Evaluation of Moroccan monoclonal antibodies for the detection of Italian sources of Citrus infection variegation virus (CVV)

Djelouah K., D'Onghia A.M.

in

D'Onghia A.M. (ed.), Djelouah K. (ed.), Roistacher C.N. (ed.).
Proceedings of the Mediterranean research network on certification of citrus (MNCC): 1998-2001

Bari : CIHEAM
Options Méditerranéennes : Série B. Etudes et Recherches; n. 43

2002
pages 71-73

EVALUATION OF MOROCCAN MONOCLONAL ANTIBODIES FOR THE DETECTION OF ITALIAN SOURCES OF CITRUS INFECTION VARIEGATION VIRUS (CVV)

K. Djelouah, A.M. D'Onghia
CIHEAM/Mediterranean Agronomic Institute
Valenzano, Bari - Italy

SUMMARY - Italian citrus sources showing infectious variegation-like symptoms in the field and in the woody indicator plants were assayed by serological means, using Monoclonal antibodies (Mabs) from Morocco and the homologous isolate. All tested sources were ELISA-positive using the commercial kit, whereas differences occurred in the assays with the other Moroccan tested Mabs. One Italian source showed absorbance values as high as the homologous.

Key words: Citrus, infectious variegation virus, diagnosis, ELISA, Italy.

RESUME - *Des sources agrumicoles italiennes montrant des symptômes de type panachure infectieuse en plein champ et sur indicateurs ligneux ont été testées par les moyens sérologiques, ceci, en utilisant des anticorps monoclonaux d'origine marocaine et l'isolat homologue. Toutes les sources ont réagi positivement à l'ELISA en utilisant le kit commercial alors que des différences ont été mises en évidence avec les autres anticorps monoclonaux marocains testés. Une source italienne a montré des valeurs d'absorbance supérieures à celles obtenues sur l'homologue.*

Mots-clés*: Agrumes, virus de la panachure infectieuse, diagnostic, ELISA, Italie.*

INTRODUCTION

Citrus infectious variegation, the presumed first experimentally-transmitted virus of citrus (Trabut, 1913) and crinkly leaf have been considered syndromes of the same disease (Majorana and Martelli, 1968; Roistacher, 1991). Infectious variegation shows typical leaf variegation or chlorotic specks, often with leaf epinasty, waved and irregular margin (Fawcett and Klotz, 1939; Roistacher, 1991), while crinkly leaf is characterized by distortion and puckering of the leaves.

Detection is mainly based on graft-transmission to woody indicators such as citron and lemon, at 24-27°C under greenhouse conditions (Roistacher, 1991). Polyclonal antibodies against CVV were raised by Davino and Garnsey (1984) and ELISA was successfully used in the virus detection (Davino and La Rosa, 1984; Davino *et al*., 1988).

In this study, serological assays were conducted in five Italian CVV sources, in the framework of the activities of the CIHEAM/Mediterranean Research Network on Certification of Citrus (MNCC). The ring tests programme was aimed at evaluating the performance of the CVV ELISA kit from Morocco, which is the only kit available in the market at evaluation.

In the same study a serological characterization of the five Italian CVV sources was also conducted using other CVV Mabs from Morocco raised against the same CVV homologous source.

MATERIALS AND METHODS

The five Italian CVV accessions (Table 1), belonging to 3 species and previously indexed by graft-transmissions, are maintained in the IAMB collection. They were serologically assayed in comparison with the Moroccan CVV isolate used to raise the tested antisera.

Table 1. Italian CVV-infected citrus genotypes

Symbols	CVV infected genotypes
IAMB 58X	Duretta sanguine orange
IAMB 60X	Gargano lemon
IAMB 225X	Lemon
IAMB 522X	Seedless lemon
Palermo 1	Pomelo
Morocco 1	Orange

TAS ELISA was processed according to the protocol of Cambra *et al.,* (1995), using the commercial Kit for CVV detection (Domaines Agricoles UCP, Morocco) and 4 CVV monoclonal antibodies, kindly offered by Dr. Zemzami, UCP, Morocco.

Coating was carried out with a polyclonal antiserum at a concentration of 1:1000, after washing the plates 3 times using PBS Tween and draining by blotting on paper. Samples (leaf tissues) were added at a 1:10 dilution using the extraction buffer**.** The plates were incubated overnight. The day after, the washing procedure was repeated and a CVV monoclonal antibody (Mab) diluted in the conjugate buffer (1:500) was added and incubated for 2 h at 37°C. The CVV Mabs were used at 1/50 for the supernatant and 1/3000 for ascetic fluid .

After washing, antimouse diluted in a 1:1000 conjugate buffer was added and plates were incubated for 2 h at 37°C.

Washing was repeated and the plates were loaded with the substrate (P-nitrophenyl phosphate) in a substrate buffer freshly prepared (1mg/ml). Incubation took place at room temperature until the yellow colour developed. Absorbance readings were done at 405nm after 15 min., 30 min., and 60 min., using a Titertek Multiskan plus MKII reader.

RESULTS

As shown in Table 2, all sources positively reacted with the ELISA kit, although the highest absorbance values, compared to the homologous isolate (Morocco 1), were given by Palermo 1 and the weakest were from source IAMB 60X.

As for the tested Moroccan Mabs, some sources (IAMB 225X, Palermo1) were ELISA-positive with all Mabs, whereas others (IAMB 58X,IAMB 522X) were not recognized by Mab 3G4. On the contrary, source IAMB 60X induced a reaction not only with the kit but also with Mab 3G4.

Table 2. Results of ELISA readings using 4 monoclonal antibodies and the commercial Kit from Morocco

Source	Species	Absorbance value (OD) Positive/Negative				
		ELISA Kit	AE/1	CF1/2	3G4	CF1/5
58X	Duretta orange	5	3	3	-	4
60X	Gargano lemon	2.5-3	-	-	3.5	-
225X	Lemon	4	2.5-3	2.5-3	2.5-3	2.5-3
522X	Seedless lemon	4	4	2.5-3	-	3
Palermo 1	Pomelo	6	12	4	10	5
Morocco 1	Orange	5	12	10	8	10

Negative: -

CONCLUSION

The Moroccan ELISA kit successfully performed with all Italian CVV sources, highly correlating with biological indexing. Its use for large scale surveys is of utmost importance. It should also be strongly recommended in the framework of a certification programmes as a preliminary and complementary assay of biological indexing by graft and mechanical transmissions.

Relatively to the serological characterization, the highest absorbance values were induced by the source 'Palermo 1' and the homologous. The serological result correlates with the severity of this Italian isolate which induces strong leaf yellowing and crinkling in the field trees and in the woody indicator plants. In the case of CVV source 60X, the weakest ELISA readings did not correlate with field symptomatology and results of biological indexing because of their severity. Beside the weakest reaction showed by this source, a very low serological affinity compared to the others was observed. It could be presumed that it is not a true CVV source but probably another ilarvirus serologically correlated with CVV.

Based on these preliminary results, further investigations should follow for a complete characterization of these sources by other means.

REFERENCES

Cambra M., M. Assensio, M. T. Gorris, E. Perez, E. Camarasa, J.A. Garcia, J.J. Moya, D. Lopez Abella, C. Vela and A. Sanz, 1995. Detection of plum pox Potyvirus using monoclonal antibodies to structural proteins. *EPPO Bulletin*, 24: 569-577.

Davino M. and S.M. Garnsey, 1984. Purification, characterization, and serology of a mild strain of citrus variegation virus from Florida. *Proc. 9th Conf. IOCV,* Argentina 1982, University Riverside (CA) Eds: 196-203.

Davino N. and R. La Rosa, 1984. Indagini sulla diffusione del virus della variegatura infettiva degli agrumi in Italia. Atti delle *Giornate Fitopatologiche:* 257-264.

Davino M., R. Areddia and S.M. Garnsey, 1988. Distribution of citrus variegation virus within citrus hosts. *Proc.10th Conf. IOCV*, Spain 1986, University Riverside (CA) Eds: 322-326.

Fawcett H.S. and L.J. Klotz, 1939. Infectious variegation of citrus. *Phytopathology* 29: 911-912.

Majorana G. and G.P. Martelli, 1968. Comparison of citrus infectious variegation and citrus crinkly-leaf virus isolates from Italy and California*. Proc. 4th Conf. IOCV*, Italy 1966: 273-280.

Roistacher C.N., 1991. Graft-transmissible diseases of citrus. Handbook for detection and diagnosis. *FAO, Rome Eds.*: 286 p.

Trabut M., 1913. A note. In*: Comptes-rendus de l'Académie des sciences, Paris,* 156*:* 243-244*.*