

Purification of Citrus Tristeza Virus

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ABSTRACT

Citrus tristeza virus (CTV) was purified from bark tissues of infected lime plants. The purification procedure consisted of extraction of powdered bark tissues, filtration, and virus precipitation by polyethylene glycol. A short Cs_2SO_4 -sucrose cushion step gradient was introduced to further purify the virus. The yield of purified virus was 4 mg/100 g bark tissue. Purified virus suspension composed of abundant threadlike particled and exhibited a UV-absorption spectrum of a nucleoprotein with a $A_{260/280}$ of 1.23. The CTV antiserum was effective for virus detection in immuno-electron microscopic tests.

INTRODUCTION

Citrus tristeza virus (CTV), the causal agent of tristeza disease is the major factor affecting citrus production worldwide (McClellan, 1974). The virus can infect all citrus varieties and be readily spread by vegetation propagation and the citrus aphid vector *Toxoptera citricidus*. In Thailand, CTV was found infecting all commercial varieties, namely som-khiewan *Citrus reticulata*, som-O *C. grandis*, som-tra *C. sinensis*, and manao *C. aurantifolia* (Attathom *et al.*, 1983). No effective control measure is available thus far to control this disease. Production of the CTV-free citrus plants has been reported (Attathom *et al.*, 1983, Attathom *et al.*, 1985), and field trials are in progress. However, the CTV in Thailand is not fully characterized due to the lack of a suitable purification method to obtain sufficient amount of the purified virus. Also, there is a need for the CTV-antiserum to be used in the immunological tests for the rapid and large scale disease diagnosis. Attempts were then made to purify this virus and the results reported in this paper.

MATERIALS AND METHODS

The virus. An isolate of citrus tristeza

virus was obtained from a naturally infected manao *C. aurantifolia*. Infected plant showed symptoms of tristeza disease consisting of severe stunting, leaf cupping and vein clearing. Bark tissue collected from an infected manao and stored at -20°C . was used for virus purification.

Virus purification. The CTV was purified according to the modified procedure described by Bar-Joseph *et al.* (Bar-Joseph *et al.*, 1985). Frozen bark tissue was powdered in a stone mortar with a pestle in the presence of liquid nitrogen. The frozen powder was extracted in 0.1 M tris-HCl buffer, pH 7.8 and filtered through glass-wool. After low speed centrifugation, the virus in the supernatant was precipitated in a solution of 30% (w/v) polyethylene glycol (PEG 6,000) as described by Bar-Joseph *et al.* (Bar-Joseph *et al.*, 1985). Precipitated virus was further purified by a short cesium sucrose cushion step gradient centrifugation (Bar-Joseph *et al.*, 1985). The light-scattering band located immediately below a green band was withdrawn from the gradient by using a 5-ml syringe and re-centrifuged in the same gradient. Virus collected from the second Cs_2SO_4 gradient was dialyzed 48 hours at 4°C . against 0.05 M tris-HCl, pH 7.8.

Production of CTV antiserum. The CTV antiserum was produced in a rabbit by

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injecting with a purified CTV preparation. Two intramuscular injections with of 1 mg/ml purified virus were given to the rabbit at 3 days interval. Two intravenous injections were done after the second intramuscular injection at 2 days interval. The immunized rabbit was bled a week after final injection.

Electron microscopy. Purified virus preparations were mounted on formvar coated grid and stained with 2% uranyl acetate for electron microscopic observation. For the decorate method used in immuno-electron microscopy (IEM), the virus was treated with a dilution of 1:1,000 of the CTV antiserum before uranyl acetate staining (Derrick, 1976). Observations were made with a JEOL 100s electron microscope.

RESULTS

Virus Purification. Purified virus preparations exhibited a UV-absorption spectrum of a nucleo-protein with a 260/280 of 1.23 (Fig. 3) Electron microscopic observation of this preparation revealed numerous thread-like particles resembling those of CTV (Fig. 1). Yield of purified virus was estimated to be 4 mg/100g bark tissue ($E = 1.20$.)

Immuno electron microscopy. Antiserum produced for purified CTV reacted positively in the Derrick and decorated methods of immuno electron microscopy. Particles of CTV were successfully detected in the crude sap of an infected plant by the Derrick method. None was observed in sap of a healthy plant. Purified

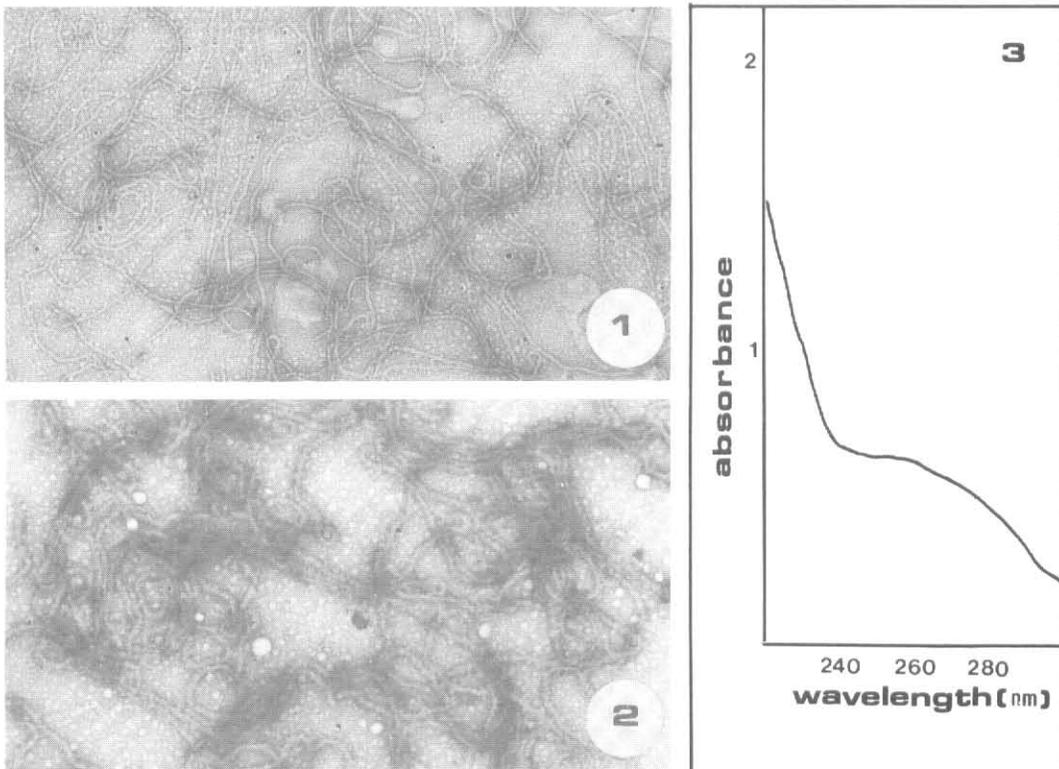


Figure 1-3 Citrus tristeza virus (CTV)

1. Purified CTV preparation
2. Decorated CTV particles
3. UV scanning profile of purified CTV preparation

CTV as well as those in saps from infected plants were very well decorated with the antiserum produced (Fig. 2). This antiserum did not decorate other viruses like TMV, watermelon mosaic virus, sugarcane mosaic virus and papaya ringspot virus, suggesting that it was specific for CTV.

DISCUSSION AND CONCLUSION

The sucrose-cesium cushion g gradient proved to be suitable for CTV purification in our study. We considered that a successful CTV purification could be derived from an effective extraction procedure through powderizing the infected tissue with liquid nitrogen. This technique may be inconvenient for the laboratory where liquid nitrogen is not locally available.

In Thailand, we feel that cross-protection in CTV infected plants has a good potential for application. Searching for mild strains is in immediate need. Therefore, CTV characterization for strain differentiation has to be further developed so that the whole process of mild strain selection can be shortened. That will be the time where the value of the study on CTV characterization can be demonstrated in this region.

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