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Serological characterization of a collection of Mediterranean *Citrus tristeza virus* (CTV) isolates

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Abstract. A collection of 68 CTV isolates from 11 Mediterranean countries were cross-tested against a panel of 8 monoclonal antibodies (MAbs), including one severe strain specific (MCA-13) and one broad pattern (17G11) from USA, four broad spectrum (1D12, 4C1, 4E5 and 4F3) and two differentials (4B1 and 4D3) from Morocco. Based on the reaction patterns, the tested CTV isolates were classified in 8 serogroups (Sgr), among which Sgr.1 had the largest number of isolates (35 in total) that reacted with all MAbs. Three broad spectrum MAbs (17G11 from USA and 4C1 and 4E5 from Morocco) were able to detect all the CTV isolates tested. They proved to be highly reliable for universal detection of Mediterranean strains of CTV. MCA-13, the severe CTV-strain specific MAb, reacted with 2/3rd of the tested isolates, indicating that some of the Mediterranean CTV strains could be of severe nature. MAbs 4B1 and 4D3 were the most selective antibodies.

Keywords. Mediterranean CTV isolates – Selective monoclonal antibodies – Serological patterns.

Caractérisation sérologique d'une collection méditerranéenne d'isolats du virus de la tristeza des agrumes (CTV)

Résumé. *Une collection de 68 isolats de CTV provenant de 11 pays méditerranéens ont été testés contre un panel de 8 anticorps monoclonaux (AcMc), comportant le MCA-13 spécifique des souches sévères et le 17G11 à large spectre tous les deux originaires des USA, 4 AcMc à large spectre (4B1, 4C1, 4E5 et 4F3) et 2 AcMc différentiels (4B1 et 4D3) originaires du Maroc. A la base des profiles des réactions obtenus, les isolats de CTV testés ont été classés en 8 séroproupes parmi lesquels le Sgr.1 comporte le plus grand nombre d'isolats (35 au total), ayant réagit avec tous les AcMc testés. Trois des AcMc à large spectre (17G11 des USA et 4C1 et 4E5 du Maroc) ont réagi avec tous les isolats testés. Ils ont ainsi prouvé leur haute performance pour la détection universelle des souches Méditerranéennes de CTV. Le MCA-13 connu pour sa spécificité aux souches sévères de CTV a réagi avec les 2/3 des isolats testés, indiquant qu'il est fort possible que certaines des souches Méditerranéennes de CTV soient de nature sévère. Les AcMc 4B1 et 4D3 ont montré les profiles de réaction les plus sélectifs.*

Mots-clés. *Isolats méditerranéens de CTV – Anticorps monoclonaux sélectifs – Profiles sérologiques.*

I – Introduction

Citriculture is of paramount strategic importance for the Mediterranean region for its socio-economic role as a source of income, a provider of employment opportunities and for its contribution to the well-being of the people in the Mediterranean countries. However, the Mediterranean citrus industry relied too much on the use of sour orange, a root-stock well adapted to numerous biotic and abiotic stresses largely prevalent in the region, but highly susceptible to CTV, a devastating virus which killed millions of trees throughout the world, including Spain and Israel (Marroquìn *et al.*, 2004; Bar Joseph *et al.*, 1989). The threat of tristeza has even worsened with the recent appearance in Northern Portugal and Spain of the brown citrus aphid "*Toxoptera citricidus*", known to be the most efficient vector of CTV. With CTV inoculum present in almost all the Mediterranean citrus producing countries, invasion of the Mediterranean region by *T. citricidus* will cause a rapid dissemination of severe CTV strains that will devastate in few years all trees grafted on sour

orange. A sudden collapse of the major part of the Mediterranean citrus industry will occur with unpredictable dramatic socio-economic consequences.

This study was conducted for the serological characterization of the Mediterranean collection isolates of CTV obtained from 11 countries, using a panel of monoclonal antibodies (MAbs), including the severe strain specific MCA-13, some broad spectrum and some selective MAbs. The aim is to identify efficient serological tools for general detection of CTV and selective MAbs for specific strain typing.

II – Materials and methods

1. The CTV collection

The CTV isolates were either collected directly in citrus orchards during surveys conducted by the Mediterranean Agronomic Institute of Bari (MAIB) in various Mediterranean countries, or obtained from local collaborating institutions as material exchange for research purposes. All the isolates were subjected to a preliminary test for CTV infection by DAS ELISA as reported by Bar Joseph *et al.* (1979), using two commercial kits; i.e. one based on polyclonal antiserum (PAs) from Agritest (Italy) and one based on a mixture of Monoclonal antibodies (3DF1+3CA5) from Ingenasa (Spain) targeting two highly preserved epitopes of CTV coat protein and able to detect all known CTV isolates (Garnsey *et al.*, 1989).

These CTV isolates were kept in their original plant material grafted onto different rootstocks (Sour orange, Troyer citrange or Rough lemon) under insect-proof screenhouse and were assigned an "IAMB-Q" number. Globally, these CTV sources included several countries as reported in Table 1.

2. Monoclonal Antibodies

The MAbs used include the severe strain specific MCA-13 (Permar *et al.,* 1990), and the large spectrum 17G11 (Lin *et al.* 2000) both from USA, four large spectrum (1D12, 4B1, 4C1, and 4E5) and two discriminating (4D3 and 4F3) from Morocco (Zemzami *et al.,* 1994). All MAbs were purified IgG's at 1 mg/ml optimized for use at a final concentration of 1 µg/ml.

3. Serological characterization

In order to assess the epitopic diversity of the different CTV isolates, TAS-ELISA assay (Cambra *et al.*, 1995) was performed using the Agritest PAs for capture. All virus sample extracts were prepared from fresh bark and leaf tissue of greenhouse positive plants. MAbs were allowed to react with the antigen for 2 hrs at 37 ºC. Anti-mouse (whole molecule) conjugated to Alkaline Phosphatase (Sigma, USA) was used for MAbs detection. The final reaction was revealed with p-Nitrophenyphosphate at 1 mg/ml and reading was done at OD_{405}. Results were recorded as positives when exceeding 3 times the mean of the negative control.

Table 1. List of the selected CTV sources maintained at the IAMB screenhouse.

Countries	Number of isolates	Numbers assigned
Albania (Alb)	11	IAMB-Q3, Q5, Q8, Q11, Q90, Q91, Q92, Q93, Q94, Q132, Q133
Algeria (Alg)	1	IAMB-Q76
Croatia (Cro)	1	IAMB-Q96
Cyprus (Cyp)	1	IAMB-Q73
Egypt (Egy)	4	IAMB-Q57, Q82, Q61, Q81
Iran (Ira)	1	IAMB-Q97
Italy (Ita)	23	IAMB-Q21, Q32, Q102, Q103, Q105, Q110, Q111, Q112, Q113, Q114, Q115, Q116, Q117, Q118, Q119, Q121, Q122, Q123, Q124, Q134, Q135, Q143, Q144
Lebanon (Leb)	10	IAMB-Q4, Q6, Q7, Q12, Q15, Q125, Q126, Q127, Q128, Q131
Montenegro (Mon)	4	IAMB-Q106, Q107, Q108, Q109
Morocco (Mor)	2	IAMB-Q74, Q75
Palestine (Pal)	8	IAMB-Q40, Q44, Q48, Q49, Q51, Q52, Q53, Q54
Syria (Syr)	2	IAMB-Q149, Q150

III – Results and discussion

The 68 CTV sources tested against the panel of 8 monoclonal antibodies exhibited 8 distinct serological reaction patterns designated here as serogroups (Tab. 2).

Table 2. Serological analysis of 68 selected CTV sources and the established serogroups.

Serogroups	CTV isolates	17G11	4C1	4E5	1D12	MCA 13	4F3	4B1	4D3	Frequency (nbr)
1	Alb (3, 5, 8, 11, 90, 91, 92, 93, 94, 132), Cro 96, Cyp 73, Egy (57, 82, 61), Ita (21, 32), Leb (6, 15, 126), Pal*, Mon*, Mor*.	+	+	+	+	+	+	+	+	**35**
2	Egy 81, Leb (128, 131), Syr	+	+	+	+	+	+	+	--	**5**
3	Leb (4, 7, 12, 125)	+	+	+	+	+	+	--	--	**4**
4	Leb 127	+	+	+	+	+	--	--	--	**1**
5	Alb 133, Alg 76, Ita (102, 105, 110, 111, 117, 118).	+	+	+	+	--	+	+	+	**8**
6	Ita (103, 113, 114, 115, 119, 121, 122, 123, 124)	+	+	+	+	--	+	--	--	**9**
7	Ita (134, 135, 143, 144)	+	+	+	+	--	--	+	--	**4**
8	Ita (112, 116)	+	+	+	--	--	+	--	--	**2**
Total	68	68	68	68	66	45	63	52	44	

Sgr.1 (35 isolates) reacted with all MAbs; Sgr.2 (5 isolates) reacted with all MAbs except 4D3; Sgr.3 (4 isolates) reacted with all MAbs except the Moroccan differential MAbs (4B1 and 4D3); Sgr.4 (1 isolate) reacted with MCA-13 and all broad spectrum MAbs except 4F3, it didn't react also with any of the differential MAbs; Sgr.5 (8 isolates) reacted to all MAbs except MCA-13; Sgr.6 (9 isolates) reacted only with the broad spectrum MAbs; Sgr.7 (4 isolates) had a negative reaction with MAC-13, 4B1 and 4F3 MAbs; Finally Sgr.8 (a single isolate) which reacted only to 4 broad spectrum MAbs (17G11, 4C1, 4E5 and 4F3).

The broad spectrum MAbs: 11G17, 4C1, and 4E5 were able to react with all CTV isolates tested, meaning that they target conserved epitopes. The MAb 1D12 which is also considered of broad spectrum did not react with 2 Italian CTV isolates of serogroup 8. MAb 4F3 of broad spectrum pattern did not react with 5 isolates. MCA-13 MAb exhibited opposite reaction patterns with all the other MAbs against 4 serogroups each. Opposite reaction pattern of MCA-13 and 4D3 has already been noticed in our previous work (unpublished data). The MAbs 4B1 and 4D3 were the most discriminating, confirming the result reported by Zemzami *et al.* (1999). MCA-13 (Permar *et al.*, 1990) described in Florida as specific for severe and CTV decline-inducing isolates reacted with 66% of the tested CTV isolates from various countries, indicating that some Mediterranean CTV isolates could be of severe types. Although positive reaction with MCA-13 is not necessarily correlated with the decline of trees in the Mediterranean Basin, it is an indication of potential aggressiveness of an isolate. Interestingly, all the tested CTV sources belonging to the following countries Croatia, Cyprus, Egypt, Lebanon, Montenegro, Morocco, Palestine and Syria gave a clear-cut positive reaction with the MCA-13 MAb, even if the number of the tested CTV sources was not always significantly large.

These results provide an initial evidence of the serological diversity of CTV isolates present throughout the Mediterranean region. They also confirm the considerable antigenic diversity existing in CTV isolates which was reported in other studies (Permar *et al.,* 1990). The fact that more than 50% of CTV isolates reacted with all MAbs could reflect the presence of multiple CTV strain mixtures in the collected individual CTV sources as already reported (Rubio *et al.,* 2001). They indicate also that broad spectrum MAbs are reliable for universal detection of Mediterranean CTV isolates. They denote clearly that potential severe CTV strains are widely distributed, thus providing favourable grounds for CTV outbreaks, if or when the brown citrus aphid "*T. citricidus*" spreads throughout the region.

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