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Short Notes

Natural incidence of tomato viruses in the North of Iran

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Summary. A survey was conducted in Qazvin province in the North of Iran, to determine the incidence of tomato viruses including: *Tobacco mosaic virus* (TMV), *Tomato yellow leaf curl* virus (TYLCV), *Tomato chlorotic spot virus* (TCSV), *Tomato bushy stunt virus* (TBSV), *Tomato spotted wilt virus* (TSWV), *Tomato ring spot virus* (ToRSV), *Tomato aspermy virus* (TAV), *Potato virus Y* (PVY), *Beet curly top virus* (BCTV), and *Cucumber mosaic virus* (CMV). A total of 742 tomato symptomatic samples were collected during the summer of 2007 in five regions of Qazvin province (Qazvin, Takestan, Boeen-Zahra, Alborz and Abiyek) and tested by enzyme-linked immunosorbent assay (ELISA). TSWV was detected in Alborz (4.4 %) and Abiyek (3.57%) regions but TMV and CMV were detected in all five regions. The greatest and least incidence of tomato viruses were recorded in Alborz (40.7 %) and Takestan (11.1 %), respectively. The presence of these viruses was also evaluated in the weed hosts as natural sources of plant viruses. The greatest and least incidence of tomato viruses in weed hosts were recorded in Boeen-Zahra (25.6 %) and Qazvin (12.8 %), respectively. TSWV was not detected in weeds. Transmission tests demonstrated that *Thrips tabaci* acts as TSWV carrier and *Myzus persicae* and *Aphis gossypii* were CMV carriers. Seed transmission tests were positive for TMV (13 tomato seedlings from 100 seedlings), but no TSWV transmission was observed through the seeds of infected tomato fruits.

Key words: ELISA, Qazvin province, tomato viruses.

Introduction

Tomato (*Solanum lycopersicum L.*) is one of the most important crops in the world with the average yield of 26 t ha^{-1} (Ganoo and Saumtally, 1998). Tomato production has suffered from many pests and disease problems, including those caused by viruses which substantially reduce yield and quality (Golnaraghi *et al*., 2004). More than 30 viruses belonging to different genera and families are reported to infect tomato naturally worldwide (Jones *et al*., 1991; Ganoo and Saumtally, 1998). The incidence and severity of tomato viruses vary from season to season because of the complex inter-relationships that exist among the pathogens, hosts, vectors, virus sources and environments (Jones *et al*., 1991; Mohammadi Hajiabadi *et al*., 2009). Symptoms produced by different viruses on tomato cultivars include leaf and fruit deformations, stunting, necrosis of stems and leaves, vein discoloration, general yellowing of leaves, systemic chlorotic and necrotic leaf spots, general mosaic and mottle on leaves, purple leaf veins, vessel browning, development of light green concentric spots with black centers on immature fruits, yellow spot discolorations on ripe fruits and subsequent wilting and complete collapse of plants (Braunt *et al*., 1990; Jones *et al*., 1991; Etebarian, 1997; Ganoo and Saumtally, 1998). These symptoms can vary based on the level of host resistance and environmental conditions (Mohammadi Hajiabadi *et al*., 2011).

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Some of the most important viruses infecting tomatoes naturally are: *Cucumber mosaic virus* (CMV, *Cucumovirus, Bromoviridae*), *Beet curly top virus* (BCTV, *Curtovirus, Geminiviridae*), *Tomato mosaic virus* (ToMV, *Tobamovirus, Virgaviridae*), *Tomato yellow leaf curl virus* (TYLCV, *Begomovirus, Geminiviridae*), *Tomato spotted wilt virus* (TSWV, *Tospovirus, Bunyaviridae*), *Tobacco mosaic virus* (TMV, *Tobamovirus, Virgaviridae*) and *Potato virus Y* (PVY, *Potyvirus, Potyviridae*). Many others can also be damaging to tomato (Jones *et al.*, 1991; Massumi *et al.*, 2009), including: *Eggplant mottled dwarf virus* (EMDV, *Nucleorhabdovirus, Rabdoviridae*); *Tomato bushy stunt virus* (TBSV, *Tombusvirus, Tombusviridae*); *Tomato yellow ring virus* (TYRV, Tospovirus, Bunyaviridae); *Alfalfa mosaic virus* (AMV, *Alfamovirus, Bromoviridae*); *Tomato ring spot virus* (ToRSV, *Nepovirus, Comoviridae*); *Tomato aspermy virus* (TAV, *Cucumovirus, Bromoviridae*); *Tomato chlorotic spot virus* (TCSV, *Tospovirus, Bunyaviridae*); and *Arabis mosaic virus* (ArMV, *Nepovirus, Comoviridae*).

Tomato cultivation in Iran covers around 160,000 ha with the mean yield of 36 t ha^{-1}. Around 80% of tomato production is field-grown and the rest is produced in greenhouses (Massumi *et al.,* 2009). Of the viruses mentioned above, AMV, CMV, ToMV, BCTV, *Sweet potato chlorotic stunt virus* (PCSV, *Crinivirus*, *Clostroviridae*), TBSV, TYLCV, TSWV, ArMV and TCSV have been previously reported to infect tomato in different regions of Iran (Farzadfar *et al.*, 2002; Massumi *et al.,* 2009*).* Some weeds can be reservoirs of some of these viruses and also can act as sources of the virus for vectors (Duffus, 1971*;* Hobbs *et al.*, 2000*).*

Recently, *Tomato yellow ring virus* (TYRV) was reported to infect tomato in Baluchestan province in the South of Iran (Jafari *et al.*, 2010). Qazvin province in the North of Iran is one of the main provinces of tomato production, which cover an area of 10,000 ha with an average yield of 40 t ha^{-1}. Because no earlier study was conducted on tomato viruses in this province, in spite of the different symptoms suggestive of virus infection, a survey was conducted to determine the incidence, distribution and relative importance of TMV, TYLCV, TCSV, TBSV, TSWV, ToRSV, TAV, PVY, BCTV, and CMV in tomato fields of Qazvin province, as well as their occurrence in weed hosts. Some vectors for the most important viruses were tested for their ability to naturally transmit these viruses. Seed transmission of prevalent viruses in tomato seed was also investigated.

Materials and methods

Field surveys

Tomato samples were collected during the summer 2007 from five regions of Qazvin province. A total of 742 symptomatic samples were collected from 36 fields located in Qazvin, Takestan, Boeen-Zahra, Alborz and Abiyek regions (Table 1). The fields were selected using a predetermined distance criterion, where distance between the fields ranged from 5 to 20 km (Golnaraghi *et al.*, 2004). Collected samples included young and fresh leaves and fruits of tomatoes with various symptoms.

Virus identification by serological assays

Samples of symptomatic tomato plants were analyzed for virus infection by enzyme-linked immuno-

Table 1. Occurrence of natural virus infections in tomato fields in five regions of Qazvin province surveyed.

Region	Samples collected	Fields surveyed	Cultivated area (ha)	Detected viruses[a]		
				TMV	CMV	TSWV
Qazvin	150	7	52	21	4	-
Takestan	180	9	75	12	8	-
Boeen-Zahra	165	6	38	11	14	-
Alborz	135	9	110	38	11	6
Abiyek	112	5	45	18	5	4
Total	742	36	320	100	42	10

[a]TMV, *Tobacco mosaic virus*; CMV, *Cucumber mosaic virus* and TSWV, *Tomato spotted wilt virus.*

sorbent assay (ELISA). The presence of TMV, TYLCV, TCSV, TBSV, TSWV, ToRSV, TAV, PVY, BCTV, and CMV was determined using the double antibody sandwich ELISA (DAS-ELISA). DAS-ELISA procedure was performed according to the general protocol (Clark and Adams, 1977). Antisera to these viruses were purchased from DSMZ (Braunshweig, Germany) and were used as 1/1000 dilution. Samples with absorbance values greater than or equal to three times the average of healthy samples were considered infected (positive) with the respective viruses.

Determination of natural hosts

Some symptomatic cultivated plants (other than tomato) and symptomless non-cultivated (weed species) plants, from different plant families were collected from locations adjacent to the surveyed tomato fields. All plant samples were tested serologically by DAS-ELISA using the antibodies for the viruses detected in each region.

Detection of viruses in potential vectors

Three aphid species (*Myzus persicae*, *Aphis gossypii* and *Aphis fabae*) and one thrips species (*Thrips tabaci* Lindeman) were collected from tomato fields and identified by N. Evazian Kari (Entomologist, Azarbaijan University of Tarbiat Moallem, Tabriz, Iran). The colonies of each species were found both in infected and non-infected fields, and then analyzed for virus infection by ELISA tests (Raccah *et al.*, 1985; Marchoux *et al.*, 1991; Chamberlin *et al.*, 1993; Kresta *et al.*, 1995; McPherson and Pappu, 1999; Raboudi *et al.*, 2002; Dheepa and Paranjothi, 2010) using 20–30 individual insects as one mixed sample in each ELISA test. Thrips and aphid colonies were collected from Abiyek, Alborz, Boeen-Zahra and Takestan regions.

Seed transmission assays

To investigate seed transmission rate, samples of tomato seeds were selected from several tomato plants infected with TMV and TSWV. The seeds were planted in plastic pots containing a mixture of pasteurized soil, sand and compost in a greenhouse with temperature set between 20–25°C and 50–70% relative humidity with at least 12 h light. All the emerging tomato seedlings (100 for each virus) at the four trifoliate leaf stage were tested individually by DAS-ELISA for the presence of these viruses (Mohammadi Hajiabadi, 2002).

Results

Virus detection using serological assays

Among the 742 collected symptomatic tomato samples collected, 152 samples (20.5 %) were infected with one virus. TMV and CMV were detected in all five regions surveyed and TSWV was only detected in tomato fields in Alborz and Abiyek regions. TMV was found in 100 samples (13.5%), CMV in 42 (5.7%) and TSWV in 10 (1.4%) samples. Incidence of TMV, CMV and TSWV was variable among the regions: the incidence of these three viruses was 14.0, 2.6 and 0 %

Table 2. Incidence of *Tobacco mosaic virus* (TMV) and *Cucumber mosaic virus* (CMV) in weeds and tomatoes in the five regions of Qazvin province.

Regions	TMV infection (%)		CMV infection (%)	
	Tomatoes	Weeds	Tomatoes	Weeds
Qazvin	14	11.11	2.66	1.71
Takestan	6.66	14.53	4.44	7.69
Boeen-Zahra	6.66	14.53	8.48	11.11
Alborz	28.14	15.38	8.14	4.27
Abiyek	16.07	14.53	4.46	5.12
Total	71.53	70.08	28.18	29.9

Table 3. Natural hosts collected during the survey for viruses in Qazvin province. Results of serological tests on with specific polyclonal antibodies of each virus are reported.

Plant species	Plant family	Collected samples[a]	Virus	Number of infected samples in five regions					
				Qazvin	Takestan	Boeen-Zahra	Alborz	Abiyek	Total
Datura spp.	Solanaceae	30	TMV	2	2	3	3	1	11
			CMV	0	2	1	0	0	3
Solanum spp.	Solanaceae	45	TMV	3	3	4	4	0	14
			CMV	0	2	2	0	0	4
Vicia faba	Fabaceae	35	TMV	0	0	0	0	0	0
			CMV	0	0	0	0	0	0
V. unguiculata	Fabaceae	20	TMV	1	0	0	2	2	5
			CMV	0	0	0	0	0	0
C. quinoa	Chenopodiaceae	50	TMV	2	1	3	2	1	9
			CMV	0	0	1	0	0	1
C. amaranticolor	Chenopodiaceae	50	TMV	0	3	4	2	2	11
			CMV	0	1	2	0	0	3
Malva spp.	Malvaceae	30	TMV	3	0	0	3	4	10
			CMV	0	0	0	0	0	0
Gomphrena globosa	Amarantaceae	25	TMV	0	0	0	0	0	0
			CMV	0	0	0	0	0	0
Cucumis sativus	Cucurbitacee	50	TMV	2	2	3	2	4	13
			CMV	2	4	7	5	6	24
Xanthium spp.	Compositae	50	TMV	0	0	0	0	3	3
			CMV	0	0	0	0	0	0
Helianthus spp.	Compositae	40	TMV	0	2	0	0	0	2
			CMV	0	0	0	0	0	0
Euphorbia spp.	Euphorbiaceae	35	TMV	0	4	0	0	0	4
			CMV	0	0	0	0	0	0
Capsella bursa-pastoris	Cruciferae	25	TMV	0	0	0	0	0	0
			CMV	0	0	0	0	0	0
Convolvolus arvensis	Convulvulaceae	50	TMV	0	0	0	0	0	0
			CMV	0	0	0	0	0	0
Centaurea depressa	Compositae	25	TMV	0	0	0	0	0	0
			CMV	0	0	0	0	0	0
Descurainia sophia	Cruciferae	25	TMV	0	0	0	0	0	0
			CMV	0	0	0	0	0	0

[a] Number of collected samples indicates the total numbers of each region that shared equally. For example, six samples of *Datura* spp. were collected from each of five regions.

in Qazvin; 6.7, 4.4 and 0 % in Takestan, 6.7, 8.5 and 0 % in Boeen-Zahra, 28.1, 8.1 and 4.4 % in Alborz and 16.1, 4.5 and 3.6 % in Abiyek, respectively (Tables 1, 2). Some of symptoms of infected plants are shown in Figure 1. TYLCV, TCSV, TBSV, ToRSV, TAV, PVY, and BCTV were not detected in any of the samples tested. No mixed infections were detected in the above mentioned regions.

Natural sources of virus infection

Of the 585 plant samples assayed from 10 families, 117 samples reacted positively in ELISA tests with specific antisera. Assays showed many weeds grown in or around tomato fields harbored TMV or CMV. TMV was identified in 14.0 % and CMV in 6.0 % of the weed samples. TMV was detected in cultivated plants including *Cucumis sativus* and *Vigna unguiculata*, grown around tested tomato fields, as well as in weed species including *Datura* spp., *Solanum* spp., *Chenopodium quinoa, C. amaranticolor, Malva* spp., *Xanthium* spp., *Helianthus* spp. and *Euphorbia* spp. CMV was detected in *Cucumis sativus*, *Datura* spp., *Solanum* spp., *Chenopodium quinoa* and *C. amaranticolor.* Other tested plants, including *Vicia faba, Gomphrena globosa, Convolvolus arvensis, Centaurea depressa, Descurainia sophia,* and *Capsella bursa-pastoris*, were not infected with any virus. There was no mixed infection in collected samples except for one sample of *Cucumis sativus* in Boeen-Zahra region that was infected both

Figure 1. Symptoms of collected samples: (a), (b) and (c) tomato fruits and leaves with symptoms suggestive of virus infection; (d), (e) and (f) TMV infected fruit and leaves; (g) and (h) CMV infected leaf and fruit; (i) and (j) TSWV infected fruit and leaf. TMV = Tobacco mosaic virus; CMV = Cucumber mosaic virus and TSWV = Tomato spotted wilt virus.

with TMV and CMV (Table 3). Incidences of TMV and CMV infection in the different regions were: Qazvin 12.8 %, Takestan 22.2 %, Boeen-Zahra 25.6 %, Alborz 19.7 % and Abiyek 19.7 % (Table 3). TSWV was not detected in any weed species tested. The incidence of other viruses (TYLCV, TCSV, TBSV, ToRSV, TAV, PVY, and BCTV) on weeds was not determined.

Detection of viruses in potential insect vectors

ELISA tests showed that two aphid species, *Myzus persicae* and *Aphis gossypii*, carried CMV. These tests also showed weak reaction with *Thrips tabaci* colonies collected from infected fields, but no positive reaction was observed with *T. tabaci* colonies collected from non-infected fields.

Seed transmission assays

Thirteen tomato seedlings out of 100 showed positive reaction with TMV specific polyclonal antibodies. No reaction was observed between seedlings grown from seeds collected from TSWV-infected tomato plants and TSWV polyclonal antibodies.

Discussion

In this study, the incidence and distribution of ten viruses in Qazvin province, one of the important regions of tomato production in Iran, is documented. Only 20% of the symptomatic collected samples were found infected with a virus. The rest of the samples could be infected with viruses not tested for, or symptoms observed could be due to reasons other than virus infection. TMV was the most prevalent virus in all surveyed tomato growing regions of Qazvin province. TSWV was detected only in tomato plants, and no other plant species were found infected with this virus, which is in agreement with what has been previously reported in north-east of Iran (Mohammadi Hajiabadi *et al.*, 2009). Other viruses, including TYLCV, TBSV, ToRSV, TAV, TCSV, PVY and BCTV which have been reported previously from other regions in Iran (Farezadfar *et al.*, 2002; Jafari *et al.*, 2010), were not detected in this study.

The green peach aphid *Myzus persicae* and the cotton aphid *Aphis gossypii* were identified as CMV carriers. These two species are known to feed on tomato and are efficient vectors of CMV (Etebarian 1997; Hobbs *et al.*, 2000; Raboudi *et al.*, 2002). Although our results showed that *Thrips tabaci* is able to carry TSWV, experimental transmission tests are needed to verify transmission of this virus by thrips. Detection of TSWV in *Thrips tabaci* collected from infected and non-infected fields suggest that thrips are the main agent for TSWV dissemination in Qazvin region, which is similar to what has been reported earlier in other regions of the world (Sakimura 1963; Cho *et al.*, 1989; Puche *et al.*, 1995; Nagata *et al.*, 2002). Seed transmission results in the present study were similar to those of Jones *et al.* (1991) and Etebarian (1997).

The results presented here suggest that tomato virus infections should be of special concern to farmers in Qazvin province, and they represent potential threats to tomato production. For this reason, additional work on characterization of tomato viruses, their modes of transmission and best means of their management should be carried out further.

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