Citrus Tatter Leaf Virus: A Review of its Properties and the Development of a Serological Detection System

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ABSTRACT. Citrus tatter leaf virus (CTLV) is a Capillovirus serologically related to the type species, apple stem grooving virus. It causes a budunion abnormality of trees on trifoliate rootstock, the main rootstock used in Japan. Research on CTLV in Japan has, therefore, been important to characterize the virus and develop suitable detection techniques. ELISA tests using both polyclonal and monoclonal antibodies have been developed which can be used reliably for indexing for CTLV.

Citrus tatter leaf virus (CTLV), the causal agent of a budunion abnormality in citrus trees on trifoliate orange rootstock, is one of the most important citrus viruses in Japan because trifoliate orange is generally used as a rootstock (7). CTLV has frequently been detected in many citrus varieties imported from China during post-entry quarantine in Japan (9). It was also found in naturally-infected lily (3). Therefore, for a number of years, research in Japan has been directed towards the characterization of CTLV, and the development of reliable detection assays for it.

For virus isolation, isolate K-1, which was obtained from Dahongtiancheng sweet orange (8), was inoculated onto *Chenopodium quinoa*. The virus was partially purified from *C. quinoa* leaves by differential centrifugation, PEG precipitation and sucrose gradient centrifugation, and then purified (10) by molecular permeation chromatography on controlled glass beads (1). Electron microscopic observations revealed flexuous particles 600-650 nm in length and 13 nm wide, with conspicuous cris-cross patterns when samples were stained with uranyl acetate and viewed with an electron microscope (10), similar to that reported for potato virus T (12). SDS-PAGE analysis of the coat protein revealed a single band of M_r 27,000 Da. The virus was shown to possess a single species of RNA of M_r 2.83×10^6. Similar results were obtained when apple stem grooving virus (ASGV), the type member of the capilloviruses, was subjected to the same treatments. Serological studies also indicated that the two were closely related (10, 13).

More recently, the complete nucleotide sequence of CTLV-RNA has been determined (11). It is 6,496 nucleotides long excluding the 3'-terminal poly (A) tract, and contains two putative overlapping open reading frames. The genome structure is homologous to that of ASGV.

Polyclonal antibodies (PAbs) to CTLV were raised in rabbits and used in ELISA (PAb-ELISA) tests (4). In PAb-ELISA, use of the modified procedure (i.e. mixing the sample and conjugate in the same well of the microplate) and horseradish peroxidase for conjugated enzyme obtained sufficient sensitivity for detection from citrus leaves (5).

Monoclonal antibodies (MAbs) were produced by using BALB/c mice immunized with purified CTLV K-1 (6). Hybridomas were screened and cloned as described in (2). Three hybridomas were selected for this study. The immunoglobulin isotype of the MAbs belonged to the class IgG1. Alkaline phosphatase (AP) conjugates of the three MAbs were obtained by the standard method.

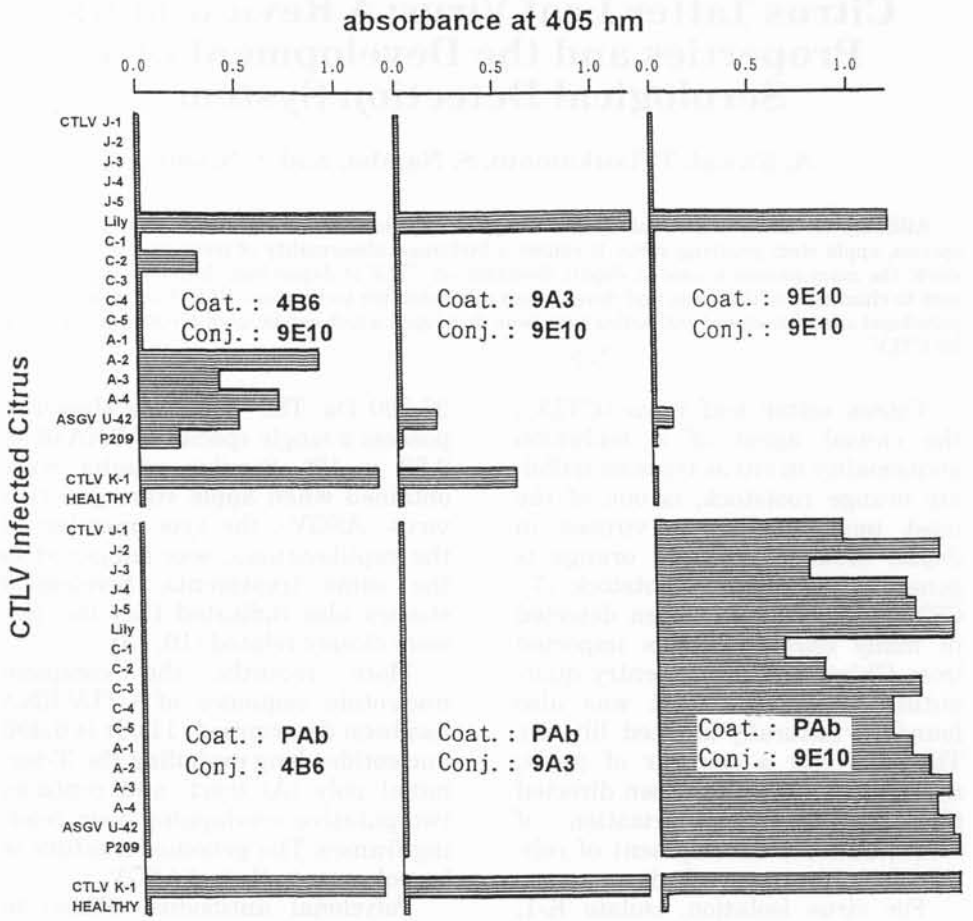

Fig. 1. Reactions of three MAbs in ELISA against virus isolates. Reactions of the three MAbs and the PAb used as coating antibodies and as conjugates assayed against the virus isolates. The antigens were prepared from infected *C. quinoa* leaves homogenized in PBS-T- PVP (0.02 M phosphate-buffered saline with 0.05% Tween-20 and 2% PVP-40). The isolates CTLV J-1 to -5 are Japanese isolates from citrus trees and 'Lily' is the isolate from a trumpet lily in Japan. Isolates C-1 to -5 were from citrus varieties imported from China. Isolates of A-1 to -5 were from United States of America by courtesy of Dr. S. Garnsey. ASGV U-42 was isolated from Japanese apricot (13) and P-209 was obtained by courtesy of Dr. H. Yanase.

The MAbs and the PAb were applied individually to microplates for coating with antibody, and the three conjugates prepared from three different MAbs were assayed using the standard ELISA test against 16 isolates of CTLV and two isolates of ASGV, all from infected *C. quinoa* leaves. When MAbs were used as both coating and conjugate, only a few isolates, including the homologous K-1, were detected. When the PAb was used for coating, all isolates were detected by ELISA with MAb 9E10 conjugate. In the case of the other MAb conjugates, only the homologous isolate was detected (Fig. 1). Twenty-five citrus trees infected with CTLV, including K-1, and five healthy trees were indexed by ELISA using PAb for coating antibody and MAb 9E10 AP-conjugate. The same samples were assayed by the sap inoculation test. All infected samples showed a positive reaction in the ELISA (Fig. 2);

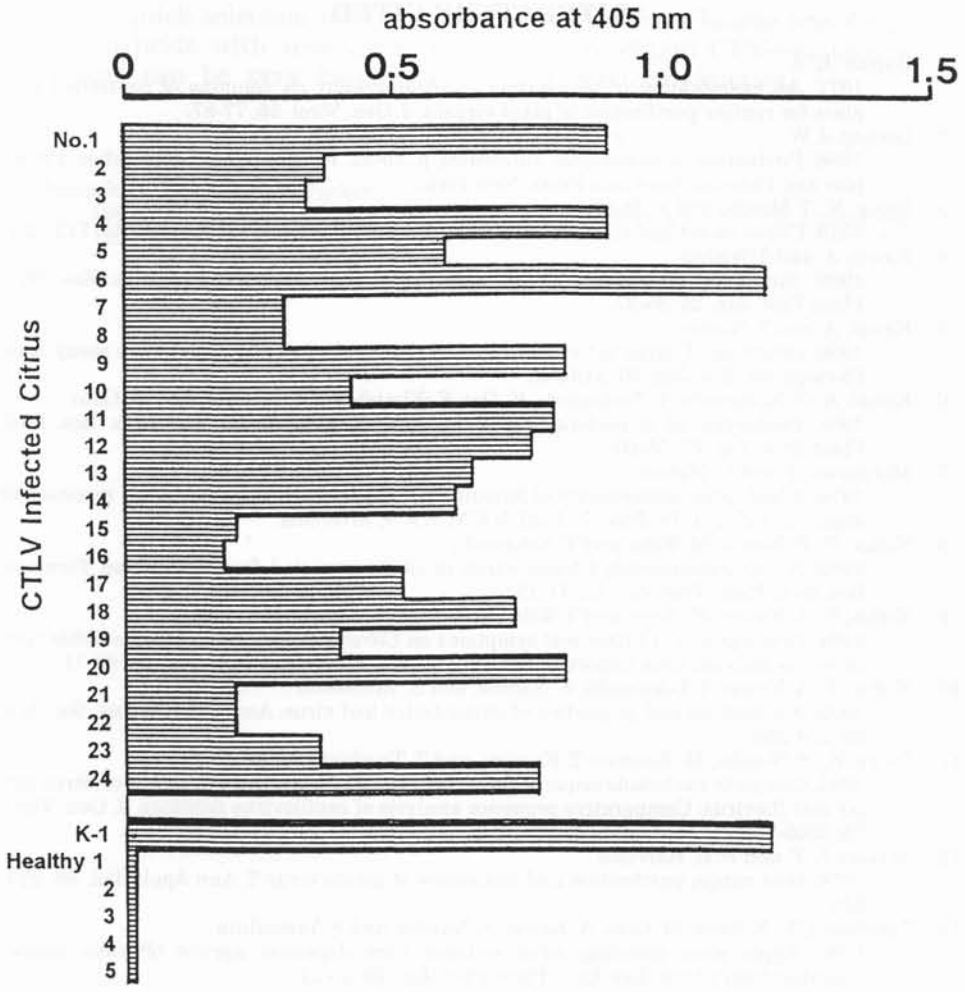

Fig. 2. Absorbance values in MAb-ELISA for citrus leaves infected with CTLV. Each sample extract of citrus leaves prepared as in Fig. 1 was assayed by MAb-ELISA. PAb-coated plates and MAb 9E10 conjugate were used in this ELISA. Samples 1 to 14 were collected from Japanese orchards. Samples 15 to 24 were detected from imported citrus varieties by post-entry quarantine in Japan. CTLV K-1 in rough lemon was used as positive control. Healthy citrus seedlings were used as negative control.

healthy control were negative. These results coincided with those of the inoculation test. These studies indicate that ELISA using PAb for coating and MAb 9E10 AP-conjugate are useful for detecting CTLV from citrus leaves. The finding that when MAbs were used as coating and conjugate, only a few isolates were detected, suggests that there were serological differences among the isolates.

Since the purification of CTLV and the development of PAbs were comparatively difficult, the development of MAbs suitable for coating would be desirable for the development of a stabilized indexing system for CTLV.

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