Evaluation of two monoclonal antibodies specific to Citrus infectious variegation virus (CVV)

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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 57-59

CITRUS PSOROSIS VIRUS (CPsV), CITRUS INFECTIOUS VARIEGATION VIRUS (CVV) AND OTHER GRAFT-TRASMISSIBLE PATHOGENS

EVALUATION OF TWO MONOCLONAL ANTIBODIES SPECIFIC TO CITRUS INFECTIOUS VARIEGATION VIRUS (CVV)

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SUMMARY - An attempt to evaluate the performance of CVV Mabs by DASI ELISA using six Italian lemon sources showing leaf variegation-like symptoms was carried out in Marocco at the UCP, Rabat where the tested Mabs were produced. Mab (CF 1-1) was much better than Mab (AE 1-2) for its higher IgM specificity or for its higher affinity and or avidity. Five of the six tested samples were CVV-positive, whereas sample S5 was probably from a non CVV-infected tree or the virus titre was below the detection threshold.

Key words: Citrus, citrus infectious variegation virus, ELISA, Monoclonal antibodies.

RESUME - *L'évaluation de la performance des anticorps monoclonaux (Mabs) dirigés contre le CVV et produits par l'UCP de Rabat (Maroc), a été effectuée au sein même de l'UCP, ceci en réalisant le test DASI ELISA sur six sources de citronnier, d'origine italienne, qui montraient des symptômes du type panachure foliaire. Le Mab (CF 1-1) s'est avéré de loin plus performant que le Mab (AE 1-2) en raison de sa plus grande spécificité pour les IgM ou de son affinité et/ou avidité plus élevée. Cinq des six échantillons testés étaient positifs au CVV, alors que l'échantillon S5 avait probablement été prélevé d'un arbre non infecté par le CVV ou bien le titre viral était inférieur au seuil de détection.*

Mots-clés*: Agrumes, virus de la panachure infectieuse des agrumes, ELISA, anticorps monoclonaux.*

INTRODUCTION

Citrus infectious variegation virus (CVV) has been found in several citrus-growing areas in Florida; however, it is not usually widespread nor a major problem. It was probably the first citrus virus transmitted mechanically from citrus to citrus and to herbaceous hosts (Trabut, 1913).

Apart from its presence in the United States, the disease has also been reported from many countries in the Mediterranean basin and from Australia. In Morocco, the virus has been identified on lemon trees in the Larache and Rabat (Nhami, personal communication) and in the vicinity of Rabat (Zemzami, unpublished data).

CVV can infect most citrus species, cultivars, and other non-citrus hosts. Lemons, sour orange, Etrog citron and grapefruit usually develop chlorotic leaf symptoms and distortions, which persist in mature foliage (Roistacher, 1993).

CVV is a typical ilarvirus with four nucleoprotein components, whose spherical particles vary in size from 26 to 32 nm.

Different techniques are used to identify the virus in the field, in the laboratory or in the greenhouse:
- field diagnosis by identification of symptoms;
- indexing by graft transmission to citrus using Etrog citron, lemon, Dweet tangor, mandarin or sour orange;
- indexing by mechanical transmission to herbaceous hosts such as Cowpea or common bean;

- serological diagnosis using ELISA;
- molecular detection by RT-PCR.

This paper describes the ELISA technique developed for CVV detection, using polyclonal antiserum for trapping the virus and a specific monoclonal antibodies for detection (UCP-Rabat).

MATERIALS AND METHODS

Six Italian samples of 'Femminello' lemons (S1, S2, S3, S4, S5 and S6) showing leaf variegation like symptoms were sent from IAM-Bari as dry tissues obtained from young leaves. S3 and S4 were brownish and seemed not well conserved.

Samples were ground with Roller Grinder ME 2000 in phosphate buffer (1/5 w/v). Extracts were left for 2 hours in a refrigerator to allow decanting of debris, the supernatant was carefully collected and used for test.

Positive and negative controls used in the study were collected as fresh tissues from plants maintained under an insect-proof screenhouse in the indexing facility.

Two monoclonal antibodies specific to CVV were used in this study: CF1-1 and AE1-2, both of IgM class, produced at the Hybridome facility of Direction des Domaines Agricoles Royaux (unpublished data) against the isolate CVV-1 (a local CVV isolate which induces variegation-like symptoms on indicators plants).

ELISA test was done according to the DASI procedure described by Cambra *et al*. (1995). ELISA plates “Nunc-Immuno Plate” were coated with a polyclonal antiserum raised in rabbit against CVV (unpublished data). After washing, sample extracts were added and the plates incubated overnight at 4°C. After washing, Mab's were added at various concentrations as shown in Fig. 1 and Fig. 2.

Mab's were detected by addition of a anti-mouse (the whole molecule), conjugated with alkaline phosphatase (Sigma) at the recommended concentration.

After addition of alkaline phosphatase substrate, the optical absorbance was measured at 405 nm using *Titertek Multiscan* reader. The reaction was stopped by adding 50 microliters of 3M NaOH after one hour's incubation in the dark at room temperature.

RESULTS AND CONCLUSION

Optical densities OD (405nm) readings obtained for each Mab are reported in Fig. 1 and Fig. 2

Mab (AE1-2) showed a high background reaction which remained significantly high at 200 ng/well. It could be concluded that samples S1, S2, S3 and S4 are positive, while S6 is doubtful and S5 negative.

Mab (CF1-1) has a lower background reaction than the previous Mab. It reacted more strongly even at the lowest concentration tested with this Mab, samples S1, S2, S3, S4 and S6 are positive, while S5 remains negative.

The results obtained indicate clearly that Mab (CF 1-1) is a much better performer than Mab (AE 1-2) for the detection of CVV. It is possible that the IgM could be the reason for its higher specificity.

However, there could also be other reasons such as higher affinity and or avidity. We conclude that samples S1, S2, S3, S4 and S6 come from trees infected with CVV, whereas S5 was collected from a non CVV-infected tree or that the virus titer was below the detection threshold.

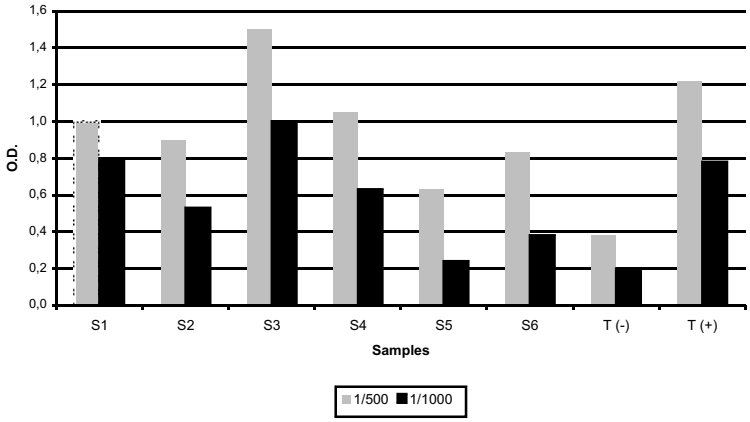

Fig. 1. Optical sensitivities of CVV isolates to Mab (AE1-2) at two concentrations

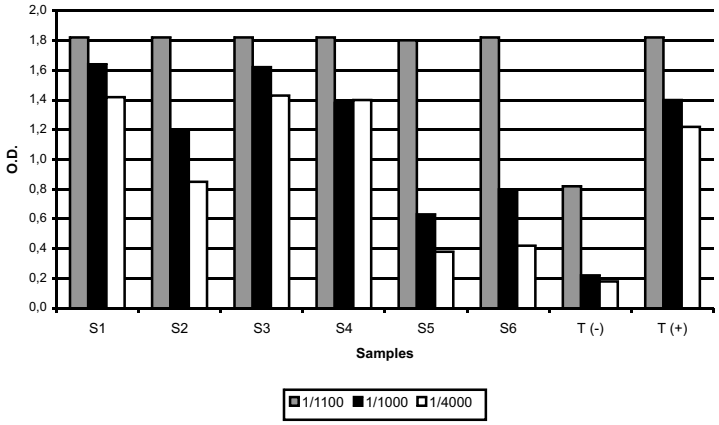

Fig. 2. Optical sensitivities of CVV isolates to Mab (CF1-1) at three concentrations

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