Widespread occurrence of Tomato ring spot virus in deciduous fruit trees in Iran

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Abstract

Despite a long tradition of fruit-tree growing in all provinces of Iran, information on tree viruses in this country is scant. In the present study, presence of *Tomato ring spot virus* (ToRSV) was surveyed in various woody plants in this country by mechanical inoculation to herbaceous hosts, ELISA using a commercial antiserum, and PCR with specific primers. ToRSV was identified in the following plant-symptom combinations: Walnut with mottling, deformation, necrosis, and yellowing of main veins from Tehran Province; plum with yellowing of main veins, peach with yellowing of major veins and marginal necrosis, and hazelnut with interveinal chlorosis and marginal necrosis from Ardabil Province; apple with yellowing of main veins, mosaic and necrotic lesions, quince with large necrotic spots, and almond with leaf deformation and rosetting from Khorasan Province; and raspberry with marginal necrosis of leaf and necrotic lesions from Mazandaran Province. Mechanical inoculation from walnut, plum, peach, hazelnut, apple, quince, almond, and raspberry to *Nicotiana tabacum* cv. Samsun resulted in systemic infection. The virus isolates induced local lesions, leaf deformation, and necrosis in *N. rustica*, chlorotic local lesions on *Chenopodium quinoa*, and large local lesions on *Gomphrena globosa*. All samples were ELISA positive. PCR with specific primers resulted in the amplification of the expected fragment (490 bp). This study shows extensive occurrence of ToRSV in Iran.

Keywords: *Tomato ringspot virus*, Fruit tree viruses, Iran

Introduction

Tomato ringspot virus (ToRSV) is primarily a pathogen of woody and semi-woody plants and has been shown to cause mild to severe economic loss in many perennial fruit crops including *Malus* species (Stouffer and Uyemoto, 1976) and *Prunus* (Schlocker and Traylor, 1976). It can also be found in herbaceous ornamental and weed species (OEPP/EPPO, 2001), raspberry, grapevine, and dogwood (Rosenberger et al., 1983). The virus is a member of the genus *Nepovirus*, tramssmited by *Xiphinema Americana*, and through seed and pollen (Stace-Smith**,** 1984). ToRSV is widespread in the temperate region of Asia, Europe, and North and South America (OEPP/EPPO, 2005). ToRSV symptoms in peach and almond are pale-green to pale-yellow blotches developing along the main vein or large lateral veins of the leaves (OEPP/EPPO, 2001). ToRSV isolated from lily shows yellow stripe symptoms (Kim and Choi, 1990). It is associated with yellow bud mosaic and stem pitting and decline in peach and other *Prunus* species, brown line in prune, graft union necrosis and decline in apple, ringspot and decline in raspberry, and decline in grapevine (Stace-Smith**,** 1984). In the present study ToRSV was surveyed in various woody plants in Iran by mechanical inoculation to herbaceous hosts, ELISA using a commercial antiserum, and PCR with specific primers.

Materials and methods

Sources of the samples: ToRSV-infected samples were collected from various parts of Iran. Collected samples included, walnut with mottling, deformation, necrosis, and yellowing of major veins from Tehran Province; plum with yellowing of major veins, peach with yellowing of main veins and marginal necrosis, and hazelnut with interveinal chlorosis and marginal necrosis from Ardabil Province; apple with yellowing of major veins, mosaic and necrotic lesions, quince with large necrotic spots, and almond with leaf deformation and rosetting from Khorasan Province; and raspberry with marginal necrosis of leaf and necrotic lesions from Mazandaran Province.

Inoculation: Symptomatic leaves of plants were used for mechanical inoculation experiments. The tissues were homogenized in 5 volumes of 0.01 M phosphate buffer containing 0.01 M sodium sulfite, PH. 7.4. The extracts were rubbed on Carborundum-dusted leaves of *Nicotiana rustica*, *N. tabacum* cv. Samsun, *Gomphrena globosa*, *Chenopodium amaranticolor*, and *C. quinoa*.

Serological tests: A commercial ELISA-kit of ToRSV (Agdia, USA) was used to detect the virus in naturally and experimentally infected plants. DAS-ELISA (Clark and Adams**,**1977) was used throughout the study.

RT-PCR: A pair of ToRSV primers specific for the putative viral polymerase gene (F: 5'- GAC GAA GTT ATC AAT GGC AGC- 3' / R: 5'- TCC GTC CAA TCA CGC GAA TA- 3') (Griesbach, 1995) was used to detect the virus. Total RNA was extracted from infected *N. tabacum* cv. Samsun according to Boom et al. (1990).

Results and discussion

Mechanical inoculation of extracts from walnut, plum, peach, almond, apple, quince, hazelnut, and raspberry to *N. tabacum* cv. Samsun resulted in systemic infection. We found Samsun tobacco a suitable host plant for initial isolation of the virus. However, it took sometimes more than a month for systemic symptoms to develop. The virus isolates induced local lesions, leaf deformation, and necrosis in *N. rustica*, chlorotic local lesions on *Chenopodium quinoa*, and large local lesions on *Gomphrena globosa*. Some infected tobacco (*N. tabacum* cv. Samsun) remained symptomless. ToRSV was identified serologically in walnut, plum, peach, almond, hazelnut, apple, quince, and raspberry. All symptomatic samples of fruit trees were ELISA positive. RT- PCR with ToRSV specific primers resulted in the amplification of the expected fragment (449 bp). However, there were many symptomatic samples which were ELISA positive but failed to show amplification in RT-PCR. This could be due to the presence of inhibitors. ToRSV was present in all Iranian provinces surveyed. It was also detected in many non-woody plants such as tomato and *Physalis* sp. However, it is considered to be economically more important in fruit crops than in other crops (OEPP/EPPO, 2005). The widespread occurrence of and severe symptoms associated with ToRSV in Iran warrants a comprehensive study on its sources and its mode of spread and survival in this country.

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