



New isolates of *Citrus tristeza virus* naturally occurring in old lemon and mandarin trees in Greece

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Citrus tristeza virus (CTV) has quarantine status in Greece. The virus was first reported in 2000 in non-certified sweet orange trees grafted on CTV-tolerant Carrizo citrange originating from Spain (Dimou *et al.*, 2002). High homology of these isolates was then found with the Spanish mild T385 isolate (bio-group 1, Garnsey *et al.*, 2005) from sweet orange grafted on sour orange rootstock, sharing 100% nucleotide sequence identity for the p20 gene (Varveri, 2006). In 2009, surveys were conducted in the area of Poros (Trizinia, Prefecture of Piraeus), where about 20,000 citrus trees grow, mainly lemon. (This was an area previously devoted to citrus cultivation for more than 100 years but now consisting virtually of a wood protected only for its beauty and historical importance). A total of 285 citrus trees, 87% of which were lemon, were tested by ELISA (Enzyme-Linked Immunosorbent Assay) in a programme lasting until September 2010. Six trees (four lemons and two mandarins) were found positive. None of the above mentioned trees showed any symptoms.

All ELISA positive samples were confirmed with reverse transcription-polymerase chain reaction (RT-PCR). Two sets of primers targeting the p20 protein (PM48 CGAGCTTACTTTAGTGTTA and PM49 TAATGTCAAACACTGACCGC) (Sambade *et al.*, 2002) and the coat protein (cp) genes (CTV1 ATGGACGACGAAACAAAGAA and CTV10 ATCAACGTGTGTTGAATTTCC) (Nolasco *et al.*, 2002) were used. PCR products were purified using the QIAquick Gel Extraction Kit (QIAGEN GmbH, Hilden, Germany) and subjected to bidirectional direct sequencing (Macrogen, Inc., Seoul, Korea). Partial sequences of the p20 (483 bp) and cp (617-632 bp) genes of two lemon (L192GR, L594GR) and one mandarin (M8GR) CTV isolates were obtained and deposited in GenBank with the Accession Numbers recorded in Table 1. Sequence comparisons of the cp gene nucleotide sequences (HQ270181, HQ270182, and HQ270183) revealed a 99% identity with CTV isolates from Brazil (I141-62, I141-59), Cyprus (CY96-18, CY93-10, CY89-507) and Portugal (28C) (Table 1), belonging to the 3b genotype group of CTV isolates (Nolasco *et al.*, 2009). Nucleotide sequences of the p20 gene (HQ270180, HQ270185, and HQ270186) showed a 99% identity with a seedling yellows (SY) isolate from Brazil (Galego 50) belonging to bio-group 3 of CTV isolates (Garnsey *et al.*, 2005). Further phylogenetic analysis involving standard, fully sequenced and well characterized natural CTV isolates showed that the newly recovered isolates share a common ancestor with the VT seedling yellows (bio-group 4) isolate from Israel, which reveals a totally different topology in the obtained phylogenetic trees from the mild CTV isolates recovered in 2000-2001 in the country (data not

shown). The immunological reaction with the MCA13 monoclonal antibody confirmed the occurrence of a virus strain more virulent than the one thus far recorded in orange groves in Greece. Natural isolation of the "lemonwood" in Poros from hills and the sea has apparently hindered the spread of these isolates in sweet orange trees further on in the same region, which however represents a threat for the local citrus industry. Surveys are being continued intensively in Poros aiming at eradicating CTV. Our findings confirm previous conclusions on the diversity of CTV isolates in the Mediterranean basin reflecting the worldwide virus diversity.

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Table 1. Origin and nucleotide sequences of *Citrus tristeza virus* (CTV) isolates used in phylogenetic analysis.

Isolate	Origin	Gene	GenBank Accession No.
L192GR	Greece (Poros)	p20	HQ270185
		cp	HQ270182
L594GR	Greece (Poros)	p20	HQ270180
		cp	HQ270181
M8GR	Greece (Poros)	p20	HQ270186
		cp	HQ270183
I141-62	Brazil	cp	EU579359
I141-59	Brazil	cp	EU579358
CY96-18	Cyprus	cp	EF491677
CY93-10	Cyprus	cp	EF491676
CY89-507	Cyprus	cp	EF491675
28C	Portugal	cp	AF184118
Galego 50	Brazil	p20	FM955991
VT	Israel	cp, p20	U56902

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