. 'White Burley': transient systemic chlorotic rings and mottling; *Chenopodium quinoa*: chlorotic/necrotic local lesions; *Phaseolus vulgaris* cv. 'La Victoire': chlorotic/necrotic local le-

sions, followed by mottling and distortion of upper non-inoculated leaves; *G. globosa*: red dark local lesions followed by mottling and distortion of upper leaves; *Cucumis sativus*: chlorotic local lesions; *Cucurbita pepo* and *Vigna unguiculata* were apparently not infected. Castor bean seedlings inoculated with OLV-2/CB or OLV-2 type strain, reacted with systemic vein clearing and mottling, thus reproducing the syndrome observed in nature.

The procedure used for OLV-2/CB purification was satisfactory, as it gave high virus yields (6-8 mg per 100 g of leaf tissue). Purified virus preparations contained quasi-spherical to bacilliform particles, *ca* 25 nm to 55-60 nm in size. These preparations reacted in gel double diffusion tests with antiserum to OLV-2 but not with antisera against AMV. Electrophoresis of denatured nucleic acid extracts showed the consistent presence of four major RNA species, which comigrated, with the comparable genomic species of OLV-2 type strain. (Fig. 1A). In polyacrylamide gel electrophoresis, protein preparations from unfractionated OLV-2/CB particles migrated as a single band with a  $M_r$  of *ca* 24,000 daltons, and at the same rate as OLV-2 coat protein (Fig. 1B).

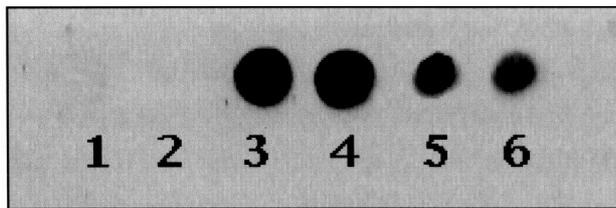
RT-PCR assay produced a DNA fragment 1696 bp in length, which corresponded to the coding portion of OLV-2 RNA3 (EMBL Database acc. no. AJ439450). When the sequence obtained was aligned with the comparable genomic portion of the OLV-2 type strain, it showed 93% identity. Pairwise comparison of the amino acid (AA) sequences of OLV-2CB coat (CP) and movement (MP) proteins, with the comparable polypeptides of the type strain, showed a high level of identity among CPs (96%) and MPs (94%) (Fig. 2). In fact, CPs differed in only seven positions and only one of them was in the exposed C-terminal region (alanine for threonine in position 188), whereas OLV-2/CB MP



**Fig. 2.** **A.** Amino acid sequence alignment of OLV-2/CB (CP-R) and OLV-2 (CP-O) coat proteins. **B.** Amino acid sequence alignment of OLV-2/CB (MP-R) and OLV-2 (MP-O) movement proteins. In the CP-R and MP-R sequences, identical amino acids are indicated by a dash (-).

showed seventeen AA changes in positions scattered along its primary structure. Dot-blot hybridisation assays using an OLV-2-specific riboprobe (Fig. 3) demonstrated the presence of OLV-2/CB in artificially infected castor bean plants.

The present results show that the OLV-2/CB is virtually indistinguishable from OLV-2 type strain from olive (Grieco *et al.*, 1992), from which it is virtually indistinguishable. This confirms that castor bean is a new host for this virus, which is recorded for the first time outside Italy (Vovlas *et al.*, 2002).



**Fig. 3.** Chemiluminescent detection of dot blot hybridisation assays using a DIG-labelled OLV-2-specific riboprobe. 1, healthy *N. occidentalis*; 2, healthy castor bean; 3, castor bean leaf locally infected with OLV-2/CB; 4, castor bean leaf systemically infected with OLV-2/CB; 5, castor bean leaf locally infected with OLV-2; 6, castor bean leaf systemically infected with OLV-2.

## REFERENCES

- Anonymous, 1994. Program manual for the Wisconsin package, Version 8, September 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711.
- Gonsalves D., Shepherd R.J., 1972. Biological and physical properties of the two nucleoprotein components of pea enation mosaic virus and their associated nucleic acid. *Virology* **48**: 709-723.
- Grieco F., Martelli G., Savino V., Piazzolla P., 1992. Properties of olive latent virus 2. *Rivista di Patologia Vegetale* **2**: 125-136.
- Grieco F., Savino V., Martelli G.P., 1996. Nucleotide sequence of the genome of a citrus isolate of olive latent virus 1. *Archives of Virology* **141**: 825-838.
- Grieco F., Alkowni R., Saponari M., Savino V., Martelli G.P., 2000. Molecular detection of olive viruses. *Bullettin OEPP/EPPO* **30**: 469-473.
- Higgins D.G., Sharp P.M., 1988. CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. *Gene* **73**: 237-244.
- Koonin E.V., Dolja V.V., 1993. Evolution and taxonomy of positive-strand RNA viruses: implications of comparative analysis of amino acid sequences. *Critical Reviews in Biochemistry and Molecular Biology* **28**: 375-430.
- Laemmli H.K., 1970. Cleavage of structural protein during the assembly of the head of the bacteriophage T4. *Nature* **227**: 680-685.
- Martelli G.P., Grieco F., 1997. Oleavirus, a new genus in the family *Bromoviridae*. *Archives of Virology* **142**: 1933-1936.
- Saldarelli P., Barbarossa L., Grieco F., Gallitelli D., 1996. Digoxigenin-labelled riboprobes applied to sanitary certification of tomato in Italy. *Plant Disease* **80**: 1343-1346.
- Sambrook J., Fritsch E.F., Maniatis T., 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York.
- Vovlas C., Parrella G., Di Franco A., 2002. Infezioni naturali del virus latente 2 dell'olivo su piante di ricino in Grecia. *Informatore Fitopatologico* **7-8**: 66-68.

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