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Partial Biological and Molecular Characterization of Tomato yellow fruit ring virus Isolates from Potato

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Eight potato-producing provinces of Iran were surveyed during the growing seasons of 2004–2006 to detect the presence of Tomato yellow fruit ring virus (TYFRV), a tentative species in the genus *Tospovirus*. A total of 1,957 potato leaf samples were collected from plants with tospovirus-like symptoms of chlorotic or necrotic spots, chlorosis and necrosis. The samples were tested by enzyme-linked immunosorbent assay using TYFRV-specific antibodies. Among those tested, 498 samples (25.4%) were found to be infected with the virus. The virus was detected in 72.4% of the potato fields in all provinces surveyed. Thirteen potato isolates of TYFRV were selected for further biological and molecular studies. Based on their reactions on *Nicotiana tabacum* plants, the isolates were separated into two groups, namely L (local infection) and N (systemic infection). The nucleotide sequences of the nucleoprotein (N) genes of the isolates were determined and compared with the homologous sequences in Genbank. No recombination evidence was found in the isolates using different recombination-detecting programs. In the phylogenetic tree, the potato isolates fell into two major groups: IRN-1 and IRN-2 corresponding to the two biologically separated groups. This study shows for the first time the biological and phylogenetic relationships of geographically distant TYFRV isolates from potatoes in the mid-Eurasian country of Iran.

Keywords : evolution, phylogeny, potato, Tomato yellow fruit ring virus

The genus *Tospovirus* includes plant viruses that cause serious losses in several economically important crops

worldwide (Pappu et al., 2009; Whitfield et al., 2005); e.g. one billion dollars are the estimated annual loss caused by *Tomato spotted wilt virus* (TSWV), the type member of the genus (Scott, 2000). Tospoviruses are characterized by quasi-spherical enveloped particles, 80–120 nm in diameter. These viruses of the family *Bunyaviridae* have a genome consisting of three negative or ambisense single-stranded RNA segments designated L (large), M (medium) and S (small) (Fauquet et al., 2005). Typically, viruses from this genus are transmitted by phytophagous thrips species in a persistent propagative manner (Mound, 2005; Wijkamp et al., 1993); however, the possible thrips vector species of some tospoviruses have not yet been determined (Jones, 2005). These viruses cause necrosis, chlorosis, ring patterns, mottling, silvering, stunting and local lesions. Symptoms vary depending on the virus, host, season and environmental conditions (German et al., 1992; Mumford et al., 1996). Virus/vector specificity, host range, serological relationships and amino acid sequence identity of the viral N protein are important criteria for the species designation of tospoviruses; to date, eight tospoviruses as species and six as tentative species have been recognized (Goldbach and Kuo, 1996).

Tomato yellow fruit ring virus (TYFRV), a tentative species in the genus *Tospovirus* (Jones, 2005; Winter et al., 2002, 2006), has been found in ornamentals, tomato, peanut, soybean and some weed species in the mid-Eurasia of Iran (Golnaraghi et al., 2004; Ghotbi et al., 2005; Golnaraghi et al., 2008; Winter et al., 2002). TYFRV is transmitted in nature by thrips vectors, namely *Thrips tabaci*, and has spherical particles of ca. 100 nm (Golnaraghi et al., 2007b). Preliminary studies showed that TYFRV isolates from soybean, originally mistaken for TSWV, were biologically distinguishable based on their host range (Golnaraghi et al., 2002).

Potato (*Solanum tuberosum* L.) is an important field crop which is susceptible to infection by several viruses belonging to different genera and families, including tospoviruses (Loenenstein et al., 2001; Stevenson et al., 2001). Different

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Tospovirus species, including TSWV, *Groundnut ringspot virus* (GRSV), *Impatiens necrotic spot virus* (INSV), *Tomato chlorotic spot virus* (TCSV) and a species serologically related to *Peanut bud necrosis virus* (PBNV) have all been found to infect this crop worldwide (Jefferies, 1998; Loenbenstein et al., 2001; Perry et al., 2005). Tospovirus infection of potato was first reported in Iran by Pourrahim et al. (2001); the virus was originally misidentified and named as TSWV. The correct identity of the virus, TYFRV, and its presence were subsequently confirmed through biological, serological and molecular assays (Golnaraghi et al., 2007a; 2008). Studies have shown that TYFRV may induce leaf and stem necrosis as well as tuber necrosis symptoms on potato plants, is transmitted through potato tubers and is widely spread in Iranian potato fields (Golnaraghi et al., 2007a; Pourrahim et al., 2007). A microarray immunoassay procedure was developed for its detection (Golnaraghi et al., 2010). Recently, INSV and TSWV have also been reported to occur in Iranian potato fields (Pourrahim et al., 2012).

In the present study, the occurrence of TYFRV in some potato producing areas of Iran is reported. Since TYFRV was only recently reported on potato in the mid-Eurasia of Iran, the available data in regard to its biological and molecular characteristics are limited. For this reason, the biological properties of thirteen isolates of the virus from naturally infected potatoes in different potato growing areas of Iran were investigated. The complete nucleoprotein (N) gene sequences of these isolates were also determined and their phylogenetic relationships with other known isolates of the same virus were analyzed.

Materials and Methods

Collection of potato samples. Potato leaf samples were collected from the potato crop-producing areas of eight provinces of Iran during the growing seasons of 2004–2006 (Table 1, Fig. 1). Samples were collected from the potato plants showing tospovirus-like symptoms of chlorotic or necrotic spots, chlorosis and necrosis. The collected leaves were immediately placed in plastic bags and transported to the laboratory and kept at 4°C until use.

Serological virus identification. Collected samples were tested for TYFRV by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), as described previously by Clark and Adams (1977), using specific polyclonal antibodies against TYFRV N protein prepared by Golnaraghi (2009) or kindly provided by S. Winter (DSMZ, Braunschweig, Germany). These antibodies had no-cross reactivity with other *Tospovirus* species. Microtitre plates (Greiner, Germany) were coated with 100 µl of the

appropriate dilution of TYFRV-IgG in carbonate coating buffer (15 mM Na₂CO₃, 35 mM NaHCO₃, and 5 mM NaN₃, pH 9.6) and incubated overnight at 4°C. Leaves were ground (1:5 wt/vol) with a mortar and pestle in phosphate buffered saline (PBS) (2.7 mM KCl, 5 mM NaN₃, 8 mM Na₂HPO₄, 1 mM NaH₂PO₄, and 0.13 M NaCl) containing 2% polyvinylpyrrolidone (PVP)-24,000 and 0.05% Tween 20, pH 7.4. Plates were washed four times at 5-min intervals with washing buffer (PBS containing 0.05% Tween 20); leaf extracts were then added to the plates in duplicate wells (100 µl in each well) and incubated overnight at 4°C. After washing the plates, 100 µl of alkaline phosphatase-conjugated TYFRV-IgG diluted in conjugate buffer (PBS, pH 7.4, containing 2% PVP-24,000, 0.05% Tween 20, 0.2% bovine serum albumin, and 1 mM MgCl₂) was added and incubated 3 h at 37°C. Wells were washed and incubated with 100 µl of substrate (9.7% diethanolamine, pH 9.8, containing 1 mg/ml of *p*-nitrophenyl phosphate and 5 mM NaN₃) at room temperature. Absorbance values at 405 nm were measured using a microtiter plate reader (Multiscan-334, Lab System, Finland or Tecan Spectra, Austria). Absorbance values, measured 1 h after adding the substrate, equal to or greater than three times those of the negative controls (healthy) were considered positive.

Virus isolates and host tests. Potato leaf samples with a positive reaction in ELISA to TYFRV were homogenized in inoculation buffer (0.05 M sodium phosphate buffer containing 1.0% 2-mercaptoethanol, pH 7.0) and mechanically inoculated onto *Nicotiana rustica* and cowpea (*Vigna unguiculata*) plants dusted with carborundum. Altogether, 25 out of 498 ELISA-positive TYFRV isolates were selected, considering differences for origin and field symptoms. Each virus isolate was serially cloned through single isolations at least twice using *V. unguiculata*, and subsequently propagated in *N. benthamiana*, *N. glutinosa* or *N. rustica* plants. Systemically infected plants from each of the TYFRV isolates were used for inoculation on young plants of *Amaranthus* sp., *Gomphrena globosa* (Amaranthaceae), *Carthamus tinctorius*, *Helianthus annuus* (Asteraceae), *Brassica rapa*, *Raphanus sativus*, *Rapistrum rugosum*, *Sysimbrium loeselii* (Brassicaceae), *Chenopodium amaranticolor*, *C. quinoa* (Chenopodiaceae), *Citrus vulgaris*, *Cucumis sativus* (Cucurbitaceae), *Arachis hypogaea*, *Glycine max* cv. Hill, *Phaseolus aureus* cv. Gohar, *P. vulgaris* cv. Talash, *Pisum sativum*, *Vicia faba*, *Vigna unguiculata* cv. Mashad (Fabaceae), *Capsicum annum*, *Datura metel*, *D. stramonium*, *N. benthamiana*, *N. glutinosa*, *N. rustica*, *N. tabacum* cv. Samsun, *N. tabacum* cv. White Burley, *Petunia hybrida*, *Solanum lycopersicon*, *S. melongena*, *S. nigrum* and *S. tuberosum* cv. Agria (Solanaceae). Inoculated plants were kept for at least four weeks in a greenhouse at 25 ± 5

°C for symptoms observation, and subsequently tested by DAS-ELISA using TYFRV-specific antibodies.

Extraction of viral RNA and sequencing. Viral RNAs were extracted from TYFRV-infected tobacco leaves using the RNA extraction solution (RNX Plus™, CinnaGen Inc., Tehran, Iran). The RNAs were reverse transcribed and amplified using RevertAid™ M-MuLV reverse transcriptase (Fermentas, Lithuania) and *pfu* DNA polymerase (CinnaGen Inc.), as previously described (Golnaraghi et al., 2007b; 2008). The amplified DNA fragments were separated by electrophoresis in agarose gels and purified using Nucleospin (Macherey-Nagel, Germany). Sequences from each isolate were determined using two independent RT-PCR products. Using the PCR amplification primers mentioned above, each RT-PCR product was sequenced in both directions by the custom sequencing service of MWG-Biotech Co. (Ebersberg, Germany).

Recombination analyses. The N gene sequences of the thirteen TYFRV isolates from potato and of two isolates available from GenBank were used for evolutionary analyses (Table 2, Fig. 1). In this study, the *Iris yellow spot virus* (IYSV) sequence (GenBank access. no. AF001367), the closest *Tospovirus* species to TYFRV, was used as outgroup. All the sequences were aligned with the outgroup using CLUSTAL X (Jeanmougin et al., 1998) and TRANSALIGN (kindly supplied by Georg Weiller, Australian National University, Canberra, Australia) to maintain the degapped alignment. The aligned sequences were evaluated for incongruent relationships, which might have resulted from recombination, using RDP (Martin and Rybicki, 2000), GENECONV (Sawyer, 1999), BOOTSCAN (Salminen et al., 1995), MAXCHI (Maynard Smith, 1992), CHIMAERA (Posada and Crandall, 2001) and SISCAN programs (Gibbs et al., 2000) in RDP3 software (Martin et al., 2005). These analyses were performed using default settings for the different detection programs and a Bonferroni corrected *p*-value cut-off of 0.05 or 0.01.

Phylogenetic analyses. Phylogenetic grouping of the sequences under study were investigated by the maximum-likelihood (ML) algorithm of TREEPUZZLE version 5.0 (Strimmer and von Haeseler, 1996; Strimmer et al., 1997) and the neighbor-joining (NJ) algorithm of PHYLIP version 3.5 (Felsenstein, 1993). For ML analyses, 1,000 puzzling steps were calculated using the Hasegawa-Kishino-Yano model of substitution (Hasegawa et al., 1985). For NJ analyses, distance matrices were calculated by DNADIST with the Kimura two-parameter option (Kimura, 1980) and trees were constructed from these matrices by the NJ method (Saitou and Nei, 1987). A bootstrap value for each

internal node of the NJ trees was calculated using 1,000 random resamplings with SEQBOOT (Felsenstein, 1985). The calculated trees were shown by TREEVIEW (Page, 1996). Multiple sequence alignment was carried out using CLUSTAL X (Jeanmougin et al., 1998) or CLUSTAL W (Thompson et al., 1994) and TRANSALIGN, as above, to ensure that the nucleotide alignment was congruent with the deduced amino acids. The IYSV sequence was used as outgroup to construct the phylogenetic trees. The Kimura 2-parameter (Kimura, 1980) and Dayhoff PAM250 matrices (Dayhoff et al., 1983) were used respectively to estimate the genetic distances among the potato isolates using nucleotide and amino acid sequence data.

Results

Surveys and detection of TYFRV by DAS-ELISA. A total of 1,957 leaf samples collected from potato plants during 2004–2006 were tested by DAS-ELISA (Table 1). Based on the results, 498 samples (25.4%) had a positive reaction in ELISA to TYFRV. The virus was detected in 92 out of 127 fields (72.4%), representing all eight provinces surveyed. Infection rates of TYFRV varied in the different regions and among fields of the same region. The highest infection rate of TYFRV (100%) was recorded in some fields of Tehran, surveyed in the year 2004. In contrast, the infection rate recorded in Ardabil and Azarbayejan-e-sharqi was the lowest, 3.9% and 8.5%, respectively (Table 1, Fig. 1). Chlorosis, necrosis, necrotic sports on leaves and stem necrosis were associated with TYFRV infections (Fig. 2).

Host range and symptoms. Twenty four out of 32 plant species and cultivars mechanically inoculated were susceptible to the virus isolates studied. All isolates systemically infected *A. hypogaea*, *C. annuum*, *D. metel*, *D. stramonium*, *G. max* cv. Hill, *N. benthamiana*, *N. glutinosa* and *N. rustica*. Local lesions were observed on inoculated leaves of *Amaranthus* sp., *C. amaranticolor*, *C. quinoa*, *C. sativus*, *H. annuus*, *P. hybrida*, *P. aureus* cv. Gohar, *P. vulgaris* cv. Talash, *V. faba*, *V. unguiculata* cv. Mashad and *S. nigrum*. Some isolates (TY-PE14, TY-PF36, TY-PKh116) induced systemic necrosis in addition to necrotic lesions on *G. globosa*. TY-PF36, TY-PT108 and TY-PL89 induced only necrotic local lesions on *S. lycopersicon*, contrary to the other isolates that induced also systemic symptoms. The isolates tested were divided into two groups according to their capability to infect *N. tabacum* cvs. Samsun and White Burley only locally (L group) or also systemically (chlorotic and necrotic symptoms, N group) (Tables 2, 3). Artificially inoculated healthy potato plants (*S. tuberosum* cv. Agria) showed necrotic lesions followed by systemic chlorosis and

Table 1. Laboratory test results of potato leaf samples collected from 127 fields in different provinces of Iran in the years 2004–2006^a

Province	Year	No. of fields	No. of samples	Infection		
				No. of infected plants (%)	Range of infection (%)	No. of infected fields (%)
Ardabil	2005	18	282	11 ^b (3.9) ^c	0.0–15.0 ^d	6 ^e (33.3) ^f
Azarbayejan-e-sharqi	2005	11	141	12 (8.5)	0.0–20.0	6 (54.5)
Esfahan	2005	19	291	32 (11.0)	0.0–40.0	13 (68.4)
Hamedan	2006	17	300	87 (29.0)	0.0–56.7	15 (88.2)
Kerman	2006	16	272	128 (47.1)	6.7–76.0	16 (100)
Khorasan	2005	18	260	54 (20.8)	0.0–36.4	14 (77.8)
Lorestan	2005	10	137	25 (18.2)	0.0–35.7	6 (60.0)
Tehran	2004	4	95	95 (100)	100	4 (100)
	2006	14	179	54 (30.2)	0.0–68.4	12 (85.7)
Total		127	1,957	498 (25.4)	0.0–100	92 (72.4)

^a Identification was based on serological reactions (enzyme-linked immunosorbent assay).^b Number of infected potato leaf samples (infection rate)^c Percent of infected potato leaf samples [infection rate (%)]^d Range of percent virus infection rate^e Number of fields with virus infection^f Percent of infected potato fields **Fig. 1.** Map of Iran showing the location of potato-producing provinces where the potato isolates of Tomato yellow fruit ring virus were obtained. 1, Azarbayejan-e-sharqi; 2, Ardabil; 3, Tehran; 4, Hamedan; 5, Lorestan; 6, Esfahan; 7, Khorasan; 8, Kerman. Courtesy of Farzadfar et al. (2002).

necrosis. However, N group isolates induced more severe symptoms (severe necrosis) on potato, similar to those observed on inoculated *Datura* plants. Infections were confirmed by positive reaction in ELISA. No infection was induced on *B. rapa*, *C. tinctorius*, *C. vulgaris*, *P. sativum*, *R. sativus*, *R. rugosum*, *S. melongena* and *S. loeselii* by the isolates studied, as ascertained by symptoms observation on inoculated herbaceous hosts, ELISA testing and bioassay on *V. unguiculata*. The host reaction of six potato isolates of TYFRV was shown in Table 3.

Fig. 2. Field symptoms associated with Tomato yellow fruit ring virus (TYFRV) infections on potato: a, necrotic spots on leaves; b, leaf and stem necrosis.

Nucleotide sequences. The N gene nucleotide sequence of the TYFRV isolates was determined and it was 825 nts long. The BLAST search results confirmed that these sequences encompass the entire N ORF encoding the nucleocapsid protein. The N protein sequences were determined to be 274 residues long with a predicted molecular weight of 30.0–30.1 kDa. The complete N gene sequences of TYFRV isolates determined in this study were deposited in the GenBank database (Table 2).

Recombination analysis. The nucleoprotein gene sequence of the TYFRV isolates was evaluated for evidence of recombination. After removing all gaps and nucleotides homo-

Table 2. Tomato yellow fruit ring virus isolates analyzed in this study

Isolate	Original host	Location (city, province)	Year of collection	Host type*	Accession no.
TY-PAr72	<i>Solanum tuberosum</i>	Ardabil, Ardabil	2005	L	JF750337
TY-PAzs121	<i>S. tuberosum</i>	Ajabshir, Azarbayejan-e-sharqi	2005	N	JF750339
TY-PE14	<i>S. tuberosum</i>	Fereydan, Esfahan	2005	N	JF750345
TY-PF12	<i>S. tuberosum</i>	Frouzkooh, Tehran	2004	L	DQ419916
TY-PF36	<i>S. tuberosum</i>	Frouzkooh, Tehran	2004	N	EU126931
TY-PH132	<i>S. tuberosum</i>	Bahar, Hamedan	2006	N	JF750344
TY-PH60	<i>S. tuberosum</i>	Kaboudar-ahang, Hamedan	2006	N	JF750346
TY-PK28	<i>S. tuberosum</i>	Jiroft, Kerman	2006	L	JF750338
TY-PK95	<i>S. tuberosum</i>	Jiroft, Kerman	2006	N	JF750342
TY-PKh116	<i>S. tuberosum</i>	Jolgeh-rokh, Khorasan	2005	N	JF750341
TY-PL89	<i>S. tuberosum</i>	Khorram-abad, Lorestan	2005	N	JF750343
TY-PT108	<i>S. tuberosum</i>	Damavand, Tehran	2006	N	JF750340
TY-PT65	<i>S. tuberosum</i>	Damavand, Tehran	2006	L	JF750336
TY-SM23	<i>Glycine max</i>	Sari, Mazandaran	2004	L	EF157855
TY-Tom	<i>S. lycopersicum</i>	Varamin, Tehran	2002	L	AJ493270

*L, isolates infecting *Nicotiana tabacum* cvs. Samsun and White Burley only locally; N, isolates infecting the same hosts systemically (chlorosis and necrosis).

logous to them from the aligned sequences, the possible recombination sites were assessed using RDP3. Finally, no 'clear' recombination (P value of $< 1 \times 10^{-6}$) was found in the N gene sequences of the isolates under study, therefore the isolates seemed not to be recombinants.

Phylogenetic relationships. As no recombination was found among the sequences studied, all of the fifteen sequences were used for phylogenetic analysis. Multiple sequence alignment using the CLUSTAL W program showed that the N gene sequence of the TYFRV isolates (Table 2) shared 85.6–100% and 90.8–100% identities at nucleotide and amino acid sequence levels, respectively. Long stretches of conserved amino acid sequence and several amino acid substitutions were found among the 15 TYFRV isolates, some of them were completely different among the isolates belonging to different phylogenetic groups described below (Fig. 3). The relationships of these isolates were investigated by maximum likelihood (ML) and neighbor joining (NJ) methods and the ML tree is shown in Fig. 4. All of the trees partitioned the sequences into two major groups, named as IRN-1 and IRN-2, which were supported by high bootstrap values. The TYFRV isolates from potato fell into both groups. As the isolates were found to be non-recombinant, we calculated the nucleotide and amino acid genetic distances among the potato isolates using Kimura 2-parameter (Kimura, 1980) and Dayhoff PAM250 matrices (Dayhoff et al., 1983). The results showed that the potato isolates in each group were most closely related to each other, than to the isolates

clustered in the other group (Table 4).

Discussion

In this study, the main potato producing areas were surveyed in the period of 2004–2006 for the presence of TYFRV and the virus was found to be widespread in the mid-Eurasia, Iran; this may reflect both a very high inoculum reservoir, i.e. a wide range of host plants and infected potato tubers, and the prevalence and efficiency of the thrips vectors (Ghotbi et al., 2005; Golnaraghi et al., 2007a; 2007b; 2008). We found the lowest rates of TYFRV infections in areas with severe winters (e.g. Ardabil). In contrast, the virus infection rate in Kerman was recorded at high levels where winters are moderate, giving rise to high populations of thrips vectors. The infection rates recorded for Tehran province significantly differed in the 2004 and 2006 surveys (Table 1). This revealed that the infection rates may dramatically change year by year (Pappu et al., 2009; Scott, 2000). Further studies on the epidemiological aspects of TYFRV are necessary. We also showed that the virus plays an important role in necrosis diseases on potatoes in Iran; such symptoms are frequently mistakenly attributed by farmers to the potato early or late blight diseases caused by *Alternaria solani* and *Phytophthora infestans*, respectively, pubescence and some environmental conditions. However, the role of other potato infecting tospoviruses/viruses in such diseases remains to be determined.

In the present work, biological and molecular characteristics of thirteen TYFRV isolates from potato plants in

Table 3. Symptoms induced by different potato isolates of Tomato yellow fruit ring virus on indicator plants

Indicator plant	Isolate					
	N group ^a			L group ^b		
	TY-PF36	TY-PE14	TY-PKh116	TY-PAr72	TY-PF12	TY-PK28
Amaranthaceae						
<i>Amaranthus</i> sp.	NL/- ^d	NL/-	NL/-	NL/-	NL/-	NL/-
<i>Gomphrena globosa</i>	NL/N	CL/N	NL/N	NL/-	NL/-	NL/-
Asteraceae						
<i>Carthamus tinctorius</i>	-/-	-/-	-/-	-/-	-/-	-/-
<i>Helianthus annuus</i>	NL/-	NL/-	-/-	NL/-	NL/-	NL/-
Brassicaceae						
<i>Brassica rapa</i>	-/-	-/-	-/-	-/-	-/-	-/-
<i>Raphanus sativus</i>	-/-	-/-	-/-	-/-	-/-	-/-
<i>Rapistrum rugosum</i>	-/-	-/-	-/-	-/-	-/-	-/-
<i>Sisymbrium loeselii</i>	-/-	-/-	-/-	-/-	-/-	-/-
Chenopodiaceae						
<i>Chenopodium amaranticolor</i>	NL/-	NL/-	NL/-	NL/-	NL/-	NL/-
<i>C. quinoa</i>	NL/-	NL/-	NL/-	NL/-	NL/-	NL/-
Cucurbitaceae						
<i>Citrulus vulgaris</i>	-/-	-/-	-/-	-/-	-/-	-/-
<i>Cucumis sativus</i>	CL, NL/-	CL/-	NL/-	CL, NL/-	CL, NL/-	CL, NL/-
Fabaceae						
<i>Arachis hypogaea</i>	-/Cl, N	-/Cl, N	-/Cl, N	-/Cl, N	-/Cl, N	-/Cl, N
<i>Glycine max</i> cv. Hill	CL, NL/Mo, Cl, N	CL, NL/Mo, Cl, N	CL, NL/Mo, Cl, N	CL, NL/Mo, Cl, N	CL, NL/Cl, N	CL, NL/Mo, Cl, N
<i>Phaseolus aureus</i> cv. Gohar	NL/-	CL/-	NL/-	NL/-	NL/-	NL/-
<i>P. vulgaris</i> cv. Talash	NL/-	NL/-	CL/-	NL/-	NL/-	NL/-
<i>Pisum sativum</i>	-/-	-/-	-/-	-/-	-/-	-/-
<i>Vicia faba</i>	NL/-	NL/-	NL/-	NL/-	NL/-	NL/-
<i>Vigna unguiculata</i> cv. Mashad	CL, NL/-	NL/-	CL, NL/-	CL/-	CL, NL/-	CL, NL/-
Solanaceae						
<i>Capsicum annum</i>	-/M	-/M	-/M	-/M	-/M	-/M
<i>Datura metel</i>	NS/M, LD, SN	NS/M, SN	NS/M, LD, SN	NS/NS, LD	NS/NS, LD, Su	NS/NS, Su
<i>D. stramonium</i>	NS/Mo, LD, N	NS/M, LD, N	NS/M, LD, N	NS/NS, LD, N	NS/NS, LD, TN	NS/NS, LD, N
<i>Nicotiana benthamiana</i>	CS/M, LD, Cl, N	CS/M, LD, Cl, N	CS/M, LD, Cl, N	CS/M, LD, N	CS/M, LD, Cl, N	CS/M, LD, N
<i>N. glutinosa</i>	NS/TN	NS/TN	NS/TN	NS/TN	NS/TN	NS/TN
<i>N. rustica</i>	CL, NL/CS, LD, Su, M	NL/CS, LD, Su, M	NL/LD, Su, M	NL/LD, M	CL, NL/CS, LD, StN, Su, M	CL, NL/CS, LD, M
<i>N. tabacum</i> cv. Samsun	CL, NL/N	NL/N	NL/N	NL/-	NL/-	NL/-
<i>N. tabacum</i> cv. White Burley	-/Cl, N	-/Cl, N	-/Cl, N	-/-	-/-	-/-
<i>Petunia hybrida</i>	NL/-	NL/-	NL/-	NL/-	NL/-	NL/-
<i>Solanum lycopersicum</i>	NL/-	NS/LD, Su	CS/LD, Su	NS/LD, Su	NS/LD, Su	NS/LD, Su
<i>Solanum melongena</i>	-/-	-/-	-/-	-/-	-/-	-/-
<i>Solanum nigrum</i>	NL/-	NL/-	NL/-	NL/-	NL/-	NL/-
<i>Solanum tuberosum</i> cv. Agria	NL/Cl, SN	NL/Cl, SN	NL/Cl, SN	NL/Cl, N	NL/Cl, N	NL/Cl

^a Isolates infecting *Nicotiana tabacum* cvs. Samsun and White Burley systemically (chlorosis and necrosis); ^b Isolates infecting the same hosts only locally; ^c Local infection; ^d Systemic infection; Cl, chlorosis; CL, chlorotic lesions; CS, chlorotic spots; LD, leaf deformation; M, mosaic; Mo, mottling; N, necrosis; NL, necrotic lesions; NS, necrotic spots; SN, severe necrosis; StN, stem necrosis; Su, stunting; TN, top necrosis; -, no infection.

Recombination plays a pivotal role in diversification and evolution of both plant RNA and DNA viruses (Ohshima et al., 2007; Prasanna and Rai, 2007). In this study, none of the TYFRV isolates seemed to be recombinants. The phylogenetic analysis of the virus isolates based on the nucleotide sequences of nucleoprotein genes showed a clustering of the isolates in two separate branches, i.e. IRN-1 and IRN-2. Four out of thirteen potato isolates studied clustered with the two isolates from soybean and tomato in one branch (IRN-1); the remainder of the potato isolates was clustered in a separate branch, IRN-2 (Fig. 4). The phylogenetic groups of the isolates, namely IRN-1 and IRN-2, closely corresponded to the L and N biological groups, respectively.

Fig. 4. A maximum likelihood (ML) phylogenetic tree showing the relationship among potato isolates of Tomato yellow fruit ring virus (TYFRV) with the other isolates. The tree was constructed from nucleoprotein (N) gene sequences of all isolates determined in this study and in previous studies (Golnaraghi et al., 2007a, 2007b, 2008; Winter et al., 2002, 2006). Numbers at each node indicate the percentage of supporting puzzling steps (or bootstrap samples) in ML and NJ methods, respectively (only values > 50 are shown). Horizontal branch lengths were drawn to scale with the bar showing 0.1 nt replacements per site. The homologous sequence of one isolate of *Iris yellow spot virus* (IYSV) was used as the outgroup. The name of each isolate, its province of origin, original host plant, year of isolation, host type and accession number in the Genbank were listed.

(Table 2). These data seem to indicate the important role of “host” in the evolution of TYFRV populations, as reported previously for other viruses (Ohshima et al., 2007). The amino acid sequence comparison of nucleoprotein genes of the isolates between the two groups showed 90.8–92.7% identities, which are close to the threshold value used for species determination in the genus *Tospovirus* (90.0%) (Goldbach and Kuo, 1996). It will be of great interest to search for more isolates of the virus from Iran and its neighboring countries for further evolutionary studies. Also, whether the two different isolate groups are vectored by different thrips species should be studied in the future.

To address the lack of information on the population of potato isolates of TYFRV, the nucleotide and amino acid sequence distances among these isolates were calculated for

the N gene (Table 4). High similarities resulted among the potato isolates in IRN-2a subgroup, as shown in the ML tree in Fig. 4; some of them came from geographically distant regions (Table 2, Fig. 1). This similarity can be due to the transmission of TYFRV through transport of propagative materials, i.e. potato tubers, between locations (Golnaraghi et al., 2007a). In Iran, certification and entry of seed tuber lots in the seed program depends mainly on visual and field inspections. Moreover, many of Iranian were potato producers use their own seed tubers of uncertain quality. The difficulties associated with postharvest ELISAs limit their use in developing a reliable seed certification procedure in Iran (Pourrahim et al., 2007). These coupled with low concentration and uneven distribution of tospoviruses in potato sprouts (Wilson, 2001) finally result in no-

Table 4. Comparison of the genetic distances among the N gene of Iranian Tomato yellow fruit ring virus isolates from potato based on nucleotide and amino acid sequences^a

Isolate	TY-PT65	TY-PAr72	TY-PK28	TY-PF12	TY-PT108	TY-PF36	TY-PKh116	TY-PK95	TY-PL89	TY-PH132	TY-PE14	TY-PH60	TY-PAzs121
IRN-1													
TY-PT65	–	0.01050	0.01050	0.01050	0.08887	0.08887	0.08494	0.08502	0.08888	0.08502	0.08502	0.08502	0.09287
TY-PAr72	0.02096	–	0.00001	0.00001	0.07711	0.07711	0.07325	0.07331	0.07712	0.07331	0.07331	0.07331	0.08104
TY-PK28	0.02223	0.01845	–	0.00001	0.07711	0.07711	0.07325	0.07331	0.07712	0.07331	0.07331	0.07331	0.08104
TY-PF12	0.02096	0.00000	0.01845	–	0.07711	0.07711	0.07325	0.07331	0.07712	0.07331	0.07331	0.07331	0.08104
IRN-2													
TY-PT108	0.16027	0.14666	0.15237	0.14666	–	0.01416	0.01060	0.01773	0.02131	0.01773	0.01773	0.01773	0.02494
TY-PF36	0.15865	0.14799	0.15077	0.14799	0.00611	–	0.01060	0.01773	0.02132	0.01773	0.01773	0.01773	0.02495
TY-PKh116	0.15718	0.14654	0.14932	0.14654	0.00488	0.00366	–	0.01415	0.01772	0.01415	0.01415	0.01415	0.02133
TY-PK95	0.14521	0.13906	0.14037	0.13906	0.02726	0.02850	0.02724	–	0.00352	0.00001	0.00001	0.00001	0.00706
TY-PL89	0.14666	0.14050	0.14182	0.14050	0.02852	0.02976	0.02850	0.00122	–	0.00352	0.00352	0.00352	0.01062
TY-PH132	0.14521	0.13906	0.14037	0.13906	0.02726	0.02850	0.02724	0.00000	0.00122	–	0.00001	0.00001	0.00706
TY-PE14	0.14521	0.13906	0.14037	0.13906	0.02726	0.02850	0.02724	0.00000	0.00122	0.00000	–	0.00001	0.00706
TY-PH60	0.14521	0.13906	0.14037	0.13906	0.02726	0.02850	0.02724	0.00000	0.00122	0.00000	0.00000	–	0.00706
TY-PAzs121	0.14812	0.14194	0.14326	0.14194	0.02978	0.03102	0.02976	0.00244	0.00366	0.00244	0.00244	0.00244	–

^aThe nucleotide (top) and amino acid (bottom) sequence distances among the potato isolates were assessed using Kimura two-parameter (Kimura, 1980) and Dayhoff PAM250 matrices (Dayhoff et al., 1983). Sources of the sequences referred are as follows: TY-PT65 (JF750336), TY-PAr72 (JF750337), TY-PK28 (JF750338), TY-PF12 (DQ419916), TY-PT108 (JF750340), TY-PF36 (EU126931), TY-PKh116 (JF750341), TY-PK95 (JF750342), TY-PL89 (JF750343), TY-PH132 (JF750344), TY-PE14 (JF750345), TY-PH60 (JF750346), and TY-PAzs121 (JF750339) studied in this work.

detection or underrating of virus infection in seed tuber lots. Therefore, further studies on the development, optimization and promotion of sensitive and accurate diagnostic tools to use in the Iranian seed potato certification program seem justifiable.

We showed the widespread occurrence and the importance role of TYFRV in necrosis diseases on potatoes in the mid-Eurasia of Iran. To our knowledge, this is the first work showing the biological properties and the evolutionary relationships of geographically distant isolates of the virus in this region. However, many questions remain unanswered that relate to epidemiological and managerial aspects of the virus disease control. Further studies on tospovirus-infections in Iranian potato crops are necessary.

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