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A simple alternative method for the purification of *Citrus tristeza virus*

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Summary. A simple method for the purification of *Citrus tristeza virus* is described, based on virus extraction from liquid-nitrogen frozen midribs and cortical tissue of infected Mexican lime, precipitation with polyethylene glicol mol. wt 6000 (PEG 6000) column chromatography on Sephacryl S-300-HR columns, and concentration of virus-containing fractions by PEG 6000, followed by low-speed (17,200 *g*) centrifugation. Virus preparations were satisfactorily clean, and were successfully used for the production of an antiserum which is now being evaluated.

Key words: Citrus, virus purification, *Citrus tristeza virus*, column chromatography.

Citrus tristeza virus (CTV, family *Closteroviridae*, genus *Closterovirus*), is one of the most serious citrus pathogens (Roistacher, 1991), especially for the Mediterranean citrus industry, which is still largely based on the highly susceptible sour orange rootstocks.

The Cukurova region of Turkey, the most important citrus growing area of the country, is no exception. To deal with the threat of CTV outbreaks, sensitive virus detection procedures are required for identification and timely eradication of infection foci. The continuous and effective monitoring of citrus groves which this entails is possible only if rapid, reliable and easy to use diagnostic techniques are available.

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In developing countries, CTV identification is based primarily on biological assays, such as indexing on Mexican lime; this procedure however, is time-consuming, expensive, and not foolproof (Roistacher, 1991), or it relies on immunoenzymatic assays (ELISA), which require a continuous supply of costly commercial kits. On the other hand, raising antisera locally requires laboratories with equipment (e.g. ultracentrifuge) whose acquisition, maintenance and operational costs represent an unbearable financial strain for most Institutions.

The relatively simple CTV purification procedures described in the past (Bar-Joseph *et al*., 1985; Lee *et al*., 1988) require at least one step of ultracentrifugation, making them impractical for poorly equipped laboratories. To overcome this disadvantage, an alternative purification method has been devised in which sucrose cushion gradient centrifugation are replaced by column chromatography.

Thirty grams of cortical tissue or leaf midribs

from CTV-infected Mexican lime, sweet orange, or Etrog citron were ground in liquid nitrogen. Extraction buffer (0.05 M Tris-HCl pH 7.8 containing 0.1 M Dithiothreitol and 5% sucrose) was added and the slurry was filtered by pressing through four layers of wet cheesecloth and a layer of paper towel. Fibers were re-extracted with the same buffer so that the final ratio of extraction buffer to fresh weight of tissue was 5 ml/g. The suspension was centrifuged for 10 min at 7000 rpm in a bench centrifuge, the supernatant fluid was collected and mixed with 6% (w/v) polyethylene glycol mol.wt 6000 (PEG 6000) and 0.125 M NaCl. After 1 h incubation at 4°C, the precipitate was collected by centrifugation at 17,200 *g* for 20 min, and the pellet resuspended in extraction buffer overnight at 4°C. Differential PEG 6000 precipitation and centrifugation were repeated once and the UV absorbance profile of the pellet resuspended in extraction buffer was determined with a spectrophotometer.

Concentrated, partially purified virus preparations were loaded on a 1.5x70 cm jacketed column of Sephacryl S-300-HR (Sigma Chemical Co., St. Louis, MI, USA) balanced with separating buffer (0.05 M Tris-HCl pH 7.8, containing 0.1 M NaCl, 1 mM $MgCl_2$ and 0.1% sodium azide). After each column chromatography run, fractions were manually collected in 1-ml aliquots and checked for the presence of CTV by ELISA (Clark and Adams, 1977). Fractions with an absorbance twice that of the control (extracts from healthy Mexican lime) or more were regarded as positive; these were pooled and treated with PEG 6000. The precipitate was concentrated by centrifugation at 17,200 *g* for 20 min, re-suspended in extraction buffer, and read with a spectrophotometer.

Column chromatography separation of CTV from healthy plant material was consistently achieved with a number of experiments. A typical chromatographic run showed a major peak between fractions 26 and 36 (Fig. 1). These fractions contained many closterovirus-like particles that were clearly decorated by a CTV antiserum in immunoelectron microscopy tests done according to Milne and Luisoni (1977) (not shown). No comparable peak was seen when healthy plant tissues were submitted to column chromatography in the same way (Fig. 1). That fractions 26-36 contained CTV was confirmed by ELISA, as shown in Fig. 2. Aver-

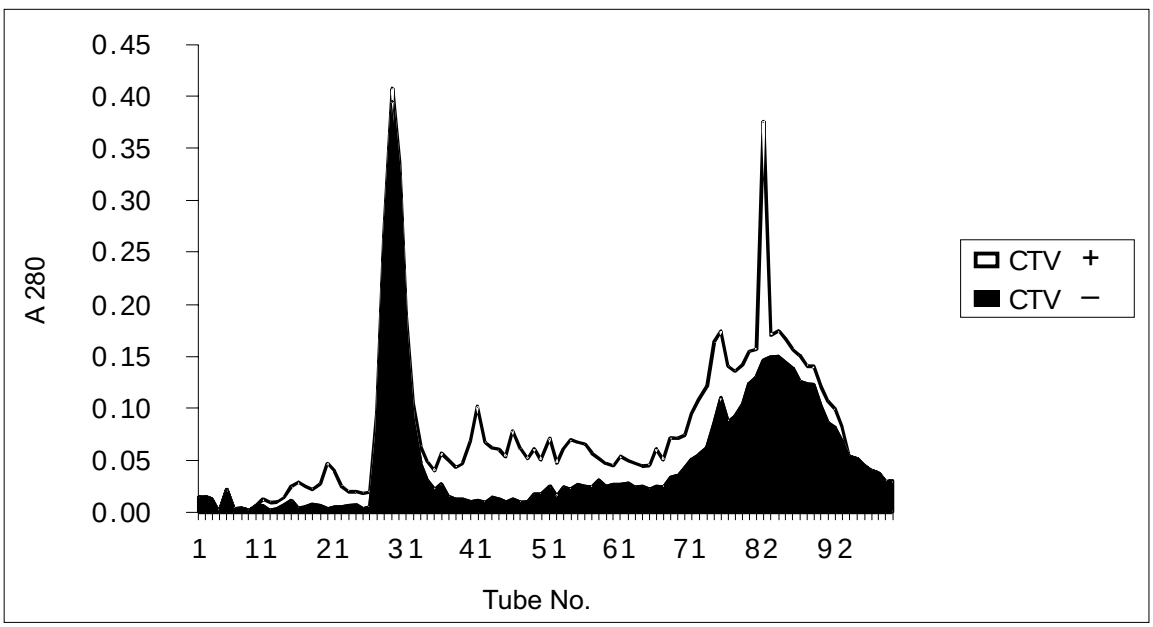

Fig. 1. UV absorption spectrum of partially purified virus preparation subjected Sephacryl 300 column chromatography (CTV+). Virus particles were present in fractions No. 26 and 36 which showed the highest absorbance. The control preparation (CTV-) show much lower absorbance values.

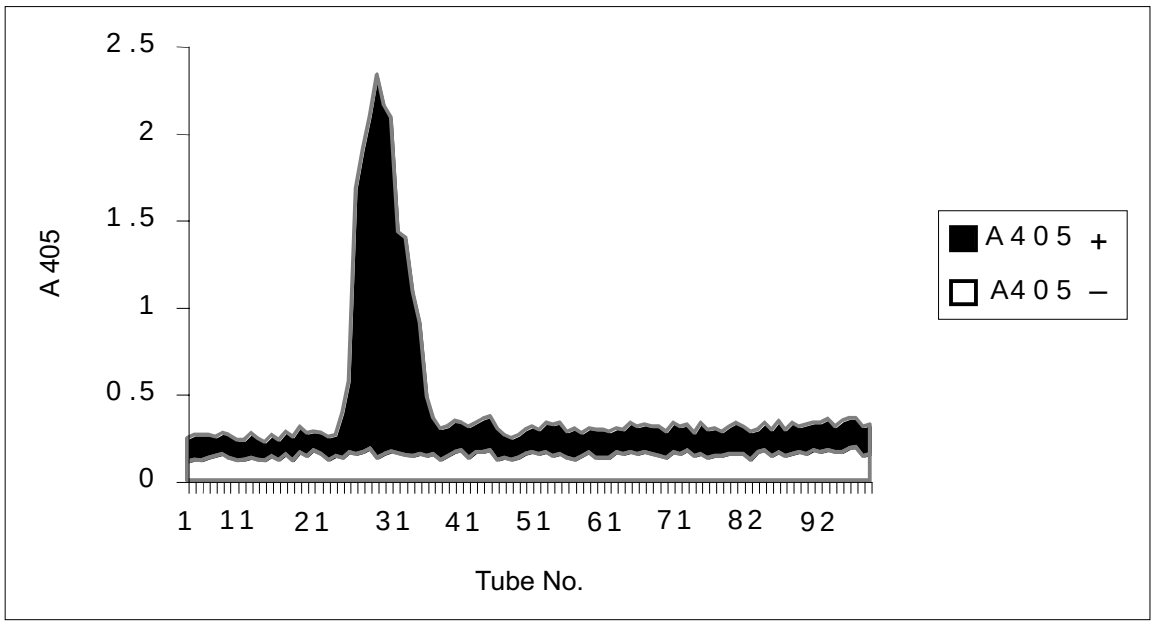

Fig. 2. Virus detection by ELISA in the same CTV preparation as Fig. 1 after Sephacryl 300 column chromatography.

age virus yield, estimated from UV readings, was ca. 450 µg/ml of the eluate consisting of the pooled virus-containing fractions. Virus preparations appeared to be fairly clean and were successfully used for production of an antiserum which is now being evaluated.

The results show that PEG precipitation followed by Sephacryl 300 column chromatography constitutes a method for CTV purification which is convenient, fairly efficient and does not require costly laboratory equipment.

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