Identification of *Tomato ringspot virus* (ToRSV) on apple in Iran

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Abstract. Apple (*Malus domestica*) showing vein yellowing was identified as a natural host of ToRSV in Iran. Mechanical inoculation of enzyme-linked immunosorbent assay (ELISA)-positive samples on *Chenopodium* spp. and *Nicotiana tabacum* induced local lesions and systemic infection, respectively. Transmission from the ToRSV-infected tobacco onto apple seedlings resulted in systemic infection and vein yellowing with positive reaction in ELISA. This is the first report of ToRSV on apple in Iran.

Tomato ringspot virus (ToRSV) is considered one of the most devastating members of the genus *Nepovirus* (Stace-Smith 1996). The virus is distributed in most parts of the world and found in woody and semi-woody hosts, but it can also be found in herbaceous ornamental and weedy species (EPPO 2001). Major diseases caused by ToRSV on fruit crops include yellow bud mosaic in peach and almond which cause pale-green to pale-yellow blotches to develop along the main vein or large lateral veins of leaves (EPPO 2005). Some strains of the virus cause stem pitting and decline in *Prunus* spp. and necrosis of the union in apple (EPPO 1991). Plants infected with ToRSV show severe distinctive symptoms as a shock reaction (Stace-Smith 1984), and strains of ToRSV have been isolated from peach, grape, tobacco, apple and eounymus (Stace-Smith 1996). Grapevine decline caused by the same virus is economically

important in New York, USA (Uyemoto *et al.* 1977) and yellow stripe of lilies is also caused by ToRSV (Kim and Choi 1990). The virus was reported on grapevines from Iran (Pourrahim *et al.* 2004).

During the 2006 growing season, symptomatic leaf samples of an apple (*Malus domestica*) tree showing yellowing of main veins, mosaic and necrotic lesions (Fig. 1a) were collected from Khorasan province, North East of Iran. The samples were tested for the presence of *Apple mosaic virus* (ApMV), *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Tobacco ringspot virus* (TRSV) and ToRSV by enzyme-linked immunosorbent assay (ELISA) using specific antibodies including positive and negative controls (Agdia, Elkhart, IN, USA). Results showed that the samples had positive reaction with ToRSV and to a lesser extent

Fig. 1. (a) Vein yellowing and necrotic lesions in naturally *Tomato ringspot virus* (ToRSV)-infected apple. (b) Vein yellowing in apple that was artificially inoculated with ToRSV-infected tobacco extract.

with TRSV antibodies (Lana *et al.* 1983). Mixed infection in fruit trees is common (Uyemoto and Scott 1992; Choueiri *et al.* 2003); however, the indicator plants used in this experiment did not show any infection when tested by TRSV antibody. There were no reactions for PNRsV, PDV and CLRV. ToRSV-infected leaves were used as the source of inoculum for host range experimentation. The tissues were ground in 5–10 volumes of 0.01 M phosphate buffer, pH 7.2, containing 0.01 M sodium sulfite and the extract was rubbed on carborundum-dusted indicator plants. Mechanical inoculation of ELISA-positive samples on *Chenopodium amaranticolor* induced local lesions and on *Nicotiana tabacum* cv. Samson resulted in systemic infection. Local lesions were observed on *N. rustica* and chlorotic local lesions on *C. quinoa*; infection of *Cucumis sativus* remained symptomless. The presence of ToRSV in the indicator plants was confirmed by ELISA. Back inoculation of systemic ToRSV-infected tobacco cv. Samson leaf extracts on to apple seedlings resulted in systemic infection and development of vein yellowing symptoms (Fig. 1b). Back inoculation of the infected seedling on tobacco induced systemic infection similar to that resulting from inoculation of naturally infected apple on tobacco. Both the artificially inoculated apple and back-inoculated tobacco were positive for ToRSV in ELISA.

The experimental results confirm the observed symptoms were caused by ToRSV in the apple. This is the first report of ToRSV on apple from Iran.

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