New Disease Reports

First record of *Citrus viroid II* (CVd-II) associated with gummy bark disease in sweet orange (*Citrus sinensis*) in Egypt

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Citrus fruit is one of the major traditional agricultural products of Egypt. Constraints to fruit production are mainly related to tree decline caused by infection with viroids (Hadidi *et al.,* 2003). The group II viroids, including *Hop stunt viroid* (HSVd) the causal agent of cachexia, have not been reported previously associated with gummy bark disease affecting sweet orange in Egypt. Gummy bark disease is a phloem discoloration affecting only sweet orange (Fig. 1), which was the first described by Nour-Eldin (1956). Affected trees are usually stunted to varying degrees and sometimes severely reduced in size.

In 2008, a total of 65 samples of sweet orange (cvs. Navel, Balady and Valencia) were collected; these samples were both from trees with gummy bark symptoms (a reddish-brown line under the bark and gum-impregnated tissue, around the circumference and especially near the bud union) and from trees of the same cultivars with no symptoms, growing in close proximity. The samples were indexed for viroids by inoculation of Etrog citron. Within three months, viroid leaf symptoms (petiole wrinkle and mid-vein browning) were observed on Etrog citron inoculated with samples from two governorates Kalyobiya and Fayoum.

Electrophoresis, under denaturing conditions of total nucleic acid extracted from the grafted Etrog citron plants with symptoms, indicated the presence of viroid-like circular low molecular weight (LMW) RNA in all the samples. These circular LMW-RNAs were used in RT-PCR using a set of HSVd specific primers (El-Dougdoug *et al.*, 2010). Amplicons of ~300 bp were obtained in all the samples with primers specific to *Hop stunt viroid* (HSVd). No amplification was obtained from trees without symptoms. In order to study the degree of sequence variation among *Hop stunt viroid*, gel-purified RT-PCR product of the viroid was cloned in the *Eco*RI site of pGEM®-T vector (Promega), then sequenced (GenBank Accession No. FJ984562). CVd-II from diseased sweet orange trees is 299 nucleotides in length and shares 100% identity with CVdIIb or Ca902 (AF131249; Reanwarakorn & Semancik, 1999). This constitutes the first isolation and identification of CVdII from sweet orange affected by gummy bark disease in Egypt.

References

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Figure 1

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