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STUDIES ON CITRUS EXOCORTIS VIROID (CEVd) IN EGYPT

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SUMMARY - The first identification of citrus exocortis viroid (CEVd) in Egypt was carried out by symptom observations in the field, biological indexing, electrophoresis analysis and electron microscopy observations.

Key words: citrus, citrus exocortis viroid, diagnosis, Egypt

RESUME - *La première identification en Egypte du viroïde de l'exocortis des agrume a été réalisée par l'observation de symptômes au champ, l'indexage biologique, l'analyse par électrophorèse et les observations au microscope électronique.*

Mots clés*: Agrumes, viroïde de l'exocortis des agrumes , diagnose, Egypte*

In the present study, the pathogen causing symptoms related to exocortis of citrus trees was identified for the first time in Egypt as the citrus exocortis viroid (CEVd).

Several criteria were used for the identification of CEVd. These included: symptomatology in the field, in woody and herbaceous indicators, physical properties, polyacrylamide gel electrophoresis (PAGE) and electron microscopy examination of ultra-thin sections of CEVd-infected leaves (Roistacher, 1991).

CEVd was detected on citrus trees in several locations in El-Qalubia, El-Bouheira, and El-Menya Governorates. The infected citrus species included Mexican lime (*Citrus aurantifolia*), Valencia, Navel, Sukkary sweet oranges (*C. sinensis*), and mandarin (*C. reticulata)* (Bové, 1995).

The infected trees in the field showed clear-cut symptoms on trifoliate rootstocks: bark cracking and severe stunting. Citron (*C. medica* cv. Arizona 861) was used as a good differential host for CEVd inducing symptoms of leaf epinasty, chlorosis and midvein browning. CEVd-inoculated tomato *(Lycopersicon esculentum* cv. Rutgers) produced reduced leaves, stunting, and malformation. Similarly, *Gynura aurantiaca* plants, mechanically infected with CEVd, exhibited leaf epinasty, stunting and malformation two weeks after inoculation (Fig. 1).

Host range studies showed that this viroid could artificially infect several plants of the following families: *Rutaceae, Solanaceae, Compositae, Chenopodiaceae, Amaranthaceae* and *Scropulariaceae*.

Mechanical inoculations were successfully carried out by the use of pestle, needle, and cotton pad dipped in CEVd-infected sap.

Physical properties of CEVd indicated an *in vitro* longevity of 5 days, dilution end-point of 10^{-5} and thermal inactivation point of 70° C.

PAGE techniques (Semancik, 1991) proved to be very successful in indexing CEVd from infected *C. aurantium, C. reticulata, C. sinensis, C. medica*, and *G. aurantiaca*. All CEVd-RNAs had similar electrophoretic mobility, different from that of potato spindle tuber viroid. Another RNA faster in PAGE migration was detected with CEVd-RNA from the infected Citron (Fig. 2).

Ultra-thin sections of CEVd-infected leaves, examined with electron microscopy (Milne, 1993), showed alteration in the membrane coupled with the presence of watchspring-like structures as well as deformed cytoplasmic vesicles (plasmalemmasomes) (Fig. 3). Infected tissues had abnormal appearance of chloroplast-grana and thylakoid membranes.

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Fig. 1. CEVd infection of *G. aurantiaca* showing leaf epinasty, rugosity, inward curling (right side), comparing to the healthy (left side).

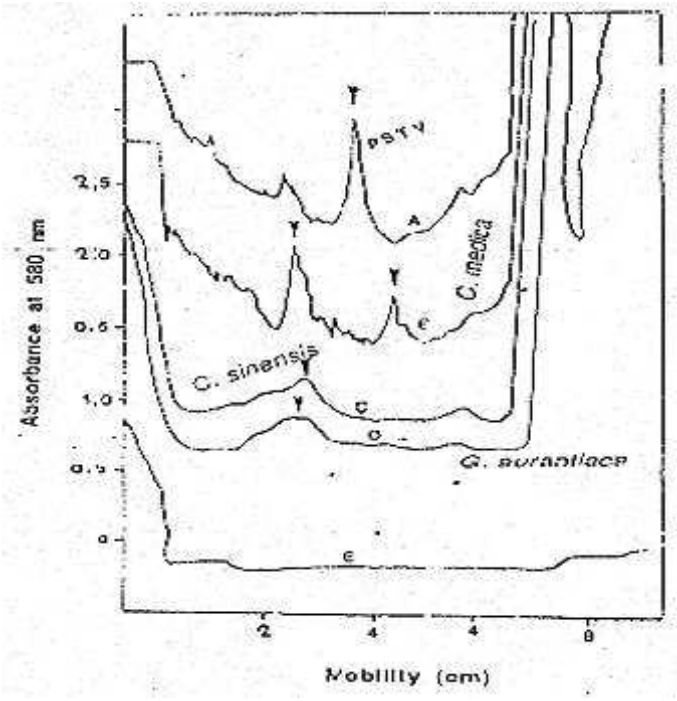

Fig. 2. A scanning profile of stained 5% PAGE containing purified nucleic acids extracted from the following plants: A. *Lycopersicon esculentum* cv Rutgers infected with PSTV; B. *Citrus medica* infected with CEVd; C. *Citrus sinensis* infected with CEVd; D. *Gynura aurantiaca* infected with CEVd; E. Healthy *C. medica*

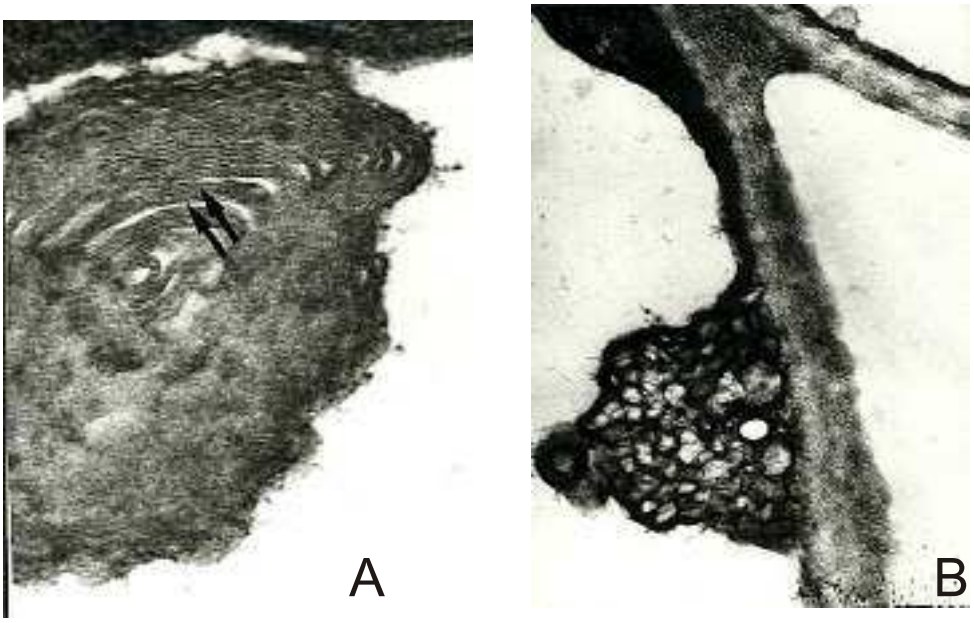

Fig. 3. An electron micrograph of CEVd-infected *C. sinensis* showing :A/ leaf cells. Arrows refer to watchspring-like structures (multilayered membrane bodies) X 73,600; B/ plasmalemmasomes (PLS) X 41,600