Plant Pathology Journal

ISSN 1812-5387

science alert

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Plant Pathology Journal 6 (3): 227-234, 2007
ISSN 1812-5387

Identification and Partial Characterization of a *Tospovirus* Causing Leaf and Stem Necrosis on Potato

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Abstract: A *Tospovirus* was isolated by mechanical sap transmission from potato plants showing leaf and stem necrosis symptoms and identified as Tomato yellow fruit ring virus (TYFRV) based on biological, serological and molecular studies. In mechanical transmission trials, a wide range of indicator plants, including members of the Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae and Solanaceae, closely similar to that of the not-infecting *Nicotiana* isolates of the same virus, was infected. The isolates under study strongly reacted with TYFRV antibodies, but not with the specific antibodies of the other tospoviruses tested (*Groundnut ringspot virus*, *Impatiens necrotic spot virus*, *Iris yellow spot virus*, *Tomato chlorotic spot virus*, *Tomato spotted wilt virus* and *Watermelon silver mottle virus*). In reverse transcription-polymerase chain reaction (RT-PCR), using specific primers for TYFRV-N gene, a DNA product with the expected size of approximately 1,200 bp was amplified from infected plants. The sequence analysis of this fragment showed a high identity with the corresponding region of TYFRV and confirmed the presence of this virus in Iranian potato crops. In this study, the transmission of TYFRV through potato tubers, even if at very low extent, was evidenced for the first time.

Key words: Potato viruses, TYFRV, detection, ELISA, serology, host range, tuber transmission, RT-PCR, sequencing

INTRODUCTION

Tospoviruses, within the family Bunyaviridae, are considered among the most common plant viruses of substantial agricultural importance, since they infect more than 1,000 plant species (Parella *et al*., 2003; Whitfield *et al*., 2005). Losses due to their infection in agricultural crops are significant worldwide (German *et al*., 1992), as in the case of *Tomato spotted wilt virus* (TSWV) that causes a total annual loss of 1 billion US$ in groundnut, vegetables, tobacco and other crops (Goldbach and Peters, 1994; Prins and Kormelink, 1998; Moyer *et al*., 1999).

Tospoviruses have quasi-spherical virions with lipid envelopes and a size of 80-120 nm in diameter, which contain three single-stranded RNA molecules (Cortez *et al*., 2001; Fauquet *et al*., 2005). There are at least 12 species of the genera *Thrips* and *Frankliniella* reported to transmit tospoviruses in nature (Mound, 2005). *Tospovirus* species are identified on the basis of their natural mode of transmission, host range, serological relationship of the N protein and their N protein sequence (Cortez *et al*., 2001; Fauquet *et al*., 2005). Tospoviruses, as well as their vectors, are worldwide distributed and have an extremely broad host range. These viruses can induce a range of symptoms, i.e., necrosis, chlorosis, ring patterns, mottling, silvering, stunting and local lesions, depending on virus species or strain, host plant, period of infection and environmental conditions (German *et al*., 1992; Mumford *et al*., 1996; Mound, 2005). TSWV is the most important *Tospovirus* species infecting potatoes (*Solanum tuberosum*) in the world (Nagata *et al*., 1995; Louro, 1996; Al-Shahwan *et al*., 1997; Khurana *et al*., 1997; Lathman and Jones, 1997; Jozsef *et al*., 2001; Wilson, 2001; Abad *et al*., 2005). Natural infections of other tospoviruses also have been previously reported in this crop: *Groundnut ringspot virus* (Granval de Milan *et al*., 1998), *Impatiens necrotic spot virus* (INSV)

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(Perry *et al*., 2005), *Tomato chlorotic spot virus* (TCSV) (Nagata *et al*., 1995; Granval de Milan *et al*., 1998) and a tospovirus serologically related to *Peanut bud necrosis virus* (PBNV) (Jefferies, 1998).

In Iran, several *Tospovirus* species of different serogroups (Moyer, 1999), including INSV, *Iris yellow spot virus* (IYSV), PBNV and TSWV, have been reported in several hosts (Moini and Izadpanah, 2001; Golnaraghi *et al*., 2002a; Ghotbi *et al*., 2005). In 2002, a distinct *Tospovirus* species infecting tomato plants in the Varamin area of Tehran province was identified and the name of Tomato yellow fruit ring virus (TYFRV) was for it proposed (Winter *et al*., 2002, 2006). A widespread occurrence of TYFRV in ornamental and soybean crops was reported in some provinces of Iran, where the virus was also detected in some weed species (Ghotbi *et al*., 2005; Golnaraghi *et al*., 2007). It seems that TYFRV isolates from soybean, originally mistook with TSWV, may be subdivided in two main groups: those readily infecting a broad range of *Nicotiana* species or cultivars and those not-infecting such indicators (Golnaraghi *et al*., 2002b). Based on ELISA tests, the thrips species *Microcephallothrips abdominalis* and *Thrips tabaci* were indicated as potential vectors for TYFRV (Ghotbi *et al*., 2005); the vectoring role of the latter was then experimentally demonstrated (Golnaraghi *et al*., 2007).

Potato is an important field crop which is widely cultivated throughout the world. Iran is the fifth largest potato producer in Asia with its 3.6 million tones/year (FAO, 2004). This crop is susceptible to infection by several viruses, which can substantially reduce yield and quality (Hooker, 1990). In recent years, *Tospovirus*-like symptoms of leaf and stem necrosis have been frequently observed in Iranian potato fields. In the present study, TYFRV was recognized as the causal agent of the disease based on host range studies and serological analysis. The identification of this virus was also confirmed by reverse-transcription polymerase chain reaction (RT-PCR) and sequencing. The information obtained in this study will hopefully aid in improving detection systems and control strategies for such infections in Iran.

MATERIALS AND METHODS

Plant sampling: In a survey done in the summer 2004, a total of 95 potato leaf samples from plants showing leaf and stem necrosis symptoms (Fig. 1a and b) were

Fig. 1: Field symptoms associated with TYFRV infections on potato: (a) necrotic spots on leaves (b) leaf and stem necrosis and (c) a field with approximately 100% of infection

Table 1: Tospovirus antibodies used in immunological assays

Virus	Cat. No.	Source	Abbreviation
GRSV-SA05	07504	Loewe, Sauerlach, Germany	GRSV-L
INSV	07505	Loewe, Sauerlach, Germany	INSV-L
IYSV	07508	Loewe, Sauerlach, Germany	IYSV-L
TCSV-BR03	07503	Loewe, Sauerlach, Germany	TCSV-L
TSWV-BR01	07501	Loewe, Sauerlach, Germany	TSWV-L
TSWV	190112, 190125	Bioreba, Reinach, Switzerland	TSWV-B
TYFRV	As-0526	DSMZ, Braunschweig, Germany	TYFRV-D
WSMoV	As-0118	DSMZ, Braunschweig, Germany	WSMoV-D
Tospoviruses[a]	07507	Loewe, Sauerlach, Germany	Tospo-L
Tospovirus-broad spectrum[b]	190315, 190325	Bioreba, Reinach, Switzerland	Tospo-B

GRSV, *Groundnut ringspot virus*; INSV, *Impatiens necrotic spot virus*; IYSV, *Iris yellow spot virus*; TCSV, *Tomato chlorotic spot virus*; TSWV, *Tomato spotted wilt virus*; TYFRV, Tomato yellow fruit ring virus; WSMoV, *Watermelon silver mottle virus*. [a]an artificial mixture of TSWV-BR01, TCSV, GRSV and INSV, [b]recognizes serogroups I, II and III within the genus *Tospovirus*

collected from four fields in Tehran province of Iran. Each plant sample was placed in a separate plastic bag, immersed in ice and stored at 4°C until tested by enzyme-linked immunosorbent assay (ELISA) for the presence of different tospovirus species (Table 1).

Enzyme-linked immunosorbent assay (ELISA): Double antibody sandwich ELISA (DAS-ELISA) (Clark and Adams, 1977) was adopted for testing different tospoviruses by using specific polyclonal antibodies. Specific antisera against TYFRV and *Watermelon silver mottle virus* (WSMoV) were kindly provided by S. Winter (DSMZ, Braunschweig, Germany), whereas those for GRSV, INSV, IYSV, TCSV and TSWV were purchased from Loewe (Sauerlach, Germany) or Bioreba (Reinach, Switzerland) (Table 1). One hour after adding the substrate, the absorbance value ($A_{405\ nm}$) was measured by a microplate reader (Tecan Spectra, Austria). Reactions were considered positive if the readings were equal to or exceeded three times the mean value for negative control.

Host reaction studies: Symptomatic leaf samples from two ELISA-positive potato plants to TYFRV were macerated in 0.05 M sodium phosphate buffer containing 0.1% 2-mercaptoethanol (pH 7.0) and mechanically inoculated onto *Nicotiana rustica* plants dusted with carborundum. Systemic infections of these plants with TYFRV were investigated by ELISA. The two virus isolates, named TY-PF12 and TY-PF28, were isolated by three serial single-lesion inoculation passages on cowpea (*Vigna unguiculata*) leaves. In the host range studies, systemically infected *N. rustica* leaves were used for inoculating Amarathaceae, Chenopodiaceae, Cucurbitaceae, Brassicaceae, Fabaceae and Solanaceae plant species (Table 2). The inoculated plants were kept under greenhouse conditions and the presence of virus was ascertained by observing eventual local and systemic symptoms induced and by ELISA tests. In host range trials, 2-10 plants of each species or cultivar were inoculated and the tests were repeated twice or more if the results were negative or uncertain. The virus isolates were maintained in freeze-dried infected *N. rustica* leaf tissue and stored at -70°C.

Serological analyses: For their serological characterization, TY-PF12 and TY-PF28 isolates were further analysed by ELISA, using the available antisera against different tospoviruses (Table 1).

Reverse transcription-polymerase chain reaction (RT-PCR): Total RNA was extracted from 100 mg healthy and infected leaf tissue using the RNA extraction solution (RNX plus™, CinnaGen Inc., Tehran, Iran), as described by the supplier. RNA was finally precipitated by isopropanol, washed in 75% ethanol, resuspended in diethyl pyrocarbonate (DEPC)-treated water and stored at -70°C until use.

In this procedure, forward (TYf: 5'-TTTTTGTTTTTTGTTGTTTTTGGTTTTTTGTTTTTTGA-3') and reverse (TYr: 5'-AGAGCAATCGAGGTATAACACATT-3') primers previously described for amplification of a DNA fragment of approximately 1,200 bp corresponding to TYFRV-N gene (Golnaraghi *et al*., 2007) were used. These primers were synthesized by MWG-Biotech Co. (Germany). First single-strand cDNA (ss cDNA) was synthesized using 5 μL of template RNA (1.2 μg), 1 μL of the primer TYr (20 pmol μL^{-1}) and 1 μL of RevertAid™M-MuLV reverse transcriptase (200 u μL^{-1}) (Fermentas, Lithuania) in 20 μL reaction volume at 42°C for 60 min and then at 70°C for 10 min to inactivate the enzyme, according to the manufacturer's instructions. For the amplification reaction, ss cDNA was used as a template for the specific PCR using *Pfu* DNA polymerase (purchased from Cinnagen, Iran) and TYFRV specific primers. The PCR program consisted of 94°C for 1 min, 30 cycles of 94°C for 30 sec, 47°C for 1 min, 72°C for 1 min and finally 72°C for 10 min (Golnaraghi *et al*., 2007). Thermocycling was done in Primus (MWG Biotech Co., Germany). PCR products and DNA ladder (GeneRuler™ 100 bp DNA Ladder Plus, Farmentas) were fractionated by electrophoresis on a 1% agarose gel in the presence of 1 μg mL^{-1} ethidium bromide (Ausubel *et al*., 1993).

Cloning and sequencing: To confirm the RT-PCR result, the expected product of approximately 1,200 bp for TY-PF12 isolate was sequenced. The amplified DNA after PCR amplification was cleaned by Nucleospin (Macherey-Nagel, Germany) and cloned into pGEM-T Easy vector (Promega Crop., Madison, WI), according to the manufacturers' instructions. Sequences from both strands of the cloned DNA in three independent clones were determined by the custom sequencing service of MWG Biotech. Co. (Germany). The nucleotide sequence obtained for N gene of this isolate was compared with other sequences available in the GenBank using Basic Local Alignment Search Tool (BLAST) (Altschul *et al*., 1997) at the National Center for Biotechnology Information. The nucleotide sequence and deduced amino acid sequence also were aligned and compared with the same region of other tospoviruses available in the GenBank by the Clustal W (Thompson *et al*., 1994) using MegAlign program ver. 5.00 from the DNASTAR package (DNASTAR, Madison, WI, USA) (Burland, 2000).

Transmission through potato tubers: To study virus transmission through potato tubers as potential inoculum sources, 50 potato plants of cv. Agria which were positive in ELISA to TYFRV were selected in a field in Tehran province, marked by wooden flags and their tubers harvested at the end of the growing season. The potato tubers were evaluated for the presence of symptoms; stored at 4°C in dark conditions for two months, planted individually in 10 cm plastic pots containing pasteurised soil and maintained in an insect-proof greenhouse. In total, 461 seedlings were separately tested by ELISA for the presence of TYFRV.

RESULTS

In the survey carried out in the summer 2004, almost all plants grown in some fields in Tehran province were damaged and showed leaf and stem necrosis symptoms (Fig. 1c). Significant populations of *Thrips tabaci* were also found in the affected fields. Based on ELISA results, 100% of symptomatic potato leaf samples were positive to TYFRV. None of the samples reacted to the other tospoviruses tested.

Host range: In the host range study, TY-PF12 and TY-PF28 isolates infected *Chenopodium amaranticolor*, *Ch. quinoa*, *Cucumis sativus*, *Gomphrena globosa*, *N. tabacum* cvs. Samsun, *Phaseolus vulgaris*, *Solanum nigrum*, *Vicia faba*, *Petunia hybrida* and *Vigna unguiculata* plants, on which they induced chlorotic and/or necrotic local lesions. In *Datura metel*, *D. stramonium*, *Lycopersicon esculentum*, *Nicotiana benthamiana*, *N. glutinosa* and *N. rustica* the appearance of systemic symptoms of necrosis followed that of necrotic or chlorotic local lesions. No local or systemic symptoms were observed on *Brassica rapa*, *N. tabacum* cv. White Burley, *Raphanus sativus*, *Rapistrum rugosum* and *Sysimbrium loeselii*. When healthy potato plants (*Solanum tuberosum* cv. Agria) were artificially inoculated with these isolates, necrotic lesions followed by chlorosis and necrosis symptoms developed (Table 2 and Fig. 2),

Table 2: Symptomatology induced by the potato *Tospovirus* (TY-PF12) on indicator plants*

Indicator plant	Local reaction	Systemic reaction
Amaranthaceae		
Amaranthus sp.	NL	-
Gomphrena globosa	NL	-
Brassicaceae		
Brassica rapa	-	-
Raphanus sativus	-	-
Rapistrum rugosum	-	-
Sysimbrium loeselii	-	-
Chenopodiaceae		
Chenopodium amaranticolor	NL	-
Ch. quinoa	NL	-
Cucurbitaceae		
Cucumis sativus	CL, NL	-
Fabaceae		
Phaseolus vulgaris cv. Talash	NL	-
Vicia faba	NL	-
Vigna unguiculata cv. Mashad	CL, NL	-
Solanaceae		
Datura metel	NS	NS, LD, Su
D. stramonium	NS	NS, LD, TN
Lycopersicon esculentum	NS	LD, Su
Nicotiana benthamiana	CS	M, LD, Cl, N
N. glutinosa	NS	TN
N. rustica	CL, NL	CS, LD, StN, Su, M
N. tabacum cv. Samsun	NL	-
N. tabacum cv. White barley	-	-
Petunia hybrida	NL	-
Solanum nigrum	NL	-
S. tuberosum cv. Agria	NL	Cl, N

*The host reaction obtained for TY-PF28 was similar to those for TY-PF12; Cl: Chlorosis; CL: Chlorotic Lesions; CS: Chlorotic Spots; LD: Leaf Deformation; M: Mosaic; N: Necrosis; NL: Necrotic Lesions; NS: Necrotic Spots; StN: Stem Necrosis; Su: Stunting; TN: Top Necrosis; -: No infection

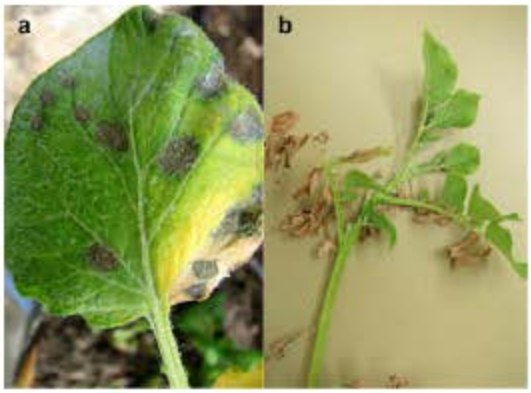

Fig. 2: Symptoms induced by mechanical inoculation of TYFRV (TY-PF12) on *Solanum tuberosum* cv. Agria: (a) necrotic lesions and (b) systemic necrosis

Table 3: Reaction ($A_{405\ nm}$) of the potato *Tospovirus* isolates (TY-PF12 and TY-PF28) with different tospovirus antibodies in double-antibody sandwich enzyme-linked immunosorbent assay formats[a]

Antibodies	Negative control	Positive control	TY-PF12	TY-PF28
GRSV-L	0.015	0.601	0.019	0.020
INSV-L	0.013	0.807	0.019	0.018
IYSV-L	0.016	1.194	0.016	0.021
TCSV-L	0.020	0.519	0.016	0.022
TSWV-L	0.015	1.080	0.018	0.016
TSWV-B	0.019	1.121	0.022	0.027
TYFRV-D	0.022	0.904	**1.021**	**1.144**
WSMoV-D	0.029	0.510	0.055	0.063

The highest absorbance value recorded for the two isolates is shown in bold. [a]The average of $A_{405\ nm}$ values 60 min after adding substrate is indicated

Table 4: Tospoviral N protein sequence identities

Virus[a]	ZLCV	CCSV	CSNV	GRSV	INSV	IYSV	MYSV	PBNV	PCFV	PhSMoV	PYSV	TCSV	TSWV	TYFRV	TY-PF12	WBNV	WSMoV	CaCV
ZLCV	100	24.2	79.6	74.5	51.9	30.0	28.1	27.3	13.5	28.5	17.9	74.1	73.7	29.2	29.2	26.2	26.9	26.9
CCSV		100	23.8	25.1	23.2	44.2	57.0	64.6	16.7	57.8	14.2	23.6	27.4	42.2	42.5	64.7	63.4	64.7
CSNV			100	73.0	53.8	30.4	30.0	26.5	16.2	30.4	15.4	74.1	75.7	29.6	29.6	25.8	26.5	26.2
GRSV				100	53.3	29.0	27.0	27.8	14.7	27.4	17.1	86.9	78.4	32.0	32.0	27.0	28.2	29.3
INSV					100	27.0	25.9	27.4	16.7	25.9	17.5	52.5	53.3	29.3	29.3	26.6	28.5	29.3
IYSV						100	46.0	43.1	16.3	46.4	14.2	31.3	33.2	72.3	71.9	40.1	40.5	42.3
MYSV							100	58.1	18.1	98.6	18.3	27.4	26.6	44.7	44.7	57.1	56.2	57.5
PBNV								100	16.7	58.8	17.5	27.4	29.3	41.8	42.2	82.9	83.3	82.2
PCFV									100	18.5	65.9	15.4	14.3	14.1	14.1	14.8	15.9	14.4
PhSMoV										100	18.7	27.8	27.0	45.5	45.5	57.8	56.9	58.5
PYSV											100	15.4	14.6	15.0	15.4	18.7	18.7	16.3
TCSV												100	79.5	32.8	32.8	26.6	26.6	29.0
TSWV													100	30.5	30.5	27.4	28.2	32.0
TYFRV														100	99.3	40.4	39.6	41.1
TY-PF12															100	40.7	40.0	41.5
WBNV																100	84.4	81.5
WSMoV																	100	86.2
CaCV																		100

[a]The tospovirus species referred to are: CCSV, Calla lily chlorotic spot virus (AY867502); CaCV, *Capsicum chlorosis virus* (DQ355974); CSNV, *Chrysanthemum stem necrosis virus* (AF067068); GRSV, *Groundnut ringspot virus* (AF513219); INSV, *Impatiens necrotic spot virus* (D00914); IYSV, *Iris yellow spot virus* (AF001387); MYSV, *Melon yellow spot virus* (AY673635); PBNV, *Peanut bud necrosis virus* (AY618564); PCFV, *Peanut chlorotic fanspot virus* (AF080526); PYSV, *Peanut yellow spot virus* (AF013994); PhSMoV, Physalis severe mottle virus (AF067151); TCSV, *Tomato chlorotic spot virus* (AF282982); TSWV, *Tomato spotted wilt virus* (AY856342); TYFRV, Tomato yellow fruit ring virus (AJ493270); WBNV, *Watermelon bud necrosis virus* (AF045067); WSMoV, *Watermelon silver mottle virus* (AY514627); ZLCV, *Zucchini lethal chlorosis virus* (AF067069) and TY-PF12 (DQ419916) in this study

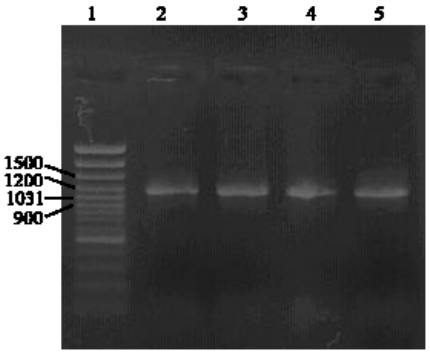

Fig. 3: Detection of TYFRV by RT-PCR. Ethidium bromide-stained agarose gel of PCR products. Lane 1, molecular weight DNA marker (GeneRuler™ 100 bp DNA Ladder Plus, Farmentas, Lithuania); lane 2-5, a fragment of approximately 1,200 bp obtained using TYf and TYr primers

resembling those observed in the field (Fig. 1). The experimental host range and the kind of symptoms observed were similar for both the isolates under study.

Serological analyses: To study the serological correlation of TY-PF12 and TY-PF28 isolates with other tospovirus species, inoculated *N. rustica* plants were tested in ELISA against antibodies to different tospoviruses (Table 1). Both isolates strongly reacted in DAS-ELISA when the TYFRV-antiserum (DSMZ, As-0526) was used; at contrary, no significant reactions were observed in ELISA tests using specific antibodies to GRSV, INSV, IYSV, TCSV, TSWV and WSMoV (Table 3).

RT-PCR and sequencing: DNA fragments with the expected size of approximately 1,200 bp were amplified by RT-PCR using the primer pairs TYf and TYr from the total RNAs extracted from *N. rustica* plants infected with TYFRV (Fig. 3). No products were obtained with RNAs from healthy plants. The nucleotide sequence of the TY-PF12 amplicon was determined (GenBank accession no. DQ419916) and compared with those in the GenBank using the BLAST algorithm. A high identity to TYFRV genome was revealed, thus confirming the results achieved by RT-PCR and that, this sequence

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1     TTTTTTTGGTTTTCATAAACTTATTTAAACACCCTTGAGTATATTTAAACACATAATTAAATGTGATAGAAAAAC
76    AAAACTTAAACACACTGATAAACTATATGGCACACAAAAAACTGAAATAAAATATTTAAATATATATAAGTAATA
151   AAACACACAATGACTAGAAGTACTTTAGTAATCTTAAATACTATATAATACTGAGTAATAGCAGAAAATGTTTTA
226   AAAACAGTAAAAGCACTCATTAAAATGCATCATTCTTTTTGGAAGATGGTTTGGGCTTTGAAGACCCAGCACCAT
                        *  F  A  D  N  K  K  S  S  P  K  P  K  S  S  G  A  G  N

301   TGTCATCAACAATTTTCCCAAAAGCACTTTCAAGAGCTTTAATTTGCTCATTGAACTTATTCAATGAAGCAGCAC
        D  D  V  I  K  G  F  A  S  E  L  A  K  I  Q  E  N  F  K  N  L  S  A  A  G

376   CAGCAGTCCCTGGAGTACAGTCATTTAGAATTTTTATGGTTTCTTCAAAAATGTCCTTGGAGACCCCAGTGAAAG
        A  T  G  P  T  C  D  N  L  I  K  I  T  E  E  F  I  D  K  S  V  G  T  F  T

451   TGAATTCTTTAGTTGCCATAACCCGGGCGATCTTGCACAGCTGTTCATAAGTTGAAAATTTGCTTATCCCCAAAG
        F  E  K  T  A  M  V  R  A  I  K  C  L  Q  E  Y  T  S  F  K  S  I  G  L  A

526   CTTCTTTTTTTACATTCTGGAAATAAGCCAATGGAAATGCAGTTGCAGCAAACTTATCAAGACTTGCCATCAAGG
        E  K  K  V  N  Q  F  Y  A  L  P  F  A  T  A  A  F  K  D  L  S  A  M  L  P

601   GGAGAGGGCCTCCTAGTGTTAGCATGACTCTAGCTGTAGTTGCATCAAATTTAGGGCTAGGTTTTAAACCATATG
        L  P  G  G  L  T  L  M  V  R  A  T  T  A  D  F  K  P  S  P  K  L  G  Y  A

676   CGTTTACCATTGGAAGCTCACAAAGTTTCTCATACATTTTCTGTTGCTCGCTTGCATTCTCCACAGCAATCAGTT
        N  V  M  P  L  E  C  L  K  E  Y  M  K  Q  Q  E  S  A  N  E  V  A  I  L  E

751   CCATGAACATTTTGGCTCTTATGAAGCCTTCCAACCTTCTGAATGTCCAATCATCTTGACCAGCGCTCACAGTTG
        M  F  M  K  A  R  I  F  G  E  L  R  R  F  T  W  D  D  Q  G  A  S  V  T  A

826   CACTGGGAACAATAATAGGATTGCCTTGGAATTTAAACTGGCCTGCTTTCACCATTTTGTAAATACCTGCTCTGT
        S  P  V  I  I  P  N  G  Q  F  K  F  Q  G  A  K  V  M  K  Y  I  G  A  R  N

901   TCCTTAAAATTGTGTAACCGTTGTTGAACGTCATTTTCATGGCTTTGTTAGCCAAAACAAACTCTTTGAAGTTGA
        R  L  I  T  Y  G  N  N  F  T  M  K  M  A  K  N  A  L  V  F  E  K  F  N  F

976   ATCCTGCAGTTTCTTCAGCTTCAATCACTACATCTGCTTCACCACCAGCAAGAAGCTTCTCAATGTTCTCTTTGC
        G  A  T  E  E  A  E  I  V  V  D  A  E  G  G  A  L  L  K  E  I  N  E  K  S

1051  TCACTCGTGCGGTAGACATGGTGTTTACTTAGGAAGCAGCTTACTTTGTGGAAGTTGTTTGAATTT
        V  R  A  T  S  M
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Fig. 4: Nucleotide sequence and deduced amino acid sequence of TY-PF12 N gene. The amino acid sequence was predicted from the complementary sense of the nucleotide sequence shown

corresponded to a tospoviral N gene. The sequence obtained (Fig. 4) was 1116 nucleotides in length and contained an ORF in the complementary strand starting with an AUG at nucleotide position 1069 and terminating with a UAA codon at position 245. The deduced N protein sequence was determined to be 274 residues long with a predicted molecular weight of about 30.1 KDa.

Computer analyses of the nucleotide sequence obtained for TY-PF12 revealed the highest identities to TYFRV (97.6%) and IYSV (75.9%). This sequence showed the lowest identities to *Peanut yellow spot virus*-PYSV (9.2%) and *Peanut chlorotic fanspot virus*-PCFV (3.9%).

Analyses of the deduced N protein showed the closest relationship of this isolate to the species TYFRV (99.3%) and IYSV (71.9%) and a distant relationship to PYSV (15.4%) and PCFV (14.1%) (Table 4).

Transmission through potato tubers: Symptoms of necrosis and malformation were observed in 7.2% of the tubers collected from TYFRV-infected potatoes. Also, in 11 cases (2.4%), tubers transmitted this virus to potato seedlings, that showed reduced growth and necrosis symptoms.

DISCUSSION

Very little information is available on the tospovirus infections of potato crops in the world. In the present study, the occurrence of a *Tospovirus* species in potato fields in Tehran province of Iran was demonstrated for the first time based on host range, serology and sequencing data. This virus caused severe leaf and stem necrosis on potatoes and sometimes the death of the whole plant. Electron microscope analysis showed the presence of enveloped spherical particles of *ca.* 100 nm in diameter in the infected material (data not shown), thus giving the first firm indication that a *Tospovirus* was involved (Fauquet *et al.*, 2005). The wide experimental host range of two isolates studied (TY-PF12 and TY-PF28) was similar to that described previously for TYFRV (Ghotbi *et al.*, 2005) and closely similar to that of not-infecting *Nicotiana* isolates from soybean (Golnaraghi *et al.*, 2002b; 2007). In serological studies, a panel of tospovirus-specific antibodies did not react with these isolates, such as it previously occurred for TYFRV (Winter *et al.*, 2006). Additional evidence of the identity of the studied viruses to TYFRV was obtained in RT-PCR,

using specific primers for amplifying TYFRV-N gene, since amplicons with the expected size of approximately 1,200 bp (Golnaraghi *et al*., 2007) were detected in infected plants. The high identity to TYFRV was further confirmed by sequencing of the amplified fragment.

During this survey, the totality of potato plants showing leaf and stem necrosis symptoms were infected with TYFRV. Similar symptoms were frequently observed in the main Iranian potato growing areas, which were often ascribed by farmers to other causal agents, e.g., *Alternaria*, or to senescence phenomena. Therefore, deeper studies devoted to the determination of the incidence and the distribution of this virus in Iranian potato fields and on its potential importance on different potato cultivars seem justified. Very low levels of virus transmission through potato tubers were also evidenced for the first time in this work, the epidemiological value of which remains anyway of great practical importance and interest since the infected tubers represent the primary foci from which TYFRV inoculum could be further spread by vectors, e.g., *Thrips tabaci* (Golnaraghi *et al*., 2007). The rate of virus transmission through potato tubers may be influenced by many factors, including the period of infection and the cultivar, as reported for other potato viruses (Hooker, 1990; Loenbenstein *et al*., 2001; Wilson, 2001); so, higher levels of such transmission can be expected. Furthermore, cutting tools also will help in getting much higher percentage of transmission to healthy tubers and this, affecting TYFRV epidemiology and wide spreading of the virus.

This research represents a preliminary step towards the characterization of tospoviruses in potato crops in Iran. Other results of the study not reported in this paper revealed considerable differences among the host range of various TYFRV-potato isolates and the lack detection with the primers TYf and TYr. Therefore, further investigation of the biological and molecular properties of this virus and improving the effective tools for virus identification will be necessary for the disease management.

ACKNOWLEDGMENTS

This research was supported by the Plant Virology Department of PPDRI (Tehran) and Science and Research Campus of Islamic Azad University (Tehran). We are grateful to Dr. K. Ohshima (Laboratory of Plant Virology, Faculty of Agriculture, Saga University, Saga, Japan) for designing primers and his kindly comments in this research. The authors also wish to thank Drs. M. Digiaro (Istituto Agronomico Mediterraneo, Valenzano, Italy) and H.R. Mojdehi for their kindly advice and to Dr. S. Winter (DSMZ, Germany) for providing some of the antisera and positive controls used in this study. This research was part of Ph.D thesis of the first author and carried out under a project of Iranian Nanotechnology Initiative entitled use of microarray technique for detection of important viruses of potato.

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