# Molecular Characterization of *Citrus tristeza virus* Isolates from Epirus (Greece)

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ABSTRACT. Since June 2000, *Citrus tristeza virus* (CTV) has been reported in Greece including Crete, in some cases introduced accidentally in budwood from Spain. Here we present a preliminary molecular characterization of some CTV isolates based on amplification of the coat protein gene and use of RFLP. Samples were collected from symptomless Washington navel and Navelina trees about 20 yr old in commercial groves in Epirus (western Greece), grafted on sour orange. Analysis of the CP gene sequences showed 98-99% identity to the T30 isolate from Florida and the Spanish isolate T385, both mild isolates. RFLP analysis confirmed the characterization of the Greek isolates as mild. A phylogenetic tree drawn using Clustal X and TREEVIEW, and including a worldwide variety of CTV isolates, again confirmed close identity with T30 and T385, bringing additional evidence that the Greek isolates came from Spain, followed by local spread of the virus, largely through human activity.

In 2005 a survey for virus and virus-like diseases conducted in the main citrus-growing areas of Epirus (western Greece) revealed the presence of *Citrus tristeza virus* (CTV) (3). In June 2000, the virus was detected for the first time in Argolis (north-east Peloponnese) and in Chania (Crete), due to accidental introduction of CTV-infected budwood from Spain (4). However little is known about the characteristics of Greek isolates, in spite of the recognized importance of isolate identities in defining disease management and control strategies in order to avoid potential epidemic outbreaks. The present study is a preliminary molecular characterization of some CTV isolates detected in Arta valley, based on amplification of the the coat protein (CP) gene by RT-PCR jointly with the use of RFLP (5).

Isolates were collected from Washington navel and Navelina orange trees, about 20-yr-old, inspected in commercial groves. Plants were symptomless although grafted on sour orange rootstock. Samples, collected from randomly selected trees, were tested by dot-blot hybridization assay to detect CTV-infected plants (2). cDNA synthesis and cloning of the CP gene were done as previously described (3). Clones were automatically sequenced (MWG Biotech, Germany) and the complete CP sequence of isolates CTV-G17, CTV-G9, CTV-G73 and CTV-G74 was deposited in the EMBL database under accession numbers AM406802, AM406803, AM746968 and AM746969, respectively. They were compared to existing sequences using the Blast (NCBI) program (1). Analysis of the CP gene nucleotide sequences, compared to published sequences, showed 98-99% nt identity to the T30 isolate from Florida (accession no. AF260651) and the Spanish isolate T385 (accession no. Y18420). Restriction analysis of the CP gene, based on *HinfI* and *RsaI* enzyme sites, revealed considerable resemblance to mild CTV isolates. When classified by the *HinfI* RFLP pattern (5), the Greek isolates fell in group 2 (nt positions 73, 111, 410, 501), which also contained mild isolates T30 and T385. When classified by the *RsaI* products, group 2 with three restriction sites (nt positions 145, 312, 576) contained the Greek isolates and also mild isolates T30 and T385. According to Mendoza et al. (6), the CP gene of CTV strains designated as mild strains does not contain the *HaeIII* and *KpnI* restriction sites; the absence of these sites in the CP gene sequence of CTV-G17, CTV-G9, CTV-

G73 and CTV-G74 confirmed their characterization as mild isolates.

Using Clustal X (9), a multiple aligment was generated with the CP gene sequences of the Greek CTV isolates and several worlwide CTV isolates, showing different biological characteristics (Table 1). The phylogenetic tree, displayed using TREEVIEW version 1.6.6 (7), showed that the Greek isolates were closely related to known mild isolates and very similar to T385 and T30 strains (Fig. 1). Interestingly, their deduced CP amino acid sequence contained, at positions 122 and 124, isoleucine (I) and tyrosine (Y) respectively; these are present at the same positions in the CP amino acid sequence of most mild CTV isolates, including T385 and T30 (8). These results are additional evidence that introduction of CTV in Greece has occurred due to infected propagative material imported from Spain (4), and suggest further local spread of the virus, and the strong influence of human activity in its dissemination.

TABLE 1
*CITRUS TRISTEZA VIRUS* (CTV) COAT PROTEIN GENE SEQUENCES SELECTED FROM
GEN BANK AND USED IN SEQUENCE AND PHYLOGENETIC ANALYSIS

| Isolates | Country | Accession number |
|---|---|---|
| B35 | California, USA | L12175 |
| CTV-G9 | Greece | AM406803 |
| CTV-G17 | Greece | AM406802 |
| CTV-G73 | Greece | AM746968 |
| CTV-G74 | Greece | AM746969 |
| CTV-0032 | Italy | AJ518842 |
| TN-2 | Iran | AY707460 |
| T6-31 | Montenegro | AY764154 |
| T30 | Florida, USA | AF260651 |
| T385 | Spain | Y18420 |
| 2-93 | Portugal | AF184116 |
| CTV-B | India | AF501867 |
| Cheju Island | South Korea | AF249279 |
| Cy95-14 | Cyprus | EF491668 |
| Jordan | Jordan | AY550252 |
| NuagA | Japan | AB046398 |
| Qaha | Egypt | AY340974 |
| SY568 | California, USA | AF001623 |
| T36 | Florida, USA | U16304 |
| VT | Israel | U56902 |
| 13C | Portugal | AF184113 |
| 19-121 | Portugal | AF184114 |
| 28C | Portugal | AF184118 |
| 446-6 | Croatia | AY791842 |

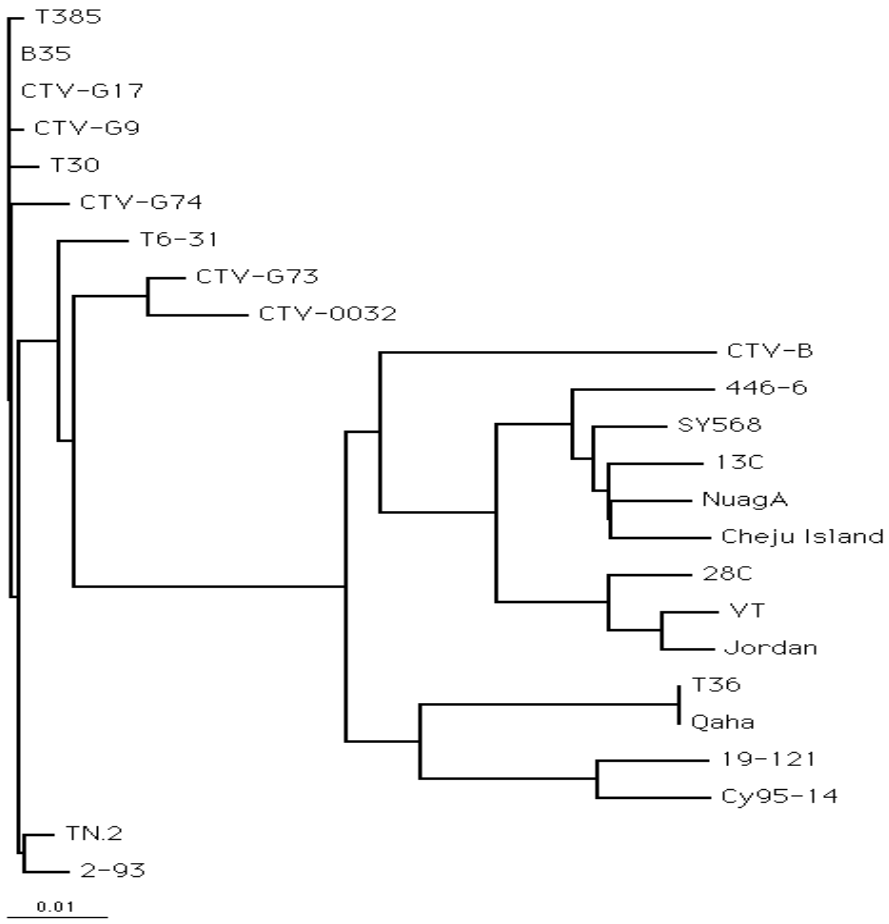


**Fig. 1 Dendrogram showing the genetic relationship among CP gene sequences of Greek and other *Citrus tristeza virus* (CTV) isolates (see Table 1). Scale bar indicates changes per nucleotide.**

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