



First record of a *Hop stunt viroid* variant associated with gumming and stem pitting on *Citrus volkameriana* trunk rootstock in Egypt

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Received: 14 Dec 2013. Published: 09 Sep 2014. Keywords: *Citrus viroid II* (CVd-II), cachexia

Citrus fruits are traditional agricultural products in Egypt. *Citrus volkameriana* is the best rootstock for growing citrus in Egypt's desert areas (newly reclaimed land). It is far superior to other rootstocks used in Egypt in its resistance to *Citrus tristeza virus* as well as its tolerance to drought. Constraints to fruit production are mainly related to tree decline caused by infection with viroids (Hadidi *et al.*, 2003). Citrus variants of *Hop stunt viroid* (HSVd) with the pathogenicity determinants of cachexia have been reported associated with gummy bark disease affecting sweet orange in Egypt (Sofy *et al.*, 2010).

In 2008, gumming and stem pitting were observed on the trunk of *C. volkameriana* rootstocks grafted with Valencia sweet orange in citrus orchards in South Sinai, Behera, Monufia and Ismailia governorates. About 75 percent of the rootstocks showed these symptoms. By removing the bark under the bud union, the phloem tissue of the trunk of the *C. volkameriana* rootstock was found to be impregnated with gum. Also, conoid pits were observed in the cambial face of the wood of the rootstock due to the suppression of xylem tissue formation at these points (Figs. 1, 2). Neither gumming nor stem pitting was seen on the sweet orange scion (Fig. 1).

Bud sticks and leaf samples of Valencia sweet orange scions growing on *C. volkameriana* rootstocks were collected. These samples were from 70 trees with gumming and stem pitting symptoms and from ten trees of the same cultivar with no symptoms on the same rootstock growing in close proximity. The samples were indexed for viroids by graft-inoculation of cv. 'Etrog' citron (*Citrus medica*) propagated on sour orange rootstock. Within three months, viroid leaf symptoms (petiole wrinkle and mid-vein browning) were observed on 'Etrog' citron using samples from the 70 symptom-bearing trees. No symptoms were observed on citron inoculated with samples from the ten trees without symptoms.

Sequential polyacrylamide gel electrophoresis (Semancik, 1991), under native and denaturing conditions was done with nucleic acid preparations enriched for viroid-like RNA extracted from ten 'Etrog' citron plants with symptoms. The results indicated the presence of circular, low molecular weight viroid-like RNA in all samples. Based on the RNA electrophoretic

patterns additional viroid species may have been present in some of the samples. However, additional experiments would be required for their identification. 'Etrog' citron plants inoculated with healthy field trees gave negative results with electrophoresis. The ten positive samples 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 the HSVd primers. In order to distinguish between cachexia and non-cachexia inducing variants, gel-purified RT-PCR product of the viroid was cloned in the EcoRI site of pGEM®-T vector (Promega), then sequenced (GenBank Accession No. KM203115). CVd-II from diseased *C. volkameriana* trees is 295 nucleotides in length and shares 100% identity with CVdIIc or Ca905 (AF131250; Reanwarakorn & Semancik, 1999). This is the first record of *Hop stunt viroid* associated with gumming and stem pitting on *C. volkameriana* in Egypt.

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Figure 1



Figure 2

To cite this report: Sofy AR, El-Dougdoug KA, 2014. First record of a *Hop stunt viroid* variant associated with gumming and stem pitting on *Citrus volkameriana* trunk rootstock in Egypt. *New Disease Reports* **30**, 11. <http://dx.doi.org/10.5197/j.2044-0588.2014.030.011>
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