Occurrence of Tospoviruses in Ornamental and Weed Species in Markazi and Tehran Provinces in Iran

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ABSTRACT

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Damage to agricultural crops by tospoviruses has occurred sporadically in Iran in the past; however, since 2000, outbreaks of tospoviruses have been recorded every year. The most affected ornamental crops were surveyed in two main cultivation areas in provinces of Markazi (Mahallat) and Tehran in 2000–01 and 2001–02. A few weed species also were collected. In all, 513 samples (with or without any conspicuous virus symptoms) were collected and analyzed by double- and triple-antibody sandwich enzyme-linked immunosorbent assay (ELISA) with polyclonal antibodies to *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV), and *Tomato Varsin virus* (ToVV), a new *Tospovirus* sp. from Iran. These viruses frequently were detected in samples of many different ornamentals and often in mixed infections, whereas *Iris yellow spot virus* (IYSV) was detected in only four samples. ToVV also was found in weeds growing in Chrysanthemum fields and in a *Cuscuta* sp. Applying double-antibody sandwich ELISA, no positive reactions were found with *Tomato chlorotic spot virus* (TCSV). Of the total of 513 samples tested, 345 samples did not react with any *Tospovirus* antisera. In Tehran, INSV was identified in 21 samples (10%), IYSV in 4 samples (2%), TSWV in 16 samples (8%), and ToVV in 22 samples (11%). In Markazi province, INSV was identified in 24 samples (8%), IYSV in 1 sample (0.5%), TSWV in 40 samples (13%), and ToVV in 36 samples (12%). ToVV was found to prevail in Tehran province and TSWV in Markazi. *Thrips* spp. present at the plant sampling sites also were collected and identified.

Tospovirus spp. are of increasing importance worldwide in crop and ornamental plants (17). In recent years, considerable diversity has been found within the genus *Tospovirus* in the family *Bunyaviridae*, with at least 12 distinct species described on the basis of serological relationships, host range and vector relationships, and comparison of nucleotide and amino acid sequences of the *L*, *M*, and *S* genes (3,6,9,13,14). Diseases caused by tospoviruses are endemic in Iran. In the past, damage has occurred sporadically in cultivated crops such as soybean (*Glycine max* L.), tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), and peanut (*Arachis hypogaea* L.) (2,8,10,15,17,19). Economic losses caused by tospoviruses in

Iran are unknown, but infection levels can reach up to 100%, as shown for *Tomato spotted wilt virus* (TSWV) in a glasshouse-grown crop of *Cineraria* spp. (15). In the Varsin (Tehran province) area, 17% yield loss to TSWV was estimated in susceptible tomato cultivars (12). Markazi (Mahallat) and Tehran provinces are the two main cultivation regions for ornamental crops in Iran (Fig. 1). These regions are located 270 km apart and are 1,747 and 1,200 m above sea level, respectively (Fig. 1). The area under cultivation of ornamentals in Iran is 4,100 ha, of which approximately 900 ha are in Markazi and 1,100 ha are in Tehran province (1). Smaller acreages of various food legumes and other vegetable crops (primarily crucifers) also are grown in these regions. The aim of the present study was to gain an insight into the occurrence and distribution of different tospoviruses and their role as disease agent in affected ornamentals, and to identify the *Thrips* spp. present in infected areas.

MATERIALS AND METHODS

Survey. In all, 513 samples were collected in 2000–02 from fields and glasshouses in Tehran (207 samples) and Markazi (306 samples) provinces (Table

1). Samples were collected from 32 plant families and 53 plant species. Samples comprised leaves and stems of ornamentals and flowering weeds that either exhibited various symptom types or were without any conspicuous symptoms. About 15% of the samples were asymptomatic.

Collection and identification of *Thrips* spp. *Thrips* populations were collected from the same areas as virus-infected plants. After collection, some thrips initially were stored in a mixture of 60% ethanol, glycerol, and acetic acid (9:1:1) for later mounting and identification. Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used to detect virus in individual adult thrips as described by Cho et al. (4).

Serological testing, sap inoculations, and electron microscopy. Standard DAS-ELISA (5) was performed with polyclonal antisera raised against the nucleocapsid (N) protein for *Iris yellow spot virus* (IYSV), *Tomato chlorotic spot virus* (TCSV), and *Tomato Varsin virus* (ToVV; synonym: *Tomato yellow fruit ring virus* [TYFRV]), a newly proposed *Tospovirus* species (21; accession nos. AJ493270 and AJ493271, EMBL/GenBank/DBJ databases). Triple-antibody sandwich (TAS)-ELISA was performed with monoclonal antiserum against TSWV and *Impatiens necrotic spot virus* (INSV) (7). All serological reagents against tospoviruses used were from the DSMZ (Braunschweig, Germany) plant virus antiserum collection: TSWV (AS-0115, AS-0106/0116 Mab-IgG), ToVV (AS-0526), INSV (AS-0115, 0117/1Mab-IgG), IYSV (AS-0528), and TCSV (AS-0195), including the respective positive controls in each ELISA. Absorbance at 405 nm was measured with a MCC-340 Multiskan Labsystem ELISA microplate reader (Finland). Healthy *N. benthamiana* sap extracted in ELISA extraction buffer was used as a negative control. A reaction was considered positive only if the absorbance was more than three times the mean of the negative control. The serological reagents used in ELISA revealed little cross reactivity with other virus species in the same genera. Mechanical transmission to test plants was done for selected ELISA

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positives; samples showing maximum absorbance in ELISA to each virus isolates were selected for mechanical inoculation to indicator host plants. Samples were prepared by grinding 1 g of leaf sap in ice-cold 0.1 M potassium phosphate buffer, pH 7.0, containing 0.15% 2-mercaptoethanol. Samples were inoculated to *N. benthamiana*, *Datura stramonium*, *D. metel*, and *Vigna unguiculata* (cv. Mashad). The test plants were kept in an insect-proof greenhouse at a constant temperature of 23 to 25°C. Symptoms on indicator hosts were recorded every 2 days for 14 days after inoculation and twice a week for the following 30 days. The indicator plants then were tested to confirm the presence of a particular virus, and to test for symptomless infections. For electron microscopy, crude plant extracts of symptomatic *N. benthamiana* test plants were prepared in 0.1 M phosphate buffer and applied to carbon-coated copper grids. The grids then were washed with distilled water, negatively stained with a few drops of 1% uranyl acetate, and examined for the presence of tospovirus-like particles using a Zeiss-906 model transmission electron microscope.

RESULTS

Presence of tospoviruses in natural hosts. All four tospoviruses—INSV, IYSV, TSWV, and ToVV—were detected in ornamentals and weed species in the two

main ornamental-growing regions of Iran (Table 1). Of the 513 samples assayed, 168 reacted positively in ELISA. In Tehran, INSV was identified in 21 samples (10%), IYSV in 4 samples (2%), TSWV in 16 samples (8%), and ToVV in 22 samples (11%). In Markazi province, INSV was identified in 24 samples (8%), IYSV in 1 sample (0.5%), TSWV in 40 samples (13%), and ToVV in 36 samples (12%). Viruses were found in 46 ornamental and 7 weed plant species collected (Table 1), ToVV (11%) was the predominant virus in samples collected from Tehran and TSWV (13%) was predominant in Markazi province. TCSV was not detected from any of these plant samples (Table 1).

Symptom descriptions on indicator test plants for these viruses are given in Table 2. A set of different plant species from two botanical families (*Solanaceae* and *Chenopodiaceae*) were inoculated from selected ELISA-positive ornamental plants with different tospoviruses. *C. amaranticolor* and *V. unguiculata* showed local necrotic lesions and *N. benthamiana* gave systemic leaf deformation and mosaic symptoms to infection with four tospoviruses. TSWV inoculation to *L. esculentum* (cv. Avand) produced necrotic local lesions, but the virus could not be recovered from uninoculated trifoliate leaves when back indexed to *V. unguiculata* indicator test plants. No distinct symptom differences were observed after inoculation of selected

isolates of the viruses to different indicator test plants. *V. unguiculata* was a good diagnostic indicator host for the tospoviruses, and symptoms expressed were informative for preliminary differentiation of the four tospoviruses. This is the first report of several new natural hosts (16) of TSWV for Iran, including an *Althea* sp. (*Malvaceae*), *Cordia crenata* (*Boraginaceae*), a *Jasminum* sp. (*Oleaceae*), *Lonicera caprifolium* (*Caprifoliaceae*), *Monihot esculenta* (*Liliaceae*), *Pelargonium odoratissimum* (*Geraniaceae*), *Rosa damascene*, a *Rosa* sp. (*Roasaceae*), a *Scindapsus* sp. (*Araceae*), a *Tagetis* sp. (*Umbeliferae*), and a *Zinnia* sp. (*Ziniaceae*) (Table 1).

Identification and testing of *Thrips* spp. *Microcephallothrips abdominalis*, *Thrips tabaci*, a *Haplothrips* sp., and *Frankliniella tenuirnis* were identified from ornamental fields (Table 3). Adults of *T. tabaci* collected from infested ornamentals in the Tehran region tested and were positive for ToVV (ELISA). *M. abdominalis* and *T. tabaci* commonly were found infesting ornamental plants collected from Mahallat areas and tested to be ELISA positive with TSWV, ToVV, and INSV.

Electron microscopy. Electron microscopical examination of sap extracts from selected host or infected *N. benthamiana* plants revealed the presence of typical enveloped tospovirus-like particles of 80 to 120 nm in diameter (T. Ghotbi, unpublished data).

Fig. 1. Map of Iran showing the location of ornamental plants surveyed during 2000–02.

DISCUSSION

Occurrence of TSWV in soybean, tomato, potato, tobacco, peanut, and *Cineraria* spp. in Iran has been reported previously (2,8,10,15,17). Occurrence of INSV on ornamentals from Mahallat and Tehran provinces also has been reported (19). Our study extends the information provided in earlier reports to the natural occurrence of at least four tospoviruses, TSWV, ToVV, INSV, and IYSV, infecting different orna-

mental crops and several weed species in Tehran and Markazi. INSV was found only occasionally, whereas ToVV, TSWV, and IYSV were collected quite frequently. These viruses also were found frequently in mixed virus infections (Table 1). The symptoms caused by TSWV and INSV in *N. benthamiana* and *V. unguiculata* were very similar to those reported by Varia et al. (20) and Gracia et al. (11). The economic impact of these viruses on ornamen-

tal production remains to be assessed; however, our findings provide preliminary evidence indicating geographical distribution of the different species of tospoviruses in ornamental and weed species in Iran. Moreover, identification of putative natural virus reservoir host plant and weed species should help in control management of the viruses. The incidence of ToVV in several crops and locations suggested that this virus is of considerable epidemiological

Table 1. Occurrence of tospoviruses in ornamentals and weed hosts in Tehran and Markazi Provinces of Iran (2000–02)^a

Plant species	Total ^b	Tehran					Markazi (Mahallat)				
		INSV	IYSV	TCSV	ToVV	TSWV	INSV	IYSV	TCSV	ToVV	TSWV
Ornamentals											
<i>Althea</i> sp.	9	–	–	–	1	–	1	–	–	–	1
<i>Anthemis</i> sp.	11	–	–	–	–	–	1	–	–	–	–
<i>Antirrhinum</i> sp.	9	–	–	–	–	–	–	–	–	1	–
<i>Bougainvillea</i> sp.	5	1	–	–	–	–	–	–	–	1	–
<i>Calendula</i> sp.	11	2	–	–	1	2	–	–	–	1	1
<i>Canna hortensis</i>	6	–	–	–	–	–	1	–	–	–	1
<i>Cheiranthus cheiri</i>	14	–	–	–	1	–	1	–	–	–	–
<i>Chrysanthemum morifolium</i>	16	4	–	–	3	–	3	–	–	2	2
<i>Chrysanthemum prutescence</i>	11	–	–	–	–	–	1	–	–	–	1
<i>Cineraria</i> sp.	15	–	–	–	2	–	1	–	–	1	1
<i>Cordia crenata</i>	5	–	–	–	–	–	–	–	–	–	1
<i>Cycas</i> sp.	8	1	1	–	–	–	–	–	–	–	–
<i>Cyclamen</i> sp.	9	–	–	–	1	1	–	–	–	–	–
<i>Dahlia</i> sp.	19	–	–	–	–	–	2	–	–	2	3
<i>Dianthus</i> sp.	8	–	–	–	–	–	1	–	–	2	3
<i>Ficus benjamina</i>	7	–	–	–	1	1	1	–	–	1	–
<i>Gazania</i> sp.	11	1	–	–	–	1	1	–	–	6	5
<i>Gladiolus</i> sp.	11	1	–	–	–	–	–	–	–	–	–
<i>Gomphrena globosa</i>	8	–	–	–	–	–	–	–	–	1	–
<i>Helianthus annuus</i>	10	–	–	–	1	–	–	–	–	–	–
<i>Impatiens</i> sp.	12	–	–	–	–	1	–	–	–	–	–
<i>Jasminum</i> sp.	5	–	–	–	–	–	–	–	–	1	1
<i>Linda</i> sp.	7	–	–	–	–	–	–	–	–	1	1
<i>Lonicera caprifolium</i>	5	–	–	–	–	–	–	–	–	2	2
<i>Magnolia</i> sp.	8	–	–	–	–	–	–	–	–	1	–
<i>Manihot esculenta</i>	6	–	–	–	–	1	–	–	–	–	1
<i>Matricaria maritima</i>	6	1	–	–	–	–	–	–	–	–	–
<i>Oenothera biennis</i>	8	–	–	–	–	–	–	–	–	–	1
<i>Pelargonium hortorum</i>	18	–	1	–	–	1	2	1	–	2	2
<i>Pelargonium odoratissimum</i>	15	–	–	–	1	–	–	–	–	2	1
<i>Petunia</i> sp.	11	–	–	–	–	–	–	–	–	–	1
<i>Rosa damascene</i>	15	–	–	–	1	1	–	–	–	–	–
<i>Rosa</i> sp.	18	1	1	–	2	1	1	–	–	1	3
<i>Rosmarinus officinalis</i>	10	–	–	–	2	2	–	–	–	–	–
<i>Rudbeckia</i> sp.	11	–	–	–	–	–	–	–	–	–	1
<i>Saintpaulia ioantha</i>	11	1	–	–	1	–	3	–	–	1	1
<i>Salvia</i> sp.	15	–	–	–	–	–	1	–	–	–	1
<i>Scindapsus</i> sp.	9	2	1	–	–	1	–	–	–	–	–
<i>Schefflera</i> sp.	7	–	–	–	–	1	–	–	–	–	–
<i>Spathiphyllum</i> sp.	6	1	–	–	–	–	–	–	–	–	–
<i>Syngonium podophyllum</i>	9	1	–	–	–	–	–	–	–	–	–
<i>Tagetes</i> sp.	10	–	–	–	–	–	–	–	–	2	1
<i>Tropaeolum majus</i>	7	–	–	–	–	1	–	–	–	1	1
<i>Verbena</i> sp.	8	–	–	–	–	–	–	–	–	1	–
<i>Viola</i> sp.	9	3	–	–	–	–	–	–	–	–	–
<i>Zinnia</i> sp.	10	1	–	–	–	1	–	–	–	–	–
Weeds											
<i>Amaranthus</i> sp.	9	–	–	–	1	–	–	–	–	1	–
<i>Chenopodium album</i>	11	–	–	–	1	–	1	–	–	–	1
<i>Cuscuta</i> sp.	8	–	–	–	1	–	–	–	–	–	–
<i>Euphorbia seguieriana</i>	9	–	–	–	–	–	1	–	–	1	–
<i>Lactuca aculeata</i>	4	–	–	–	–	–	–	–	–	1	–
<i>Lavandula</i> sp.	5	–	–	–	–	–	1	–	–	–	–
<i>Portulaca oleracea</i>	8	–	–	–	1	–	–	–	–	–	–
Total incidence	513	10%	2%	0%	11%	8%	8%	0.5%	0%	12%	13%

^a Number of samples in which virus was found; – = negative in all samples tested. INSV = *Impatiens necrotic spot virus*, IYSV = *Iris yellow spot virus*, TCSV = *Tomato chlorotic spot virus*, ToVV = *Tomato Varamin virus*, TSWV = *Tomato spotted wilt virus*.

^b Total number of plants tested.

Table 2. Host range studies of different *Tospovirus* spp.^a

Indicator host plants	TSWV		ToVV		IYSV		INSV	
	L	S	L	S	L	S	L	S
<i>Nicotiana benthamiana</i>	NL, NRS	LD, Mo	...	LD	...	LD	NL	CHL, LD, De
<i>N. rustica</i>	SNL, NRS	VCL, Mo	NL	LD	...	...	...	...
<i>N. tabacum</i> (cv. Samsun)	NL	CL, Mo	...	LD	...	...	...	CL, Mo
<i>Vigna unguiculata</i>	NL, NRS	LD, MNL	MNL	...	...	MNL, NRS	MNL	...
<i>Datura metel</i>	NL, NRS	LD	CL, CRS	LD	...	...	CL, CRS	LD, Mo
<i>D. stramonium</i>	NL, NRS	LD, Mom, De	...	LD	...	...	CL, CRS	CHL, Mo
<i>Petunia hybrida</i>	NL	...	NL	...	NL	...	NT	NT
<i>Lycopersicon esculentum</i> (cv. Avand)	NL	(Mo)	NL	Mo	...	...	...	...
<i>Chenopodium amaranticolor</i>	NL	...	NL	...	...	...	NL	NL, NRS

^a One sample from each of four different *Tospovirus* isolates showing maximum absorbance in enzyme-linked immunosorbent assay was selected for mechanical inoculation to indicator host plants. TSWV = *Tomato spotted wilt virus*, ToVV = *Tomato Varamin virus*, IYSV = *Iris yellow spot virus*, INSV = *Impatiens necrotic spot virus*, L = local symptom, S = systemic, CHL = chlorosis, CL = chlorotic lesion, CRS = chlorotic ring spot, De = plant death, LD = leaf deformation, MCL = mild chlorosis, MNL = mild necrotic lesion, Mo = mosaic, NL = necrotic lesion, NT = not tested, NRS = necrotic ring spot, SNL = severe necrotic lesion, and VCL = vein clearing.

Table 3. Vector status of populations of *Thrips* spp. in Tehran and Markazi provinces of Iran^a

Region, plant species	<i>Thrips</i> spp.	ELISA test result ^b
Markazi		
<i>Althea</i> sp.	<i>Microcephalothrips abdominalis</i> Crow., <i>Thrips tabaci</i> Lind.	TSWV*
<i>Antirrhinum</i> sp.	<i>T. tabaci</i> Lind.	–
<i>Calendula</i> sp.	<i>M. abdominalis</i> Crow., <i>T. tabaci</i> Lind.	ToVV-TSWV*
<i>Chrysanthemum</i> sp.	<i>T. tabaci</i> Lind.	INSV-TSWV
<i>Chrysanthemum</i> sp.	<i>M. abdominalis</i>	–
<i>Cropsis</i> sp.	<i>T. tabaci</i> Lind.	–
<i>Dahlia</i> sp.	<i>T. tabaci</i> Lind.	TSWV
<i>Dianthus</i> sp.	<i>T. tabaci</i> Lind.	TSWV
<i>Gaillardia grandiflora</i>	<i>M. abdominalis</i> Crow., <i>T. tabaci</i> Lind.	–
<i>Gladiolus</i> sp.	<i>T. tabaci</i> Lind.	–
<i>Gomphrena globosa</i>	<i>T. tabaci</i> Lind.	–
<i>Pelargonium</i> × <i>hortorum</i>	<i>T. tabaci</i> Lind.	ToVV
<i>Petunia hybrida</i>	<i>T. tabaci</i> Lind.	TSWV
<i>Rosa</i> sp.	<i>M. abdominalis</i> Crow.	TSWV
<i>Rudbeckia</i> sp.	<i>T. tabaci</i> Lind., <i>Haplothrips</i> sp.	TSWV*
<i>Tagetes</i> sp.	<i>T. tabaci</i> Lind.	ToVV
<i>Tropaeolum majus</i>	<i>T. tabaci</i> Lind.	ToVV- TSWV
Tehran		
<i>Chrysanthemum</i> sp.	<i>T. tabaci</i> Lind.	ToVV
<i>Polianthes</i> sp.	<i>T. tabaci</i> Lind., <i>Frankliniella tenuirnis</i> Uzel.	–
<i>Rosa</i> sp.	<i>T. tabaci</i> Lind., <i>F. tenuirnis</i> Uzel.	ToVV*
<i>Tagetes</i> sp.	<i>T. tabaci</i> Lind.	ToVV

^a Plant species from which thrips were sampled are indicated.

^b ELISA = enzyme-linked immunosorbent assay, TSWV = *Tomato spotted wilt virus*, ToVV = *Tomato Varamin virus*, INSV = *Impatiens necrotic spot virus*, * = positive ELISA results obtained from a mixed population of *Thrips* spp. (extract was a mixture from both the thrips spp.), and – = negative.

significance. ToVV, which was characterized only recently (21), has been largely overlooked in earlier studies because antiserum was not available. Although the host range of this virus appears to be as broad as that of TSWV, it is easily distinguished from TSWV by ELISA and host plant reaction. Further studies are needed to describe the epidemiological characteristics, including vector transmission of this new virus from Iran. ToVV was present in *T. tabaci* from ornamentals in Tehran and TSWV, ToVV, and INSV were present in *T. tabaci* and *M. abdominalis* from ornamentals in Mahallat. These results coincide with the rate of occurrence of these viruses in these two regions. The two species have been reported as vectors of tospoviruses elsewhere (9,18). *T. tabaci* was the more common species. However, *F. occidentalis*, a primary *Tospovirus* vector species (18), was not identified from thrips populations

from Iran, suggesting that ornamental plants in these areas are not yet infested with *F. occidentalis*. We sampled only two regions in Iran for thrips and, therefore, do not understand why the prevalence of *Tospovirus* spp. differs in separate geographic areas. The presence of more than one virus species in each geographical area might be attributed to the presence of different thrips species acting as vectors of these viruses. The lack of information on vector species and their distribution, virus transmission efficiencies, and further biological characteristics of the vectors in Iran warrant further investigation. The detection of a new virus species in Iran, ToVV, encourages the use of virus identification approaches using multiple indexing methods, including biological and infectivity assays. This will aid in a comprehensive understanding of the complex virus situation present and of

putative unknown virus species, strains, and variants.

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