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THE DETECTION OF CITRUS TRISTEZA VIRUS BY ENZYME-LINKED IMMUNOSORBENT ASSAY (*)

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SUMMARY : *Citrus tristeza virus (CTV) in extract from citrus tissues was detected by the double antibody sandwich ELISA (DAS-ELISA) with polyclonal IgG 1053 antibody. Partially purified gamma-globulin (IgG) from antiserum to purified CTV was conjugate with alkaline phosphate enzyme.*

TURUNÇGİL TRISTEZA VİRUSUNUN ELİSA (ENZYME-LINKED IMMUNOSORBENT) TEKNİĞİ İLE SAPTANMASI

ÖZET : *Turunçgil trizteza virusu, turunçgil dokularından elde edilen ekstrakstlarda polyclonal IgG 1053 antibody kullanılarak DAS-ELISA (double antibody sandwich ELISA) yöntemi ile saptanmıştır. CTV antiserumundan kısmen purifiye edilerek elde edilen gamma-globulin (IgG) alkalin fosfat enzimi ile konjuge edilmiştir.*

INTRODUCTION

An enzyme-linked immunosorbent assay (ELISA) method, which is based on the double antibody sandwich technique used by Voller et al. (1976) and Clark and Adams (1977), has been shown to be an inexpensive, reliable, and sensitive assay for several plant viruses. In contrast with the detection of many other plant viruses, conventional serological techniques were introduced only recently for detection of CTV. CTV had been especially difficult to work on serologically because of lack of specific antisera and presence in low concentration in woody systemic hosts. In the recent years, the virus has been purified (Lee et al., 1987) and antisera produced for development of serology-based sodium dodecyl sulfate (SDS) - immunodiffusion (Gonsalves et al., 1978), enzyme-linked immunosorbent assays (ELISA) (Bar-Joseph et al., 1979) and

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radioimmunosorbert assays (Lee et al., 1981). After these, serological tests for CTV have been developed and ELISA have been used widely for survey, centrification and eradication work as well as for research (Cambra et al., 1982, Garnsey et al., 1981). The polyclonal and monoclonal antibodies have been produced and they have reacted to a wide range of CTV isolates of differing symptom severity (Gonsalves et a., 1978, Vela et al., 1986).

The double antibody sandwich method (DAS-ELISA) has been most commonly used for CTV detection (Bar-Joseph et al., 1979). ELISA procedures can provide rapid, sensitive, and economical detection of CTV in crude extracts. Rapidity of identification is especially desirable for eradication attempts, because infected trees cannot be identified and removed before additional natural spread occurs.

Roche-Pena et al. (1989) reported that CTV could be detected by DAS-ELISA, using polyclonal atibody. Baloğlu and Yılmaz (1988) reported that antisera to CTV had been produced by density-gradient centrifugation in CsCl. They tested 112 citrus trees by ELISA for the detection of CTV in Mediterranean region of Turkey.

The purpose of this study is to confirm that CTV can be detected by DAS-ELISA, using polyclonal antisera 1053.

MATERIAL AND METHOD

Sample preparation

Citrus tristesa virus (CTV) infected 52 citrus samples collected from Belsville (Maryland, USA) were used in this study. One mild T-26 (Florida CTV isolate) was included as positive control. Healthy plants of *Citrus excelsa* and 0.05M Tris were used for negative controls.

The leaf midribs and the young bark tissue samples were stripped, chopped, and homogenized, using a tissuemixer at a 1:20 dilution in PBS-Tween PVP 0.02M phospate, 0.15 NaCI, 0.02 % sodium azide pH 7.4 containing 0.05 % Tween 20 and 2 % polyvinyl pyrrolidone)

DAS-ELISA

The double antibody sandwich ELISA procedure as described by Garnsey et al. (1981) for CTV was used. Polyclonal antisera 1053 which prepared to T-26 CTV isolate was obtained by Dr. R.F.Lee. The immunoglobulin (IgG) fraction was collected using a protein-A affinity column (Miller and Stone, 1978). Alkaline phosphatase conjugated IgG was prepared according to the techniques of Clark and Adams (1977).

The tests were done in Immulon 11 microtiter plates (Dynatech laboratories) in two replications for per sample. Two hundred microliters per well were used throughout the tests. Three washings with PBS-Tween were applied between every step.

The plants were coated with 2 µg/ml of IgG from polyclonal antisera 1053 in carbonate buffer for 6 hr at 37 °C. After the plant samples had been added, plates were incubated overnight at 5 °C. IgG conjugated with alkaline phosphatase in the presence of gluteraldehyde was used at a 1:1000 dilution in PBS-Tween-PVP with 0.02 % ovalbumin. After enzyme-conjugated IgG was incubated with homogenate of healthy plants for 1 hr at 37 °C, the plates were covered with enzyme conjugated IgG, and incubated for 4 hr at 37°C, P-nitrophenyl phosphate (sigma) substrate, used at 1 mg/ml, in 10 % diethanolamine was incubated in each well for 1-2 hr at room temparature. The optical density at 405 nm (OD 405) was determined at 30. and 60 minutes intervals using a Bio-Tek EL 307 ELISA plate spectrophotometer. Samples with readings 0.10 above that of the healthy control were considered positive. A histogram from ELISA results was constructed according to Gillett et al. (1986).

RESULTS AND DISCUSSION

Histogram of DAS-ELISA results for citrus tristesa virus (CTV) in crude extract from citrus plants was shown (Fig 1). The DAS-ELISA by using the 1053 polyclonal antibody gave positive reaction with 36 plant samples, and negative reaction with 17 plant samples (Fig 1). OD 405 values for positive samples were in the range of 0.14 and

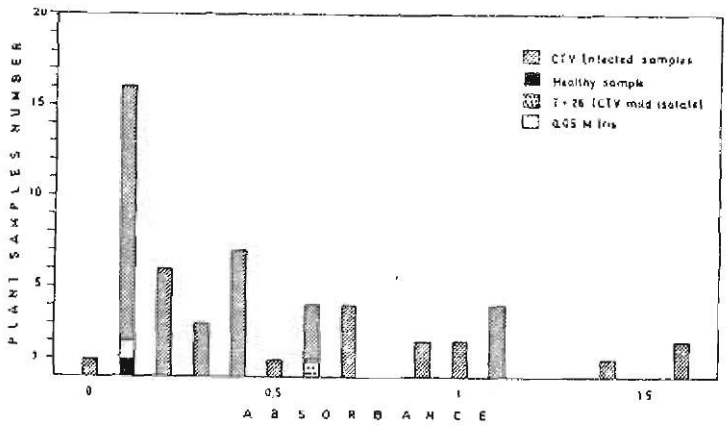

Fig. 1. Histogram of DAS-ELISA results for citrus triztesa virus (CTV) in crude extract from citrus plants.

1.57. Healthy controls showed consistently values between 0.010 and 0.072. The CTV isolate T-26 as mild control for the 1053 polyclonal antibody gave consistently OD 405 values 0.55.

After two hours of substrate reaction., the OD 405 values of healthy controls were never higher than 0.072. These low background valucs were achieved by cross absorbing routinely the enzyme conjugate with healthy sap prior to their use.

The results of this work provide evidence of the usefulness of the 1053 polyclonal antibody for CTV detection. DAS-ELISA with polyclonal polyspesific antisera has previously been used to evaluate virus titer on citrus plants (Gansey et al., 1985). A high titer in a plant infected with a mild CTV isolate may be a relative estimate of the protecting ability of mild CTV isolates in cross protection experiments (Lee et al., 1987). However, polyclonal antibodies are polyspesific and do not distinguish between severe and mild CTV isolates. Permar et al. (1990) developed MCA-13 monoclonal antibody capable of differantiating between severe and mild strains from Florida using ELISA.

DAS-ELISA with 1053 polyclonal antibody can be used for CTV detection. It is especially food where large number of samples must be assayed and, where suitable indicator plants and glasshouse facilities are not available.

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