

Citrus Viroids in Turkey

N. Önelge

Çukurova University, Agriculture Faculty, Plant Protection Department, Adana, 01330, Turkey

ABSTRACT. A survey was carried out in the Çukurova region of Turkey which was the most important citrus growing area for presence of viroids. Samples were taken from orange, mandarin, lemon and grapefruit varieties of citrus scions are displaying gum pockets, stunting, reducing yield or no symptoms. Buds from these varieties were grafted on citron seedlings (*Citrus medica* L.) for viroid amplification. Citron leaves were used for nucleic acids extraction and purification. Sequential polyacrylamide gel electrophoresis (sPAGE) was used for viroid detection. CEVd, CVd-I, CVd-II and CVd-III Group viroids and CVd-IV were found in these citrus varieties. Specific primer pairs were used on RT-PCR analysis to confirm these viroids. All citrus varieties generally produced a viroid profile of CEVd, CVd-II and CVd-III. These viroids were found generally in mixed infections.

Citrus is one of the most important agricultural products in Turkey. The total annual citrus production is 2.5 million tons, with 80% of this in the Çukurova region (1). Citrus viroids are present in almost all citrus growing areas of the world and have a broad host range infecting species of citrus as well as citrus relatives. Because of the lack of use certified nursery budwood which is free from graft transmissible disease (GTD) pathogens, citrus viroids are one of the main reasons for low productivity in the Çukurova region.

A survey was carried out in the Çukurova region of Turkey for the presence of viroids. Samples were taken from orange, mandarin, lemon and grapefruit varieties, whether or not they displayed gum pockets, stunting or were without any symptom. Buds from these varieties were grafted on to citron seedlings for viroid amplification, and Parson's Special mandarin for biological indexing. Viroids were extracted from citron according to Semancik et al. (3). Viroid RNAs were detected and isolated by sequential polyacrylamide gel electrophoresis (sPAGE), followed by staining with ethidium bromide or silver nitrate (2). Specific primer pairs were used on RT-PCR analysis to confirm viroid identity.

Since the sour orange rootstock is used in Çukurova region, no conspicuous symptoms of *Citrus exocortis* viroid

(CEVd) were seen in commercial orchards. However, mandarin varieties displayed gum induced phloem discoloration, undulating stem pitting or bumps on the bark, which fit into depressions in the wood, symptoms characteristic of citrus cachexia viroid (CCaVd). Etrog citrons which were inoculated from field trees showed leaf epinasty, bent leaf and epinasty leaf symptoms. The underside of the leaves showed brown, necrotic and cracked veins, especially in the midvein. Citrus viroid sPAGE patterns of selected citrus trees are shown in Fig. 1.

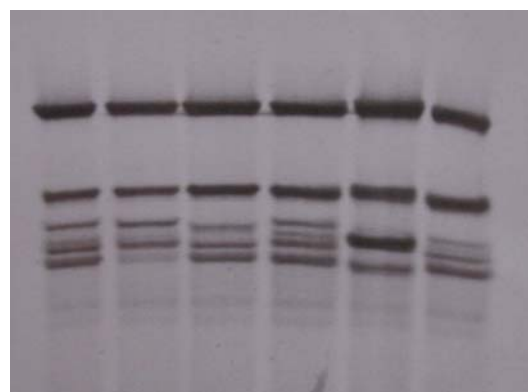


Fig.1. sPAGE of nucleic acid extracts from different citrus varieties: Lanes 1 and 4 infected with CEVd, CVd-I, CVd-II, CVd-III and CVd-IV ; Lane 2, infected with CEVd, CVd-I, and CVd-II; Lane 3, infected with CEVd, CVd-I, CVd-III, and CVd-IV; Lane 5, infected with CEVd, CCaVd and CVd-IV; Lane 6, infected with CEVd, CVd-II, CVd-III and CVd-IV.

The infected citrus sources mostly contained a viroid complex consisting of CEVd, *Citrus bent leaf viroid* (CBLVd), *Citrus viroid II* (cachexia)(CVd-II), *Citrus viroid III* (CVVd-III) group and *Citrus viroid IV* (CVd-IV). Many Washington navel and Valencia orange trees were infected with CBLVd and CVd-IV, which were rarely seen in Mediterranean countries. RT-PCR amplifications allowed detection of CEVd, CBLVd, CVd-II, CVd-

III and CVd-IV (Fig.2). Since the citrus viroids are distributed widely in the Çukurova region, the propagation and distribution of tested budwood should be emphasized in control of citrus exocortis and citrus cachexia disease. The new citrus plantations have begun with trifoliolate and its hybrids as rootstocks, and citrus exocortis disease could become an important GTD in Çukurova region.

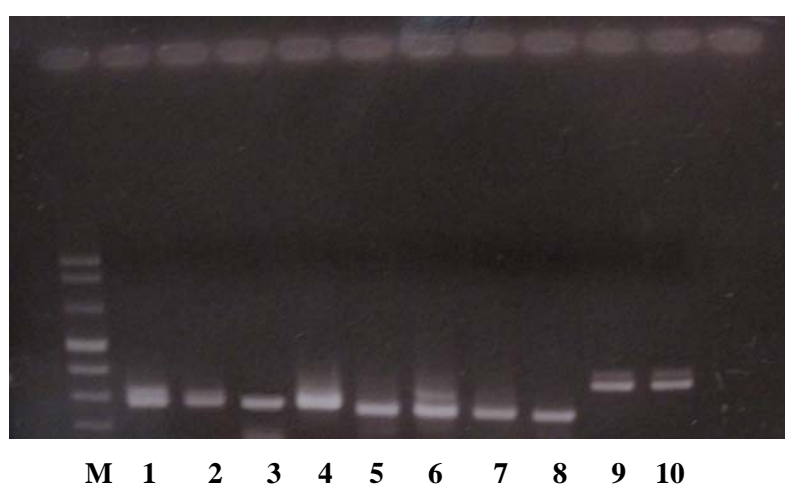


Fig. 2. RT-PCR products of citrus extracts amplified with primers: Lanes 1 and 2 CBLVd; Lanes 3 and 4 CCVd (HSVd); Lane 5 and 6 CVd-III; Lanes 7 and 8 CVd-IV; Lanes 9 and 10 CEVd; M = DNA Marker.

LITERATURE CITED

1. Anonymous
2007. <http://www.fao.org/es/ESC/en/20953/20990/highlight>
2. Semancik, J. S., C. N. Roistacher, and N. Duran Vila
1988. A viroid RNA associated with cachexia (xyloporosis) disease of citrus. In: *Proc. 10th Conf. IOCV*, 126-136. IOCV, Riverside, CA
3. Igloi, G. L.
1983. A silver stain for the detection of nanogram amounts of RNA following two-dimensional electrophoresis. *Analyt. Biochem.* 134: 184-188.