

SHORT COMMUNICATIONS

Comparison of the Capsid Protein Gene Sequences of Five Turkish Isolates of Citrus Tristeza Virus

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ABSTRACT. Capsid protein genes of five Turkish field isolates of citrus tristeza virus (CTV) were cloned and sequenced. Cluster analysis indicated that the sequences are closely related to one another and to a known severe stem pitting isolate (B53) from Japan. They also reacted with the monoclonal antibody MCA13, which reacts predominantly with severe CTV isolates. The biological characteristics of these isolates have not been assessed, and stem pitting was not obvious in orange trees in the field.

Citrus tristeza virus (CTV) causes significant losses in most citrus-producing areas of the world (5). CTV, a member of the closterovirus group, has the largest RNA genome among positive sense RNA plant viruses. The nucleotide sequence (19,296 nt) of the entire genome of the Florida CTV strain, T36, has now been completed (11). There are many isolates of CTV which differ in their biological properties (5), causing a wide range of symptoms depending on the citrus species or cultivar. Characterization of the isolates is important because it will determine the economic impact of the disease and the cultivars and rootstocks which can be safely grown in each particular production area. Effective control measures also will depend on the accurate and rapid detection and identification of the CTV isolate(s) present.

The capsid protein genes (CPGs) of several biologically distinct isolates of CTV have been cloned and sequenced (13,15). Cluster analysis of nucleotide sequences of isolates with known biological properties indicated a correlation between biological properties of the isolates and their CPG sequences (15). This relationship, however, needs further

analysis for some isolates (13). Serological diversity also has been demonstrated among CTV isolates. The monoclonal antibody MCA13 reacts predominantly with severe isolates of CTV from the Americas (17), and the epitopes of two monoclonal antibodies, MCA13 and 3DF1, have been characterized (16, 14).

Citrus production in Turkey is increasing to accommodate both internal demands and export markets. The total citrus production is about 1,500,000 tons per year, which places Turkey in tenth place of world citrus production, and fourth in the Mediterranean area (1). CTV is not yet causing a major citrus disease in Turkey, and the presence of the most efficient aphid vector, *Toxoptera citricida* (Kirkaldy), has not been reported. However, the majority of the Turkish citrus plantations consist of sweet orange grafted on sour orange rootstock, and because CTV and *Aphis gossypii* are present, CTV is a major threat to the Turkish citrus industry (3, 4, 7, 21). The objective of this research was to compare the CPGs of Turkish isolates of CTV to those previously characterized.

The Turkish isolates (TR12, TR13, TR18, TR22, TR23) were collected near Mersin and Adana from tissue

of naturally infected sweet orange trees grafted on sour orange rootstock which tested positively by ELISA, using both polyclonal and the MCA13 monoclonal antibodies. Endemic Florida strains, T26 and T30, produce mild symptoms and little stunting on Mexican lime (20). Florida strain T36 produces quick decline in sweet orange grafted on sour orange rootstock (20). Isolate B53 (T388) was collected in Spain from a Satsuma mandarin intro-

duced from Japan and produces stem pitting and vein corking in Mexican lime (2). Isolate B128 was, collected at Ginebra, Colombia from red grapefruit with severe CTV stem pitting (SP) symptoms (15). Isolate B227 was collected in India from field trees of 2-yr-old Coorg mandarin on rough lemon rootstock with stem pits on both the rootstock and scion portions (12). Sequences for the CPG of T26, T30, B53, B128 and B227 have been published previously (12, 15).

B53	MDETRKLNKKNKETKEGDDVVAESSFSSINLHIDPTLIAMNDVRQLGT
TR13	----K-----G-----
TR23	----K-----G-----
TR18	----K-----G-----
TR12	----K---T-----G-----
TR22	----K-----G-----V---GH---
B53	QQNAALNRDLFLTLKGYPNLPDKDKDFHIAMMLYRLAVKSSSLQSDDDT
TR13	-----H-----A
TR23	-----H-----A
TR18	-----H-----A
TR12	-----H-----A
TR22	-----H-----A
B53	TGITYTREGVEVDLPDKLWTDVVFNSKGIIGNRTNALRVWGRTNDALYLAF
TR13	-----
TR23	-----
TR18	-----
TR12	-----I-----
TR22	-----
B53	CRQNRNLSYGGRPDLDAIIPAGYHYLCADFLTGAGLTDLECAVYIQAQEQL
TR13	-----
TR23	-----
TR18	---H-----
TR12	-----
TR22	-----
B53	LKKRGADEVVVTNVRQLGKFNTR
TR13	-----
TR23	-----
TR18	-----
TR12	-----
TR22	-----

Fig. 1. Multiple alignment of deduced amino acid sequences of the CP of five Turkish CTV isolates compared to isolate B53. Shaded letters indicate the divergent amino acids. Dashes indicate identical amino acid residues.

The CTV CPGs were cloned and sequenced as described by Cevik et al. (6) and Pappu et al. (15). A minimum of three clones were sequenced in both directions for each isolate. Sequence analyses were performed using the programs Seqaid II (18), Clustal V (10) and UWGCG (8).

The open reading frames of the CPGs of the five Turkish isolates were 672 nucleotides in length, and the deduced amino acid sequences were 223 residues, which is in agreement with previously published CTV CPGs (15). There was 98% or higher nucleotide identity among these isolates and more than 97% identity between them and B53, a known stem pitting isolate from Japan (2). There were 19 nucleotides (data not shown) and seven amino acid differences between B53 and TR22, the most divergent Turkish isolate (Fig. 1). Four of these amino acid differences were conserved in all five Turkish isolates. The deduced amino acid sequences of the CPs of the five Turkish isolates and six other geographically and biologically distinct CTV strains were used to construct a dendrogram using UWGCGs pileup program. This analysis showed that the CP sequences of the Turkish isolates most closely resembled those of B53 and B227, two known severe stem pitting isolates, and T36, a quick decline strain (12, 15), but were distinct from stem pitting isolate B128 and mild strains T26 and T30 (Fig. 2). These data plus the presence of the MCA13 epitope in the CPG sequence, suggested that potentially severe CTV isolates may already be present in Turkey. The five Turkish isolates also showed a high level of similarity, indicating that their origins and biological properties may be similar.

Epidemics of tristeza have not been reported in Turkey, but existence of CTV and the vectors *A. gossypii*, and *T. aurantii* have been reported (7, 21). Further adaptation of CTV to its vector and increase in

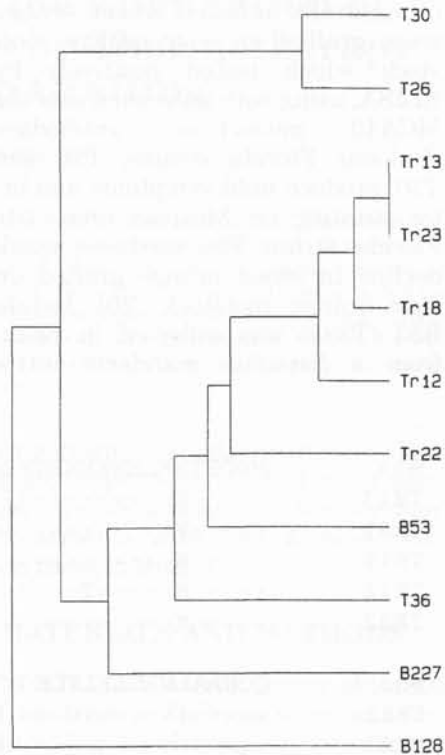


Fig. 2. A dendrogram showing the relative relationships of the deduced amino acid sequences of the CPs of five isolates of CTV from Turkey and six strains of known biological activity (12, 15). Distances along horizontal axes are proportional to the difference between sequences.

transmissibility may lead to epidemics of CTV decline in this area as have occurred in California (19) and Spain (9). Introduction of severe strains of CTV or the efficient vector, *T. citricida*, could also cause serious losses in citrus plantations where the majority of citrus trees are on sour orange rootstock.

It is important to know the characteristics of the CTV isolates already present in Turkey. However, since the CPG is only 3.5% of the CTV genome, it alone may not be adequate to reliably predict the biological properties of a CTV isolate. A systematic serological survey fol-

lowed by biological indexing of selected isolates would be a prudent measure to determine impact of CTV isolates on the Turkish citrus industry.

ACKNOWLEDGMENTS

This research was supported in part by grants from USDA-ARS/IFAS Cooperative Agreement 58-6617-102 and Florida Citrus Production Research and Advisory Council

#1594 to C. L. N. and R. F. L. CTV isolates with B numbers were obtained from the Exotic Collection of Citrus Pathogens maintained at BARC, Beltsville, MD. We thank C. Gouin-Behe and Neil Berger for technical assistance. We are grateful to the Interdisciplinary Center for Biotechnology Research for providing oligonucleotide synthesis and computing facilities. This paper is Florida Agricultural Experiment Station Journal Series No. R-05215.

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