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Comparative trials for the detection of CTV by antisera from different sources

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SUMMARY – Four types of commercial antisera to citrus tristeza virus (CTV), two of which were monoclonal and two polyclonal, were compared in enzyme–linked immunosorbent assay (ELISA) tests against different CTV isolates from four Mediterranean countries: Italy, Turkey, Morocco and Cyprus. With the exception of one monoclonal antiserum, which failed to recognize a few virus isolates from Turkey and Cyprus, the four antisera were effective in CTV diagnosis which implies that the ELISA procedure can be employed for routine testing in certification programmes.

Key words: citrus, tristeza, closterovirus, ELISA, diagnosis, Mediterranean countries

RESUME *- Quatre types d'antisérums commerciaux contre le virus de la tristeza des agrumes (CTV), dont deux monoclonaux et deux polyclonaux, ont été comparés en ELISA contre différents isolats de CTV provenant de quatre pays Méditerranéens: Italie, Turquie, Maroc et Chypre. A l'exception d'un antisérum monoclonal, qui n'a pas réussi à reconnaître certains isolats viraux de Turquie et de Chypre, les quatre antisérums se sont avérés efficaces dans le diagnostic du CTV, ce qui démontre que le test ELISA pourrait remplacer l'indexage biologique dans les programmes de certification des agrumes.*

Mots-clés: *agrumes, tristeza, closterovirus, ELISA, diagnostic, Pays Méditerranéens.*

Introduction

Citrus tristeza virus (CTV), a closterovirus transmitted in nature by aphids, is considered to be responsible for one of the most severe virus diseases of citrus, which has caused the death of millions of trees on sour orange (*Citrus aurantium*) rootstock in several Latin and North American countries, including Brazil, Argentina, California, Florida.

The disease has also affected citrus in the Mediterranean area including Spain (Moreno *et al.*, 1988), Israel (Raccah *et al.*, 1976), Egypt (Nour-Eldin and Bishay, 1958), Turkey (Norman, 1963) and Cyprus (Kyriakou *et al.*, 1993). Isolates of CTV were also reported from Morocco and Italy (Bové, 1995; Davino *et al.* 1983; A.M. D'Onghia and V. Savino, unpublished information).

The presence of CTV in the Mediterranean area in combination with the use of sour orange, the most sensitive rootstock to the disease, and the existence of *Aphis gossypii*, a vector of the virus, are a serious threat to citrus industry in this area. There is a large variation in CTV strains with regard to severity of symptoms induced. Infections may range from symptomless to severe causing decline and death of the plants. The seedling yellows strain, which is considered as the most severe has been found recently in Israel (Bar-Joseph and Nitzan, 1991) and Spain (Ballester *et al.*, 1988).

Several techniques have been employed for the diagnosis of CTV, including biological indexing through the use of indicator plants, basically Mexican lime (*C. aurantifolia*), the serological method ELISA, molecular hybridization, polymerase chain reaction (PCR) and electron microscopy (Roistacher, 1991). However, biological indexing and ELISA are the methods which have been most commonly applied on a routine basis for CTV detection in citrus certification programmes (Bar-Joseph *et al.*, 1979).

Several commercial antisera to CTV, either polyclonal or monoclonal, are being used in different laboratories for diagnosis of the virus. However, the effectiveness and reliability of each of the above antisera in detecting different Mediterranean isolates are not well known. Within the context of the activities of the Mediterranean Network on Certification of Citrus (MNCC), four sources of antisera, two of which were polyclonal and two monoclonal, were compared for their effectiveness in the diagnosis of several local CTV isolates in four Mediterranean laboratories.

Materials and methods

Samples of four commercial antisera produced in Switzerland, France, Spain and Morocco, respectively, were tested almost concurrently in the Plant Pathology laboratories of Bari (Italy), Adana (Turkey), Rabat (Morocco) and Nicosia (Cyprus), against different isolates of CTV using ELISA (Table I). The antisera which came from the commercial companies of Switzerland and France were polyclonal, whereas the Spanish and Moroccan antisera were monoclonal. The ELISA procedure in all four laboratories for each set of antisera was that suggested by the respective manufacturer.

Table I - CTV infected isolates tested in ELISA

Laboratory	Total samples	Code
Department of Plant Protection Cukurova Üniversity, Adana-TURKEY	**16**	
- Izmir		H1, H3, H4
- Mersin		T13, T12, T21
- Kozan		KTR1, KTR4
- Dörtyol		KAM3, KAM4, KAM5
- Kadirli		42A, 42B
- Sera		SER24, SER19, SER36
Agricultural Research Institute, Nicosia-CYPRUS	**12**	CY1-12
Istituto Agronomico Mediterraneo, Bari-ITALY	**5**	T4, LG1, LG2, AP1, AP2
Direction de la Protection des Végétaux, des Controles Téchniques et de la Répression des Fraudes, Rabat-MOROCCO	**12**	M1-12

Samples were prepared using cortical scrapings from cuttings of citrus plants, known to be CTV-infected, collected in the field or from the greenhouse. Different sources of negative controls were used in each laboratory.

Tissues were ground with extraction buffer (dilution 1:10 w/v) and two wells were filled with plant sap of each isolate.

Results were evaluated by comparing the mean absorbance values at 405 nm after the addition of the alkaline phosphatase substrate. Samples were considered positive when their mean value was at least twice as high as that of the healthy or CTV-negative control.

Results and conclusion

All four antisera were effective in the detection of most CTV isolates tested. The only exception were two out of 16 isolates from Turkey (Ser 36 and Kam 5) and one out 12 isolates from Cyprus (CY-3) which were not detected by the monoclonal antisera from Morocco (Figure I). Most of the isolates reacted weakly with the monoclonal antiserum from Spain. The performance of the four antisera in different laboratories is presented in Figure II. In Bari and Rabat the best results were obtained with the monoclonal antiserum from Morocco, whereas in Adana and Nicosia the French polyclonal antiserum gave the highest absorbance values at 405 nm. In general, both polyclonal antisera appeared very effective in the diagnosis of all CTV isolates. However, the high specificity and the consistency

in quality of monoclonal antibodies are advantages which cannot be ignored. A parallel use of a monoclonal and a polyclonal antiserum may be considered for routine testing of citrus material in certification programmes.

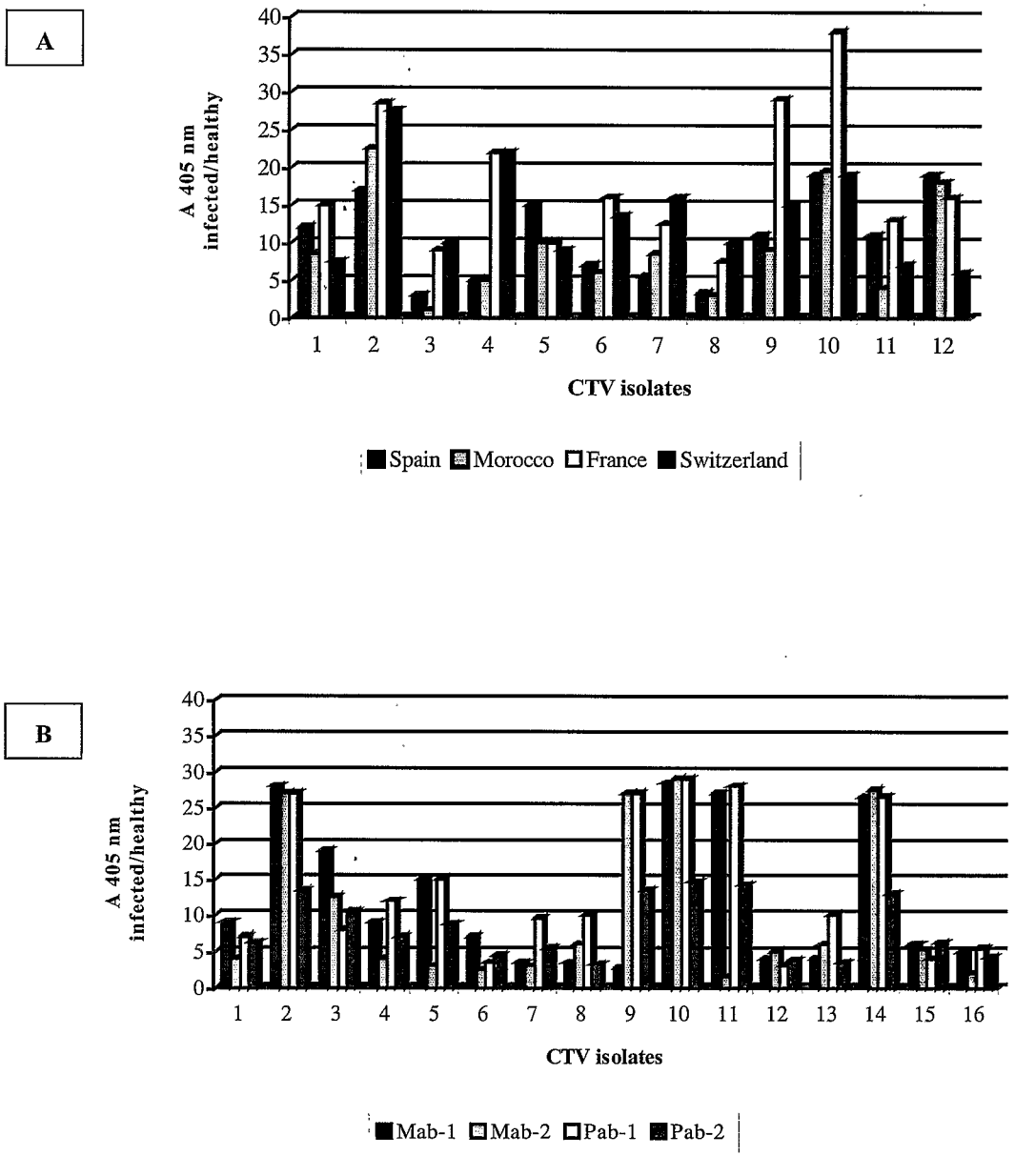

Figure I – Performances of four commercial antisera against several CTV isolates from Cyprus (**A**) and Turkey (**B**) in comparative ELISA tests.

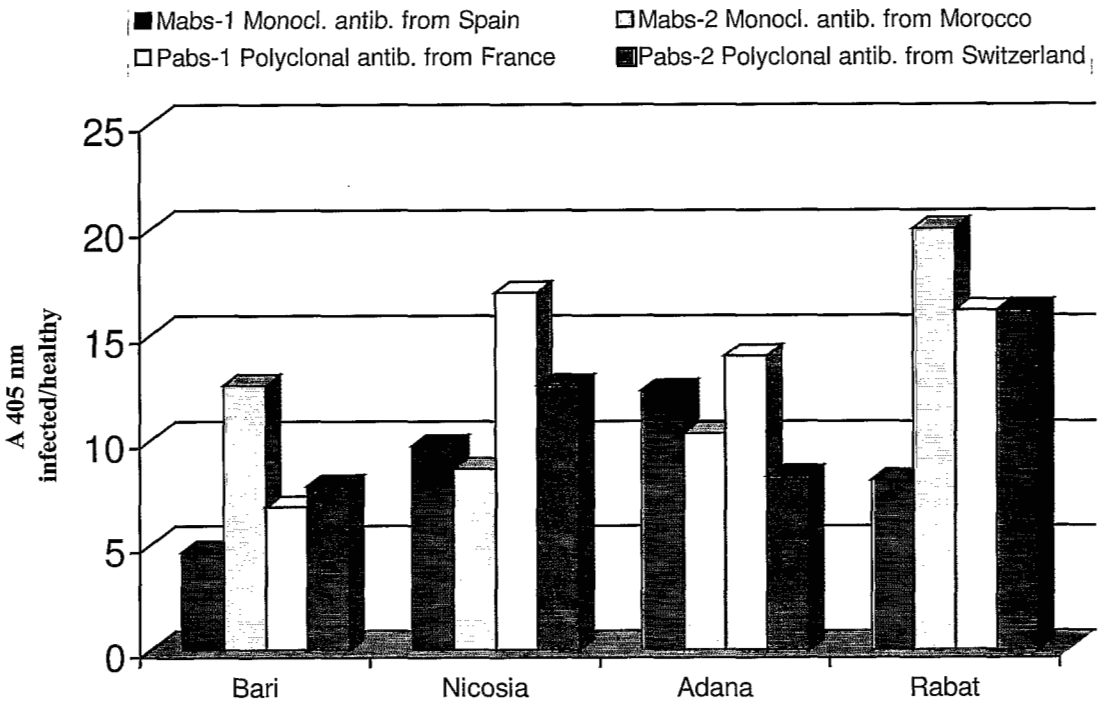

Figure II – Comparative ELISA performances of four commercial antisera for CTV detection in four Mediterranean laboratories

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