Diversity of the coat protein gene of Citrus tristeza virus (CTV) in the Mediterranean region

Djelouah K., Cerni S., Fonseca F., Santos C., Silva G., D'Onghia A.M., Nolasco G.

in

D'Onghia A.M. (ed.), Djelouah K. (ed.), Roistacher C.N. (ed.).
Citrus tristeza virus and Toxoptera citricidus: a serious threat to the Mediterranean citrus industry

Bari : CIHEAM
Options Méditerranéennes : Série B. Etudes et Recherches; n. 65

2009
pages 159-163

Diversity of the coat protein gene of *Citrus tristeza virus* (CTV) in the Mediterranean region

Djelouah K.[1], Cerni S.[2], Fonseca F.[3], Santos C.[3], Silva G.[3], D'Onghia A.M.[1], Nolasco G.[3]

[1] CIHEAM - Mediterranean Agronomic Institute, Valenzano (BA), Italy
[2] University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia
[3] Center for Biodiversity Functional and Integrative Genomics, Universidade do Algarve-Faro, Portugal

Abstract. Sequence data of the coat protein gene of CTV were retrieved from the GenBank or from tested infected tissues and compiled. A comparison with a comparable set of sequences from worldwide isolates showed a very similar value for the nucleotide diversity and a very similar genetic structure in which the same seven groups of variants are conspicuous. A geographic speciation is not apparent and, probably, differences in the distribution of CTV groups are a result of trade. In countries that usually import propagating material from Spain, the group M predominates while group 3b predominates in countries that import material from Israel.

Keywords. Coat protein gene – CTV – Group 3b – Group M – Nucleotide.

Diversité du gène de la protéine capsidique du Citrus tristeza virus dans la région méditerranéenne

Résumé. *Les données des séquences du gène de la protéine capsidique du CTV ont été récupérées de la GenBank ou des tissus infectés analysés et élaborées. Une comparaison avec un ensemble de séquences d'isolats de diverses origines a révélé une similitude élevée pour la diversité des nucléotides et une structure génétique très similaire dans laquelle les mêmes sept groupes de variantes sont importants. Une spéciation géographique n'est pas évidente et, probablement, des différences en termes de distribution des groupes de CTV sont imputables aux échanges commerciaux. Dans les pays qui importent normalement du matériel de multiplication de l'Espagne, le groupe M prédomine alors que le groupe 3b est le plus présent dans les pays qui importent le matériel végétal d' Israël.*

Mots-clés. *Gène de la protéine capsidique – CTV – Groupe 3b – Groupe M – Nucléotide.*

I – Introduction

Despite the eradication programmes that have been undertaken, *Citrus tristeza virus* (CTV) can now be found in most of the Mediterranean countries. In this work, we present a snapshot of the circulating strains in the citrus-producing areas of the Mediterranean region. Only isolates obtained from plants grown in field conditions were considered. In marginal Mediterranean countries such as Portugal, the isolates were restricted to regions which, by climate, cultural practices or commercial exchanges, can be considered as an extension of the Mediterranean basin. In total, information from sequence variants obtained from 108 isolates from 17 countries was gathered (Tab. 1).

Table 1. Number of isolates harbouring haplotypes of each group.

	Gp 1	Gp 2	Gp 3a	Gp 3b	Gp 4	Gp 5	Gp M
Israel	1			8			
Palestine				6			
Jordan				1			
Syria				1			
Turkey		1					
Cyprus	2	1		3	1	4	
Croatia		7	4		2	2	1
Montenegro		1	1				1
Albania	3						
Epirus							2
Italy	2		2	1	2		19
Spain		1	1				2
Portugal			2	2			7
Egypt	1				2		
Tunisia	3					1	
Algeria							1
Morocco					1	2	3
Percentage	**11 %**	**10 %**	**9 %**	**20 %**	**7 %**	**8 %**	**33 %**

II – Material and Methods

Coat protein (CP) gene accessions of isolates from the Mediterranean region were retrieved from the GenBank or from unpublished work carried out in the last years at the Mediterranean Agronomic Institute of Bari, Italy and at the Universidade do Algarve, Faro, Portugal. In these cases, the CP gene was amplified from infected tissue by RT-PCR from total RNA extractions or by Immunocapture RT-PCR procedures using primers CTV 1 and CTV 10 which are specific for the 5' and 3' terminal CP gene parts (Nolasco *et al*., 2002). The amplified products were TA cloned and bacterial clones harbouring the CP gene were analysed by SSCP prior to sequencing. Several isolates harboured more than one CP gene variant as determined by SSCP. Variants originating different SSCP patterns were sequenced.

The CP gene sequences were excised from the first and last 20 nucleotides in order to exclude the region which might correspond to the primers used to obtain them. After alignment the set of sequences was scanned for the presence of recombination events using the RDP software (Martin *et al*., 2005) which implements several algorithms for recombination detection. Three sequence variants which showed recombination evidence by more than one algorithm were excluded from further analysis as they probably originated by an evolutionary mechanism which is not compatible with the evolutionary model (Kimura 2- parameter) used to derive the remaining phylogenetic relationships. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 (Tamura *et al*., 2007) on the set of 143 CP gene sequence variants.

III – Results and discussion

The nucleotide diversity (π) of the set of 143 sequences was estimated as 0.072 (SE: 0.006) nucleotide changes per site which is very close to the value attained with a set of 213 CP gene sequences obtained from worldwide isolates π = 0.074, SE: 0.007 (Nolasco *et al*., 2007).

A phylogenetic tree (Fig. 1) was constructed in which additional sequences from worldwide reference isolates previously used in other studies were included (13C from Madeira Island, AF184113; 15-118 from Madeira Island, GenBank accession AY660009; T3 from Florida; T30

from Florida, GenBank accession AF260651; T36 from Florida, AY170468) to help identifying the phylogenetic relationships. A structure containing 7 well defined groups was obtained. These groups were supported by a bootstrap value (1000 repetitions) higher than 90%. This confirms and generalizes the structures obtained previously (Papayiannis *et al*., 2007; Cerni *et al*., 2007; Lbida *et al*., 2004; Amin *et al*., 2006; Zemzami *et al*., 2002) from studies using subsets of the sequence haplotypes presented here. These same 7 groups are also present when the whole set of worldwide CP gene sequences are considered (results not shown). Most of the nucleotide diversity of the Mediterranean population is due to the inter-group diversity, which accounts for a coefficient of differentiation Nst = 0.73. Intra-group diversity ranges from 0.012 (Group 1) to 0.030 (Group 2). In all the groups purifying selection was detected at 5% significance level.

The deduced aminoacid sequences showed the presence of Tyrosine in the position 124 for all the variants of group M, while the variants of all the other groups showed the presence of Phenylalanine in that position. Phenylalanine at position 124 originates the epitope responsible for the reaction of the MCA 13 antibody (Pappu *et al*., 1993), which has been used in Florida to distinguish between mild and severe strains (Permar *et al*., 1990). Biological data of isolates harbouring a master sequence belonging to group M obtained from Italy (Daden, 2006), Portugal (Bonacalza, 1998) or Spain (Moreno *et al*., 1991) showed that these isolates are of mild type, not originating stem-pitting on sweet orange or decline on trees grafted onto sour orange. The absence of reaction to the MCA13 antibody is thus valid to identify mild isolates in the Mediterranean region. However, a positive reaction does not necessarily mean a severe isolate: isolates whose master sequence belongs to Group 2, as is the case of the Turkish isolates analysed in this work and by Korkmaz *et al*. (2007) originate mild symptoms on Mexican lime and do not induce decline on trees grafted onto sour orange or sweet orange stem pitting.

No clear relationship could be established with geographic origins. As can be seen in Table 1, isolates harbouring haplotypes from the same phylogenetic groups are found in regions thousands kilometres apart and in countries with small citrus producing areas as Croatia and Cyprus five out of the seven phylogenetic groups are found. However, the sequence variants from groups 3b and M predominate in the Mediterranean. This probably reflects the major sources of CTV dissemination in the Eastern and Western Mediterranean area. In the Near East countries, there is a predominance of isolates harbouring Group 3b. Israel being the country which has the most developed citriculture in the region and in which CTV is endemic is probably the source for dissemination of Group 3b. In the western countries, Portuguese isolates harbouring Group M haplotypes clearly predominate. Besides the isolates analysed here, others which were introduced illegally from Spain and characterized by a set of hybridization probes in asymmetric PCR ELISA assays (unpublished), showed also the prevalence of group M. In Italy there is also the prevalence of Group M isolates, being the Spanish origin very probable. In Morocco the isolates harbouring Group M had also Spanish origin (Lbida *et al*., 2004). Thus, it appears that Spain represents a source for dissemination of Group M in the western Mediterranean basin.

In conclusion, the population of CTV variants circulating in the Mediterranean basin does not appear qualitatively different from the worldwide CTV population. Variants from group M predominate in countries which introduce plant material from Spain and variants from group 3b in countries which introduce plant material from Israel.

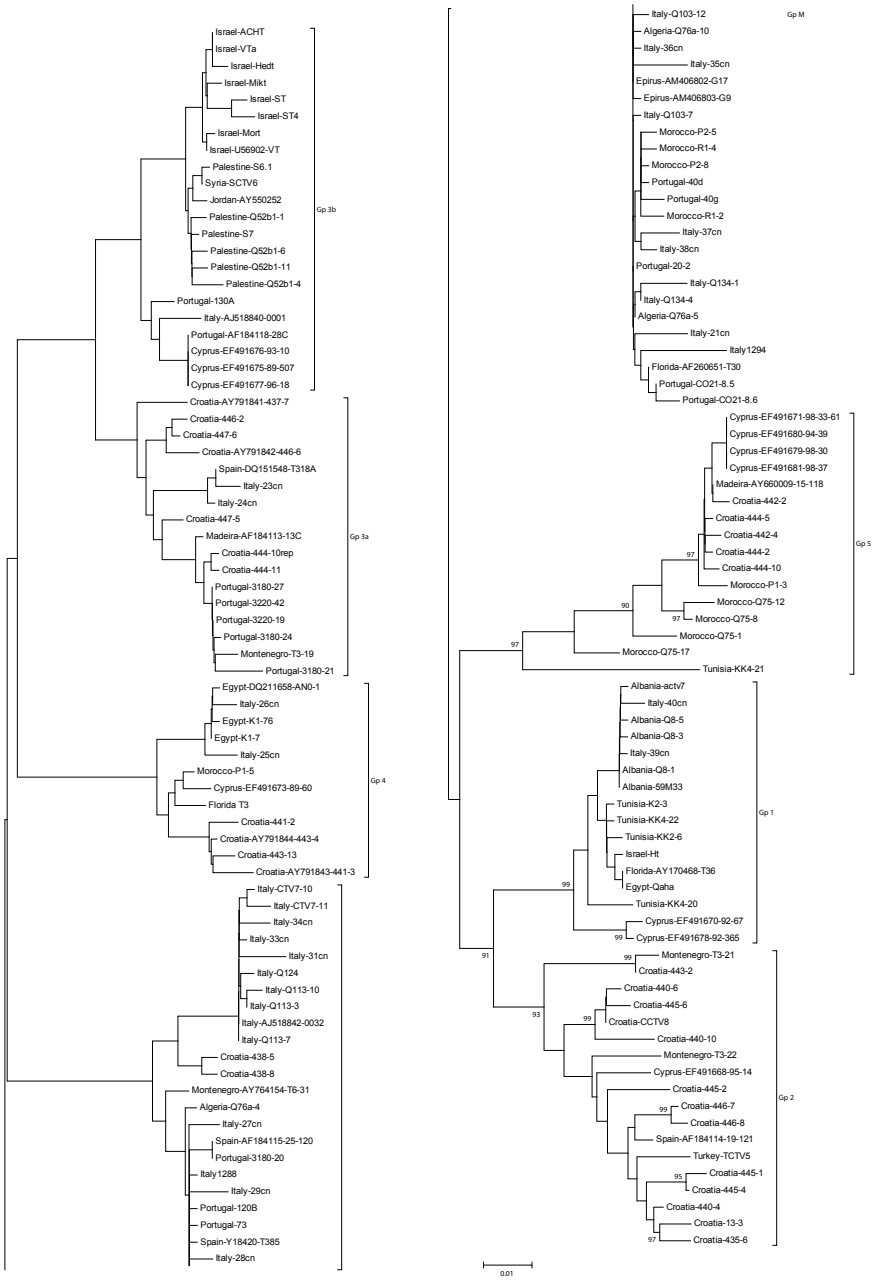

Figure 1. Phylogenetic tree obtained by the Neighbour-joining method applied to the matrix of pairwise distances (kimura 2 parameters) between sequence variants obtained from 108 Mediterranean isolates. Numbers close to the branches represent the bootstrap values when greater than 90%. The 7 groups referred to in the text are represented. Each sequence variant is designed by the country of origin, haplotype designation and GenBank accession number if previously submitted

References

Amin H.A., Fonseca F., Santos C., Nolasco G., 2006. Typing of Egyptian Citrus tristeza virus (CTV) isolates based on the capsid protein gene. *Phytopathologia Mediterranea* 45: 10-14.

Bonacalza B., 1998. Molecular typification of isolates of (CTV) in Portugal. MSc Thesis, Instituto Superior de Agronomia, Universidade Técnica de Lisboa. Lisbon (PT) (in Portuguese): 112p.

Černi S., Ruscic J., Nolasco G., Gatin Z., Krajacic M., Skoric D., 2007. Stem pitting and seedling yellows symptoms of *Citrus tristeza virus* infection may be determined by minor sequence variants. *Virus Genes*. Online First. DOI 10.1007/s11262-007-0183-z.

Daden M., 2006. Characterization of Mediterranean *Citrus tristeza virus* (CTV) isolates. MsC Thesis, CIHEAM-IAMB.

Korkmaz S., Çevik B., Onder S., Koç N.K., 2007. Detection and identification of Citrus tristeza virus isolates from different citrus growing regions of Turkey. *Abstract 17th Conf. IOCV* Reverside: 59.

Lbida B., Fonseca F., Santos C., Zemzami M., Bennani A., Nolasco G., 2004. Genomic variability of Citrus tristeza virus (CTV) isolates introduced into Morocco. *Phytopathologia Mediterranea* 43: 205-210.

Martin D.P., Williamson C., Posada D., 2005. RDP2: recombination detection and analysis from sequence alignments. *Bioinformatics* 21: 260-262.

Moreno P., Guerri J., Ballester-Olmos J.F., Martinez M.E., 1991. Segregation of citrus tristeza virus strains evidenced by double stranded RNA (dsRNA) analysis. In *Proc. 11th Conf. IOCV*, IOCV Riverside: 20–24.

Nolasco G., Fonseca F., Silva G., 2007. Occurrence of genetic bottlenecks during *Citrus tristeza virus* acquisition by Toxoptera citricidus in field conditions. *Archives of Virology*. Online First. DOI 10.1007/s00705-007-1089-8.

Nolasco G., Sequeira Z., Soares C., Mansinho A., Bailey A., Niblett C. L., 2002. Asymmetric PCR ELISA: Increased sensitivity and reduced costs for the detection of plant viral and other nucleic acids. *European Journal of Plant Pathology* 108: 293-298.

Papayiannis L.C., Santos C., Kyriakou A., Kapari T., Nolasco G., 2007. Molecular characterization of *Citrus tristeza virus* (CTV) isolates from Cyprus based on the coat protein gene. *Journal of Plant Pathology.* 89 (2): 281-285.

Pappu H.R., Pappu S.S., Manjunath K.L., Lee R.F., Niblett C.L., 1993. Molecular characterization of a structural epitope that is largely conserved among severe isolates of a plant virus. *Proc. National Academic of Science* USA 90: 3641-3644.

Permar T.A., Garnsey S., Gumpf D.J., Lee R.F., 1990. A monoclonal antibody that discriminates strains of Citrus Tristeza Virus. *Phytopathology* 80: 224–228.

Tamura K., Dudley J., Nei M., Kumar S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596-1599.

Zemzami M., Soares C.M., Bailey A.M., Niblett C.L., Nolasco G., 2002. Molecular characterization and classification of Moroccan isolates of Citrus tristeza closterovirus. In: *Proc. 15th conf. IOCV*, IOCV Riverside: 8-12.