Complete sequence of the RNA1 of an European isolate of tomato chlorosis virus

Annotated Sequence Record

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Introduction

Tomato chlorosis virus (ToCV, genus *Crinivirus*, family *Closteroviridae*) is the causal agent of a “yellow leaf disorder” of tomato (*Solanum lycopersicum* L.) that is present in Florida at least since 1989 [6]. Nowadays, the presence of ToCV has been confirmed in many countries of the Caribbean, the Far East, southern Africa, and northern and southern sides of the Mediterranean Basin. In Spain, ToCV has been present in tomato since at least 1997 [3] and also has been reported to infect sweet pepper (*Capsicum annuum* L.) [1]. ToCV is a typical crinivirus with a bipartite genome (RNA1 and RNA2) of single-stranded RNA of positive polarity. Recently, the complete nucleotide sequence of RNA1 and RNA2 of a ToCV isolate from Florida [5], and of RNA2 of an isolate from Spain (AT80/99) [2], have been reported. The sequence of the RNA1 of isolate AT80/99 reported here completes the genomic sequence of an European isolate of ToCV.

Provenance of the virus material

Isolate AT80/99 of ToCV was obtained during 1999 from a naturally infected tomato plant from Málaga (southern Spain) and was maintained in tomato cv. Moneymaker by periodic transmission using the whitefly *Bemisia tabaci* (Q biotype) as vector. Total RNA or double stranded RNA-enriched preparations from infected tomato plants were used in RT-PCR reactions to clone and sequence the RNA1 of isolate AT80/99 following a strategy similar to that used for the RNA 2 [2]. The sequence obtained has been deposited in the GenBank database under the accession number DQ983480.

Sequence properties

The sequence of ToCV RNA1, isolate AT80/99 consists of 8,594 nucleotides (nt) and contains four open reading frames (ORFs) (Fig. 1). The first two ORFs encode proteins involved in replication of viral RNA based on homology to other proteins of criniviruses. ORF 1a extends from nt 302 to 6139, and encodes a 221 kDa protein containing protease, methyltransferase, and helicase (Pro-MT-Hel) domains, conserved in all members of the family *Closteroviridae*. ORF 1b, from nt 6141 to 7655, encodes a 59 kDa protein containing the conserved motifs identified in the RNA dependent RNA polymerases (RdRp) of positive-strand RNA viruses. ORF 1b is supposed to be expressed through a +1 ribosomal frame-shift at the end of ORF 1a, as suggested for other closteroviruses. ORF 2, from nt 7662 to 8243, encodes a putative 22 kDa protein of unknown function. The predicted small ORF 3, from nt 8263 to 8418, encodes a putative protein of 6 kDa with a transmembrane domain similar to other 3'-end proteins of criniviruses. In addition to these four ORFs, RNA1 contains a 301 nt 5'-untranslated region (UTR) and a 176 nt 3'-UTR. Overall, the sequence of the RNA1 of isolate AT80/99 from Spain has a nucleotide sequence identity of 97 % with the equivalent RNA of an isolate of Florida recently reported [5]. Table 1 shows the percentages of nucleotide and aminoacid identity of ORFs of the AT80/99 isolate compared with homologous ORFs of the Florida isolate of ToCV and all the crinivirus species sequenced to date. Phylogenetic analysis of the ORFs 1a+1b of isolate AT80/99 (Fig. 2) confirmed the position of ToCV in the genus *Crinivirus*, most similar to SPCSV and CYSDV, and closely related to the ToCV isolate from Florida.

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Table 1. Percentage nucleotide and amino acid (in parenthesis) identities between the putative proteins encoded by the ToCV (isolate AT80/99 from Spain) ORFs compared to equivalent ORFs of the ToCV isolate from Florida and other species in the genus *Crinivirus*.

Virus	ORFs (putative proteins)			
	ORF1a (Pro-MT-Hel)	ORF1b (RdRp)	ORF2 (P22)	ORF3 (P6)
ToCV (Florida)	97 (97)	98 (99)	96 (96)	98 (100)
SPCSV	50 (38)	64 (66)	38 (14)	34 (15)
CYSDV	49 (33)	63 (62)	35 (12)	26 (18)
PYVV	46 (33)	61 (60)	-	31 (14)
BYVaV	50 (37)	63 (62)	-	-
SPaV	48 (35)	60 (58)	-	-
BPYV-str	50 (36)	63 (60)	-	34 (16)
BPYV-cuc	50 (36)	64 (62)	-	-
LIYV	48 (29)	57 (55)	29 (10)	-

0.1

FIGURE LEGENDS

Fig. 1. Schematic representation of the genomic structure of the RNA1 of the Spanish isolate AT80/99 of ToCV. Boxes represent ORFs, with the putative protein products indicated inside.

Fig. 2. Phylogenetic relationship of ToCV (isolate AT80/99) ORFs 1a+1b, with the corresponding genomic regions of sequenced members of the genus *Crinivirus*. Abbreviations and GenBank accession numbers: BPYV-cuc, beet pseudo-yellows virus (“cucumber yellows” strain), AB085612; BPYV-str, beet pseudo-yellows virus (“strawberry” strain), AY330918; BYVaV, blackberry yellow vein associated virus, AY776334; CYSDV, cucurbit yellow stunting disorder virus, AY242077; LIYV, lettuce infectious yellows virus, U15440; PYVV, potato yellow vein virus, AJ557128; SPaV, strawberry pallidosis associated virus, AY488137; SPCSV, sweet potato chlorotic stunt virus, AJ428554; ToCV-Fl, tomato chlorosis virus (“Florida” isolate), AY903447. Beet yellows virus (BYV, type species of the genus *Closterovirus*) (X73476) was used as outgroup. Phylogenetic tree was constructed using the CLUSTAL X program [4]. Bootstrap (1000 replicates) values are shown as percentage value and only the nodes over 50% are labeled. The bar below the tree indicates 0.1 nucleotides substitutions per site.