Detection of Tomato Ringspot Virus on Stone Fruit Trees in Jordan

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ABSTRACT

Field surveys were conducted to asses the incidence of *Tomato ringspot virus* (ToRSV , genus *Nepovirus*, Family *Comoviridae*) infection in stone fruit trees in Jordan during 2000-2002. A total of 2546 samples, collected from commercial orchards, a mother block, nurseries and a varietal collection were tested for ToRSV infection by DAS-ELISA. Results showed that 16% of the tested samples were infected with the virus. The disease incidence percentages in apricot, almond, peach, nectarine, plum and cherry trees were 10, 14, 15, 19, 22 and 28% of the tested trees, respectively. The level of viral infection was highest in the commercial orchards (20%) and lowest in samples obtained from nurseries (5%).

Keywords: ELISA, ToRSV, Survey.

INTRODUCTION

In 2007, the total area of stone fruit in Jordan was 3953.7 ha which produced 36799 metric tons (Anonymous, 2007). Peaches formed the most common species of stone fruits grown in the country with an area of 1764.4 ha and a production of 18982 metric tons in 2007 followed by apricot, plum, almond and cherry trees with 898.4, 554.9, 313.3 and 130.4 ha, respectively.

In recent years, stone fruit cultivation has increased with a number of diseases associated with it, particularly virus infection (Nèmeth, 1986; Mink, 1998; FAO, 1999). Among the viral diseases affecting stone fruits is the disease caused by *Tomato ringspot virus* (ToRSV, genus *Nepovirus*, Family *Comoviridae*). It is one of the most economically destructive viral diseases of stone fruits (Uyemoto and Scott, 1992; Zaitlin and Palulcaitis, 2000). ToRSV causes economically significant losses in many woody deciduous fruit crops, including peach, almond, cherry, plum, apple and grape crops (Senevirtne and Posnette, 1970; Bellardi and Marani, 1985). It has also been reported in strawberry, raspberry, sweetberry, elderberry crops and many ornamental hosts in many parts of the world (Powell *et al*., 1984; Ghotbi and Shahraeen, 2009). The virus is transmitted by mechanical inoculation to herbaceous plants, but it is transmitted more efficiently by graft inoculation and by the dagger nematodes (*Xiphinema* spp.) (Forer *et al*., 1984).

In Jordan, little is known about the occurrence and distribution of ToRSV in stone fruits. Since stone fruit cultivation is increasing in Jordan, the present survey was conducted to estimate the incidence of ToRSV in commercial stone-fruit orchards, nurseries, a mother block, a varietal collection and in weed species in stone fruits orchards in Jordan.

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MATERIALS AND METHODS

Field Surveys

A total of 2546 samples from stone fruit trees showing virus-like symptoms as well as symptomless trees were collected during 2000-2002 from the most important growing areas. These include Ajlun, Amman, Ash-Shawbak, Al-Mudawwara, Irbid, Jarash, the Jordan Valley, Madaba, Al-Mafraq, As-Salt, Al-Tafila, and Az-Zarqa. The samples were collected from nurseries, commercial orchards, a mother block of stone fruits located in Al- Tafila (Al- Hassan Station) and varietal collection in Ash-Shawbak (National Center for Agricultural Research and Technology Transfer (NCARTT)) in various locations in Jordan.

Leaf samples were collected from almond (*Prunus dulcis*), apricot (*P. armeniac*a), peach (*P. persica*), plum (*P. domestica*) and sour cherry (*P. cerasus*). All sample extracts were analyzed by standard double- antibody sandwich DAS-ELISA (Clark and Adams, 1977). Occurrence, distribution and relative incidence of ToRSV were recorded. Disease incidence was determined as the percentage of ToRSV trees with respect to the total number of tested trees.

ELISA Test

DAS-ELISA was performed using commercially available ToRSV-IgG and conjugated alkaline phosphatase (Bioreba AG, Switzerland). A sample was considered infected if the absorbance (A_{405nm}) reading was greater than at least twice the absorbance reading of two healthy controls. The detection of ToRSV antigens was enhanced by extending the substrate incubation period from 1hr to approximately 2 hrs.

Mechanical Transmission

Representative disease samples were biologically assayed on cucumber (*Cucumis sativus* cv. National Pickling). The seedlings were held in complete darkness for at least 12 hrs before inoculation. The inoculum was prepared from ToRSV-infected leaf tissues, by macerating leaf tissue (1:1 w/v) with sterilized and chilled mortar and pestle in 0.01M phosphate buffer, pH 7.2, containing 0.001M sodium-diethyldithiocarbamate (Na-DIECA) and 2.5% nicotine in the presence of activated charcoal (100 mg^{-1}). Inoculated plants were kept in a temperature-controlled greenhouse at 20- 24^{0}C for symptom development. Inoculated leaves and shoot tips were then tested by DAS-ELISA.

Transmission of ToRSV by Nematode

Xiphinema americanum sensu lato and *X. index* were used to transmit ToRSV into cucumber plants. Nematode species were obtained from peach trees orchard in As-Salt area and maintained on cucumber plants in the glasshouse. Nematode transmission by the above-mentioned *Xiphinema* was assessed. Large numbers of *Xiphenema* spp. were hand-extracted and placed directly on exposed root systems of virus-infected cucumber seedlings (plants mechanically inoculated in the cotyledon stage with ToRSV isolates, at 6-10 days prior to use), or healthy seedlings in 100 cm^3 plastic pots partially filled with 1:1 mixture of sterilized sandy loam soil. The pots were then filled with the same soil mixture and placed in a growth chamber at 20-24^{0}C with a 14hr light period. The nematodes were allowed an acquisition access period of 14 days on roots of ToRSV-infected cucumber plants. After that, they were extracted from the soil by sieving method, counted and separated into groups of 10 (mixture of larvae and adults). The nematodes were placed directly on exposed root system of healthy 10-14 days old cucumber seedlings in partially filled 100 cm^3 plastic pots. The control treatment consisted of five

cucumber seedlings exposed to nematodes extracted from healthy cucumber seedlings. Root samples were collected from all cucumber plants and assayed for ToRSV infection by mechanical inoculation to healthy seedlings and by DAS-ELISA.

Occurrence of ToRSV in Other Hosts and Weeds

Leaf samples were also obtained from fruit trees other than stone fruits from different orchards in Ajlun, Ash-shawbak, Jarash, Madaba, Al- Mafraq and Al-Mudawwara locations showing symptoms suggestive of virus infection. These fruit trees include apple (*Malus domestica*), grapevine (*Vitis vinifera*), fig (*Ficus carica*) and pear (*Pyrus communis*). In addition, rose (*Rosa* spp.) and tomato (*Solanum lycopersicum*) and dandelion weed were also surveyed.

RESULTS

Field Surveys

In this study, a total of 2546 leaf samples of fruit trees showing virus-like symptoms were collected and tested by DAS-ELISA for ToRSV infection (Table 1).

Peach trees infected with ToRSV showed a reduction in growth and the leaves appeared drought-stressed, especially in late summer. Interveinal yellowing was accompanied by drooping and upward rolling of leaves. Drooping of leaves usually started from the branch tip back to the base. Infected trees usually had small sized, poor quality fruits which dropped prematurely. However, the presence of a narrow strip of brown to dark-brown cambial tissue (brown line) at the junction of the scion and rootstock and pits or grooves were the diagnostic symptoms of ToRSV infection.

DAS-ELISA

Tomato ringspot virus (ToRSV) was detected in all stone fruit orchards surveyed in this study, with an incidence range of 10-28%. The rates of ToRSV infection were as follows: apricot (10%), almond (14%), peach (15%), nectarine (19%), plum (22%) and cherry (28%) (Table 1). The mean level of infection considering all tested samples was about 16%. Results of type orchard irrespective of cultivars showed that the highest rate of ToRSV infection was recorded in commercial orchards (20%), while the rates of infection in the varietal collection and the mother block were 15% and 13%, respectively (Table 1). The virus was detected in samples collected from one- two-years-old seedlings in nurseries of Madaba and Al-Tafila with an incidence rate of 10%, while no ToRSV was found in the samples collected from nurseries in Baqa' and Jarash areas.

The ToRSV was detected from all locations surveyed. Al-Mudawwara area (southern desert region) showed the highest disease incidence (42%), followed by Jarash (Middle upland region) (38%), whereas the lowest virus infection occurred in the Jordan Valley (3%) (Table 2).

Mechanical Transmission

Tomato ringspot virus produced numerous chlorotic and necrotic ringspots on mechanically inoculated leaves of cucumber seedlings within 8-10 days post inoculation. A week after symptom appearance, systemic chlorotic ringspots, tip necrosis, mottling and oak leaf pattern were observed on subsequent growth. In addition, the infection was confirmed in mechanically inoculated plants by DAS-ELISA.

Vector-Virus Relationships

Results of nematode transmission of ToRSV showed that *X. americanum sensu lato* acquired the virus from ToRSV-infected roots of mechanically inoculated cucumber plants and transmitted it to healthy cucumber plants. The virus was isolated from roots of cucumber plants 15-20 days after exposure to *X. americanum sensu*

lato and symptoms appeared on leaves of cucumber bait plants, 15-25 days after exposure. However, ToRSV could not be recovered from the plants after 22-24 days after exposure to individuals of *X. index* and no symptoms appeared on leaves after 30 days of nematode exposure. ToRSV was not detected in any cucumber seedlings exposed to nematode given an acquisition feeding period on healthy plants. The presence of ToRSV in all tests was also confirmed by DAS-ELISA.

Occurrence of ToRSV in Other Hosts and Weeds

In addition to stone fruit trees, results of field surveys showed that apples, pears and grapevines were infected with ToRSV. High disease incidence occurred in grapevines (36%), followed by apples and pears with disease incidence rates of 23% and 19%, respectively (Table 2). However, ToRSV could not be detected in fig (12) and rose (19) samples. The virus was also found in one (6%) tomato plant grown neighboring two stone fruit orchards. Similarly, ToRSV was detected in two samples (18%) of dandelion (*Taraxacum* sp.) weed.

DISCUSSION

In this study, 2546 fruit trees were individually inspected for virus infection. These trees were located in commercial orchards, a mother block, nurseries and a varietal collection. Associating defined field symptoms with the presence of a specific virus was generally difficult due to the poor conditions of growth of many plantings, mainly because of water shortage. However, all orchards inspected in this study were found infected with ToRSV. Data of DAS-ELISA showed that ToRSV was widespread in many regions and affected many fruit trees, including almond, apricot, cherry, nectarine, peach and plum trees. The virus might be introduced to Jordan through the importation of infected nursery stock materials from the United States in the early eighties of the past century (Shatat, personal communication), where ToRSV was epidemic throughout many stone fruit growing areas (Powell *et al*., 1982; 1984; Powell *et al*., 1990). Results of DAS-ELISA showed that ToRSV incidence rate in *Prunus* spp. was 16%. In the light of the absence of a certification program in Jordan, this level of ToRSV incidence is acceptable. The present incidence of ToRSV represents a high risk for the Jordanian stone fruit industry. Besides the strict quarantine regulations to be applied to new imports, the need for a national certification program is highly desirable. Certification should be implemented with the least possible delay to minimize the risk of virus dissemination through infected propagating material. The occurrence of ToRSV in the mother block for the production of budwood and in seedlings in nurseries confirmed the necessity for such approach. The present survey provided basic information for further investigations on strain identification and management of ToRSV.

Nematode transmission of ToRSV isolate was achieved using *X. americanum sensu lato* but not *X. index*. This result is in agreement with previous results reported by Te'liz *et al.* (1966). As ToRSV is readily nematode transmissible, it is possible that natural vectors could transmit the virus to plant species in the field (Forer *et al*., 1984).

The surveys conducted in this study showed that plants other than stone fruits are infected with ToRSV. Such results suggest that these host plants can act as a reservoir for the virus. Furthermore, the weed dandelion was found not only a host for ToRSV but also for the nematode vector *X. americanum*.

Table 1: Detection of ToRSV in stone fruit trees/seedlings in Jordan by DAS-ELISA.

Host plant	Source of tested samples				Total
	Commercial orchards	Mother Block	Nurseries	Varietal collection	
Peach	117/709[a] (17)[b]	10/69 (15)	7/106 (7)	26/184 (14)	160/1068 (15)
Apricot	39/296 (13)	3/40 (8)	5/121 (4)	-	47/457 (10)
Plum	78/276 (28)	3/15 (20)	0/60 (0)	8/49 (16)	89/400 (22)
Nectarine	44/191 (23)	-	4/69 (6)	-	48/260 (19)
Almond	16/68 (24)	13/100 (13)	1/47 (2)	4/21 (19)	34/236 (14)
Cherry	30/58 (52)	-	5/67 (8)	-	35/125 (28)
Total	324/1598 (20)	29/224 (13)	22/470 (5)	38/254 (15)	413/2546 (16)[c]

[a] Number of infected plants over number of tested plants from different sources.
[b] Number in parentheses represent percentages.
[C] overall average disease incidence on seedlings/trees in all regions in Jordan.
[-] No samples were collected.

Table 2: Distribution of ToRSV in stone fruit trees/seedlings in different locations in Jordan .

Region	***Source of samples***				***Total***
	Commercial orchards	Nurseries	Mother block	Varietal collection	
Salt	27/183[a] (15)[b]	-	-	-	27/183 (15)
Baqa'	-	0/48 (0)	-	-	0/48 (0)
Ajlun	13/59 (22)	-	-	-	13/59 (22)
Jarash	47/124 (38)	0/209 (0)	-	-	47/333 (14)
Mafraq	61/323 (19)	-	-	-	61/323 (19)
Zarqa	9/109 (8)	-	-	-	9/109 (8)
Amman	2/12 (17)	-	-	-	2/12 (17)
Jordan Valley	3/91 (3)	-	-	-	3/91 (3)
Madaba	53/336 (16)	19/184 (10)	-	-	72/520 (14)
Tafila	-	3/29 (10)	29/224 (13)	-	32/253 (13)
Ash -Shawbak	61/236 (26)	-	-	38/256 (15)	99/490 (20)
Al-Mudawwara	27/65 (42)	-	-	-	27/65 (42)
Irbid	12/60 (20)	-	-	-	12/60 (20)

[a] No. of infected plants over number of tested plants from different sources.
[b] Number in parentheses represent percentages.
[-] No samples were collected.

Table 3: Results of DAS-ELISA for ToRSV infection tests in some crop species and a common orchard weed species.

Host Plant	Number of collected samples	Number of infected samples	Infection (%)
Apple	65	38	23
Pear	32	6	19
Grapevine	63	23	37
Fig	12	0	0
Rose	19	0	0
Tomato	17	1	6
Dandelion	11	2	18

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فيروس التبقع الحلقي للبندورة على اللوزيات في الأردن

عائدة النسور[1]، عقل منصور [2] ، لما البنا [2] ، و نداء سالم[3]

ملخص

أجري مسح لتقصي انتشار فيروس التبقع الحلقي للبندورة في 2546 عينة من بساتين اللوزيات التجارية، ومجمع للأمهات، ومشاتل، ومجمع وراثي موزعة في مختلف محافظات المملكة خلال المدة من 2000–2002 باستخدام الفحص المناعي المباشر (DAS–ELISA). بلغ متوسط إصابات العينات المختبرة 16%، بينما كانت إصابات أشجار المشمش، واللوز، والدراق، والنكتارين، والخوخ، والكرز 10، 14، 15، 19، 22، و28% على التوالي. وسجل الانتشار الأكبر للإصابة الفيروسية بفيروس التبقع الحلقي للبندورة في البساتين الخاصة (20%)، بينما سجلت أقل نسبة إصابة في المشاتل (5%).

الكلمات الدالة: التبقع الحلقي للبندورة، اللوزيات، الفحص المناعي المباشر.

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