Characterization and Genetic Structure of a *Tospovirus* Causing Chlorotic Ring Spots and Chlorosis Disease on Peanut; Comparison with Iranian and Polish Populations of Tomato yellow fruit ring virus

A. Golnaraghi,[†] Department of Plant Protection, Faculty of Agricultural Sciences and Food Industries, Science and Research Branch, Islamic Azad University, P.O. Box 14515-775, Tehran, Iran; N. Shahraeen, Department of Plant Virus Research, Iranian Research Institute of Plant Protection, Agricultural Research, Education & Extension Organization, P.O. Box 19395-1454, Tehran, Iran; and H. D. Nguyen, Department of Plant Pathology, Faculty of Agronomy, Vietnam National University of Agriculture, Trau Quy, Gia Lam, Hanoi, Vietnam

Abstract

A *Tospovirus* species was isolated from peanut plants showing chlorotic ring spots and chlorosis, and identified as Tomato yellow fruit ring virus (TYFRV) on the basis of its biological, serological, and molecular properties. In host range studies, a broad range of indicator plants was infected by the five isolates studied; all the isolates systemically infected *Nicotiana tabacum* cultivars and, thus, they were classified into the N-host-infecting type isolates of the virus. These isolates strongly reacted with TYFRV antibodies but not with the specific antibodies of other tospoviruses tested. Recombination analyses showed that the nucleoprotein gene of the peanut isolates and other isolates studied were nonrecombinant. In phylogenetic trees, the virus isolates were clustered in three

genogroups: IRN-1, IRN-2, and a new group, POL; the peanut isolates fell into IRN-2 group. Multiple sequence alignments showed some genogroup-specific amino acid substitutions among the virus isolates studied. The results revealed the presence of negative selection in TYFRV populations. Also, the Iranian populations had higher nucleotide diversity compared with the Polish population. Genetic differentiation and gene flow analyses indicated that the populations from Iran and Poland and those belonging to different genogroups were partially differentiated populations. Our findings seem to suggest that there has been frequent gene flow between some populations of the virus in the mid-Eurasian region of Iran.

Tospoviruses (family *Bunyaviridae*) can infect more than 1,000 plant species belonging to different plant genera and families; some of them are agriculturally important crop plants (Parella et al. 2003; Whitfield et al. 2005). These viruses have quasisphepherical, enveloped particles of approximately 80 to 120 nm in diameter. Their genome consists of one negative and two ambisense single-stranded RNA segments; namely, L, M, and S, respectively. Natural transmission of tospoviruses by different species of the genera *Ceratopthripoides*, *Frankliniella*, *Scirtothrips*, and *Thrips* in a circulative and propagative manner have been previously indicated; however, no data are available on the possible thrips transmission of some *Tospovirus* species (King et al. 2012; Wijkamp et al. 1993). Furthermore, these viruses can be transmitted through propagating plant materials (e.g., seed tubers) (Wilson 2001) and even true seed (Groves et al. 2016). Plant host range, vector transmission, serological properties, and sequence identity of viral nucleoprotein (N protein) have been approved by the International Committee on Taxonomy of Viruses (ICTV) for the species demarcation of these viruses; 23 confirmed or tentative *Tospovirus* species have been listed until now (King et al. 2012).

In 2002, a new *Tospovirus* species infecting tomato plants in Varamin County (Tehran Province of Iran) was identified and named as Tomato yellow fruit ring virus (TYFRV) or Tomato Varamin virus (ToVV) (Winter et al. 2002) (GenBank accession numbers AJ493270 and AJ493271). The virus is also referred to as Tomato fruit yellow ring virus in some literature (Winter et al. 2006). TYFRV has quasisphepherical virions (approximately 100 nm in diameter) and can

infect pepper, potato, soybean, ornamental plants, and weeds under natural conditions (Ghotbi et al. 2005; Golnaraghi et al. 2007a,b, 2008, 2013); however, the virus was originally mistaken for *Tomato spotted wilt virus* (TSWV) in some earlier reports (Golnaraghi et al. 2002b, 2004; Pourrahim et al. 2001). Serological assays, using antibodies produced against TYFRV N protein, detected the virus in adults of *Microcephallothrips abdominalis* and *Thrips tabaci* (Ghotbi et al. 2005); the latter was then determined as the insect vector of the virus through greenhouse experiment studies (Golnaraghi et al. 2007b). More importantly, it was also shown that TYFRV could be transmitted through propagative plant materials (i.e., potato tubers) (Golnaraghi et al. 2007a) which may, in turn, facilitate long-distance spread of the virus. No significant serological relationship between TYFRV and some confirmed *Tospovirus* species was found in enzyme-linked immunosorbent assays (ELISA). Moreover, the isolates belonging to different genogroups (virus isolates in the same phylogenetic cluster) or host types seem to have very close serological relationships (Golnaraghi 2009; Winter et al. 2002, 2006). Preliminary studies demonstrated that soybean isolates of the virus could be categorized based on their reactions on different host plants (Golnaraghi et al. 2002b). Further investigations confirmed these results and showed the biological classification of TYFRV isolates into two host-infecting types: host type L (local infection; isolates infected *Nicotiana tabacum* plants only locally [by producing local lesions]) and N (systemic infection; isolates infected such plants systemically). Interestingly, phylogenetic analyses using different Iranian isolates of the virus indicated two main TYFRV genogroups, IRN-1 and IRN-2, which were related to their biological host types L and N, respectively, suggesting the possible role of “host” in the virus evolution (Pourrahim et al. 2012b). A closely similar virus but with a different name (i.e., Tomato yellow ring virus [TYRV]) also has been reported from the mid-Eurasian region of Iran (Hassani-Mehraban et al. 2005) and, recently, from Africa (Kenya) and Europe (Poland) (Birithia et al. 2012; Zarzyńska-Nowak et al. 2016).

Peanut (*Arachis hypogaea*) is considered to be an important agricultural crop in Iran and is mostly cultivated in the north parts of the country. The area covered by this crop in Iran has increased from 1,311 ha in 2000 to 1,443 ha in 2014, with 3,444 and 3,748 tons of production, respectively (FAO 2000, 2014). Peanut crops can be

[†]Corresponding author: A. Golnaraghi; E-mail: agolnaraghi@yahoo.com

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infected by several plant viruses under natural conditions, including six tospoviruses: *Groundnut bud necrosis virus* (GBNV), *Groundnut chlorotic fan-spot virus* (GCFSV), *Groundnut ringspot virus* (GRSV), *Groundnut yellow spot virus* (GYSV), *Impatiens necrotic spot virus* (INSV), and TSWV (Sreenivasulu et al. 2008). These viruses are considered to be the most devastating viruses in peanut worldwide (Adkins 2000).

In recent years, *Tospovirus*-like symptoms of chlorotic ring spots, chlorosis, and necrosis have been frequently observed in Iranian peanut fields. Tospovirus infection of peanut (i.e., TSWV) was first reported in Golestan Province in 2001. Severe stunting, mosaic, bud necrosis, and chlorosis symptoms were associated with these infections (Golnaraghi et al. 2001). GBNV also was detected in plants showing mosaic, mottle, ring mosaic, stunting, and bud necrosis symptoms (Golnaraghi et al. 2002a). Although greenhouse studies have shown that TYFRV can infect and produce severe disease symptoms on peanut plants (Golnaraghi et al. 2007a,b, 2008; Pourrahim et al. 2012b), no data are available on the disease caused under natural conditions and about different characteristics of the peanut isolates of the virus.

In this work, a new tospovirus disease on peanut plants was described. The causal agent of the disease was recognized as TYFRV on the basis of host range studies and serological analysis. Complete nucleotide sequencing of the nucleoprotein (N) gene for five peanut isolates followed by phylogenetic analysis confirmed the virus species identification. In these studies, the corresponding sequences of TYRV isolates also were used. We compared these sequences and other sequences from Iran, Poland, and Kenya for the evolutionary characterization of the virus. Our studies, to our knowledge, provide the first in-depth information about the genetic structure of the virus populations. These studies are important to design and develop new strategies to control the virus disease, and to optimize diagnostic systems for the fast and accurate detection of different isolates of the virus.

Materials and Methods

Plant sampling. During the growing seasons of 2004 to 2005, surveys were done and 102 peanut leaf samples were collected from plants showing chlorotic and ring spots, chlorosis, necrosis, mosaic, mottle, bud necrosis, and stunting symptoms in eight fields in Golestan and Mazandaran, the two peanut-producing provinces in the north of Iran (Table 1). The samples were transported to the laboratory and kept at 4°C until tested by ELISA using different specific and broad-spectrum tospovirus antibodies (Supplementary Table S1).

Serological virus identification. The occurrence of tospovirus infections in the collected leaf samples was first analyzed by double-antibody sandwich ELISA (Clark and Adams 1977) using polyclonal antibodies produced against N protein of different tospoviruses belonging to different serogroups. Specific antibodies to GRSV, INSV, *Iris yellow spot virus* (IYSV), *Tomato chlorotic spot virus* (TCSV), and TSWV were purchased from Loewe (Sauerlach, Germany) or Bioreba (Reinach, Switzerland). Moreover, TYFRV- and *Watermelon silver mottle virus* (WSMoV)-specific antibodies were kindly gifted by S. Winter (DSMZ, Braunschweig, Germany). Broad-spectrum antibodies reacting with *Tospovirus* serogroups I, II, and

III, provided by Bioreba, also were used in ELISA tests. Serological assays were done according to the manufacturers' instructions using buffers and controls provided by the suppliers. Finally, a sample was considered infected (positive) if its optical density (OD) value at 405 nm was equal to or exceeded three times the mean value of the healthy plant (negative controls) 1 h after adding the substrate (*p*-nitrophenyl phosphate). The OD values were measured using a microtiter plate reader (BioTek ELx 800 [United States] or Tecan Spectra [Austria]).

Host range studies. Peanut samples that positively reacted in ELISA to TYFRV but not to the other tospovirus-specific antibodies were mechanically inoculated onto *Nicotiana rustica* plants; ELISA assays were subsequently used to test their systemic infection. Finally, five TYFRV isolates, different for origin or field symptoms, including TY-PeG14, TY-PeG28, TY-PeG53, TY-PeM55, and TY-PeMz61 (Supplementary Table S2), were selected and used for the detailed host-range studies. The virus isolates were biologically purified by at least two single-local lesion transfers using cowpea (*Vigna unguiculata*), propagated in *Nicotiana* plants (*N. rustica* or *N. benthamiana*), and inoculated onto young indicator plants belonging to seven plant families (Table 2) described previously (Golnaraghi et al. 2008; Pourrahim et al. 2012b). All of the indicator plants were grown from their seed or tuber. Inoculation was done at two- to six-leaf stages, depending on the indicator plant used. Inoculated plants were kept at greenhouse conditions (with at least 12 h of light, 25 ± 5°C, and 50 to 70% relative humidity) for more than 4 weeks and the symptoms were periodically observed and recorded during this time; after that, the TYFRV infection of these plants was tested by ELISA using the specific antibodies. In these studies, three to six young plants of each indicator host were inoculated. The experiments were repeated at least two times in case of negative or unclear results. Insecticides were applied periodically to prevent possible plant infestations by insects. Systemically infected *Nicotiana* leaf tissues were used for the long-term storage of these isolates at -70°C.

Serological studies. To evaluate the possible presence of serological relationships between the five aforementioned isolates and other tospoviruses, the isolates were analyzed by ELISA using different available tospovirus antibodies. To this end, *N. rustica* plants infected with each of the peanut isolates were used.

Reverse-transcription polymerase chain reaction and sequencing. The nucleotide sequences of the N gene of the five TYFRV isolates from peanut were determined. This procedure was performed according to previous recommendations (Golnaraghi et al. 2007b, 2008). Briefly, RNX-Plus solution (CinnaGen Inc., Tehran, Iran) was used to extract total RNA from TYFRV-infected *Nicotiana* leaves (*N. rustica* or *N. benthamiana*). The extracted RNA for each isolate was used for cDNA synthesis and polymerase chain reaction (PCR) amplification using RevertAid M-MuLV reverse transcription (Fermentas, Vilnius, Lithuania) and *pfu* DNA polymerase (CinnaGen Inc.), respectively. PCR products and a DNA ladder were separated by agarose gel electrophoresis and visualized by ethidium-bromide staining and UV illumination. The expected DNA fragments were then excised from the gels and purified using the Nucleospin (Macherey-Nagel, Duren, Germany). To obtain nucleotide sequences of each isolate studied, two independent reverse-transcription (RT)-PCR products were used. Sequencing was done in both directions using PCR amplification primers by the custom sequencing service of MWG-Biotech Co. (Ebersberg, Germany). Bio-Edit software (version 7.2.5) (Hall 1999) was applied for trimming and assembling of the nucleotide sequence data obtained in this study. BLAST analysis was then used to search GenBank databases (available at the National Center for Biotechnology Information; <https://www.ncbi.nlm.nih.gov/>) to find the sequences showing the highest identities with the nucleotide and deduced amino acid sequences.

Recombination studies. For evolutionary analyses, the N gene sequences of the five peanut isolates of TYFRV obtained in this study and 20 isolates from the GenBank sequence database were used. The highly similar N gene sequences from 24 isolates of a tospovirus referred to as TYRV were also used in these studies. The results of BLAST searches showed that the sequence of *Polygonum ringspot*

Table 1. Serological detection of different tospoviruses in symptomatic peanut leaf samples^a

Province	Year	Number of		Tospoviruses ^b		Overall
		Fields	Samples	TYFRV	WSMoV	
Golestan	2004	5	74	43 (58.1)	11 (14.9)	43 (58.1)
Mazandaran	2005	3	28	25 (89.3)	0 (0.0)	25 (89.3)
Total	...	8	102	68 (66.7)	11 (10.8)	68 (66.7)

^a A double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was used to detect different tospoviruses.

^b Number of infected peanut leaf samples and percentage of infected peanut leaf samples in parentheses. TYFRV = Tomato yellow fruit ring virus and WSMoV = *Watermelon silver mottle virus*.

virus (PolRSV), GenBank accession number EF445397, closely matched that of TYFRV; therefore, this sequence was used for an out-group in these studies. We aligned all 49 sequences, with or without that of one PolRSV isolate, using Clustal X (Jeanmougin et al. 1998) and TRANSALIGN (Weiller 1998) to maintain the degapped alignment, according to procedures described earlier (Farzadfar et al. 2009). Different recombination detection programs, including BOOTSCAN, CHIMAERA, MAXCHI, RDP, and SISCAN programs in RDP 4.39 software (Martin et al. 2015), were then used to find evidence for recombination events in the aligned sequences. A sequence was considered “recombinant” if the recombination event was detected by at least three of the methods mentioned above (P value $< 1 \times 10^{-6}$); these procedures were performed according to the earlier recommendations (Farzadfar et al. 2009; Posada 2002). Default settings for all of the programs and a Bonferroni corrected P value cut-off of 0.05 or 0.01 were utilized in these analyses.

Phylogenetic studies. Multiple sequence alignment was performed, as described above, using the PolRSV sequence as the outgroup. Phylogenetic analyses were carried out using maximum-likelihood (ML) and neighbor-joining (NJ) algorithms (Nei and Kumar 2000) implemented in Mega 6.0 software (Tamura et al. 2013). For ML analysis, a Tamura three-parameter model with a gamma distribution of site-rate variants across five categories was used. Having the lowest values

for Bayesian information criterion (BIC) and Akaike information criterion, corrected (AICc) scores (Nei and Kumar 2000), this model was found to be the best nucleotide substitution model and used to estimate phylogenetic relationships between the sequences under study. The ML tree was found by subtree pruning and regrafting trees. We also did NJ analysis using a Kimura two-parameter model. Both ML and NJ methods were done using 1,000 bootstraps to evaluate node supports (Efron et al. 1996).

RT-PCR using genogroup-specific primers. For further studies, RNA of 26 peanut leaf samples that reacted positively in ELISA to TYFRV also were tested by RT-PCR using TYf and TYr primers (Golnaraghi et al. 2007b) to study the possible presence of group IRN-1 isolates of the virus in the collected samples. This primer pair has previously been shown to be able to produce the expected fragments only for these isolates of TYFRV (Golnaraghi et al. 2008; Pourrahim et al. 2012b).

Variation analyses. These studies were done using the program Mega 6.0 (Tamura et al. 2013). The Tamura three-parameter model, as described above, was used to estimate nucleotide distances. Moreover, amino acid distances were estimated using the best-fitted model (Jones-Taylor-Thornton). In this study, within-group mean distance and net between-group mean distance also were estimated. Nonsynonymous (d_n) and synonymous (d_s) differences were calculated

Table 2. Host reaction of three peanut isolates of Tomato yellow fruit ring virus^a

Indicator plant	TY-PeG53		TY-PeM55		TY-PeM61	
	Local	Systemic	Local	Systemic	Local	Systemic
Amaranthaceae						
<i>Amaranthus</i> sp.	NL	–	NL	–	NL	–
<i>Gomphrena globosa</i>	NL	N	NL	N	NL	N
Asteraceae						
<i>Carthamus tinctorius</i>	–	–	–	–	–	–
<i>Helianthus annuus</i>	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	–
<i>C. quinoa</i>	NL	–	NL	–	NL	–
Cucurbitaceae						
<i>Citrus vulgaris</i>	–	–	–	–	–	–
<i>Cucumis sativus</i>	CL	–	NL	–	CL	–
Fabaceae						
<i>Arachis hypogaea</i>	–	CS, Cl, N	–	CS, Cl, N	–	CS, Mo, N
<i>Glycine max</i> ‘Hill’	NL	Mo, Cl, N	NL	Mo, Cl, N	NL	Mo, Cl, N
<i>Phaseolus aureus</i> ‘Gohar’	NL	–	NL	–	NL	–
<i>P. vulgaris</i> ‘Talash’	CL	–	CL	–	NL	–
<i>Pisum sativum</i>	–	–	–	–	–	–
<i>Vicia faba</i>	NL	–	NL	–	NL	–
<i>Vigna unguiculata</i> ‘Mashad’	NL	–	CL, NL	–	NL	–
Solanaceae						
<i>Capsicum annum</i>	–	M	–	M	–	M
<i>Datura metel</i>	NS	M, LD, SN	NS	M, SN	NS	M, LD, SN
<i>D. stramonium</i>	NS	Mo, LD, N	NS	Mo, N	NS	Mo, LD, N
<i>Nicotiana benthamiana</i>	CS	M, LD, Cl, N	CS	M, LD, Cl, N	CS	M, LD, N
<i>N. glutinosa</i>	NS	TN	NS	TN	NS	TN
<i>N. rustica</i>	NL	CS, Su, M	CL, NL	CS, LD, Su, M	NL	CS, LD, M
<i>N. tabacum</i> ‘Samsun’	NL	N	NL	N	CL, NL	N
<i>N. tabacum</i> ‘White Burley’	–	Cl, N	–	Cl, N	–	Mo, N
<i>Petunia hybrida</i>	NL	–	NL	–	NL	–
<i>Solanum lycopersicum</i>	NL	–	NL	–	NL	–
<i>S. melongena</i>	–	–	–	–	–	–
<i>S. nigrum</i>	NL	–	NL	–	NLL	–
<i>S. tuberosum</i> ‘Agria’	NL	Cl, SN	NL	Cl, SN	NL	Cl, SN

^a 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, Su = stunting, TN = top necrosis, and – indicates no infection.

using the Pamilo-Bianchi-Li method implemented in this program; the d_p/d_s ratios were subsequently estimated. A bootstrap procedure (1,000 replicates) was performed to estimate standard errors.

Genetic differentiation and gene flow analyses. In these studies, three permutation-based statistical tests, including K_s^* , Z , and S_{nn} , were used to evaluate genetic differentiation between populations (Hudson 2000). Moreover, the absolute value of the standardized variance in allele frequencies across populations (F_{ST}) was utilized to estimate genetic differentiation and the gene flow level between populations. F_{ST} values greater than 0.33 and less than 0.33 indicate infrequent and frequent gene flow, respectively. Moreover, the value of F_{ST} ranges from 0 to 1 for undifferentiated to fully differentiated populations (Librado and Rozas 2009; Wei et al. 2009; Weir and Cockerham 1984). All of these statistical tests were carried out using DnaSP, version 5.10.01 (Librado and Rozas 2009).

Neutrality tests and haplotype analysis. We used Tajima's D , Fu and Li's D^* , and F^* statistical tests (Fu and Li 1993; Tajima 1989) to evaluate neutrality. Tajima's D test is based on the differences between the number of segregating sites and the average number of nucleotide differences. Fu and Li's D^* test is based on the differences between the number of mutations occurring in just one sequence (i.e., singletons) and the total number of mutations. Fu and Li's F^* test is based on the differences between the numbers of singletons and the average number of nucleotide differences between every pair of sequences. We also calculated haplotype diversity based on the frequency and number of haplotypes in each virus population. All of these analyses were done using DnaSP, version 5.10.01 (Librado and Rozas 2009).

Results

Surveys and ELISA tests. The results of serological assays revealed that 68 of 102 samples (66.7%) collected from the two peanut-growing provinces of Iran were infected with TYFRV. The percentage of viral infection differed among collection regions (i.e., 58.1%

and 89.3% in Golestan and Mazandaran Provinces, respectively). Except for one field in Golestan, the occurrence of the virus in peanut was recorded in all other seven fields surveyed. Symptoms associated with TYFRV infections were chlorotic ring spots, chlorosis, necrosis, mosaic, bud necrosis, and stunting (Fig. 1). The affected plants also showed severe stunting and significant decreases in their growth in early infections. Moreover, 11 samples (14.9%) collected from a field in Golestan Province positively reacted in ELISA to WSMoV antibodies (Table 1); none of the samples had positive reactions to other tospovirus antibodies tested (GRSV, INSV, IYSV, TCSV, and TSWV).

Host range. The five isolates under study produced only local infections in *Amaranthus* sp., *Chenopodium amaranticolor*, *C. quinoa*, *Cucumis sativus*, *Helianthus annuus*, *Petunia hybrida*, *Phaseolus aureus* 'Gohar', *P. vulgaris* 'Talash', *Vicia faba*, *Vigna unguiculata* 'Mashad', *Solanum nigrum*, and *S. lycopersicum*, whereas *Capsicum annuum*, *Datura metel*, *D. stramonium*, *Glycine max* 'Hill', *Gomphera globosa*, *N. bentamiana*, *N. glutinosa*, *N. rustica*, and *S. tuberosum* 'Agria' were systemically infected by these isolates. The isolates also induced systemic symptoms on *N. tabacum* cultivars. When healthy peanut plants (*A. hypogaea*) were artificially inoculated with these isolates, chlorotic spots, mosaic, and chlorosis followed by necrosis symptoms were developed which were similar to the field symptoms associated with the virus infections (Fig. 1). The inoculated plants died completely about 3 to 4 weeks postinoculation. Infections were confirmed by ELISA using TYFRV-specific antibodies. Moreover, *Brassica rapa*, *Carthamus tinctorius*, *Citrulus vulgaris*, *Pisum sativum*, *Raphanus sativus*, *Rapistrum rugosum*, *S. melongena*, and *Sysimbrium loeselii* were found to be nonhost plants on the basis of symptom expression, ELISA testing, and bioassay on *V. unguiculata*. Although some minor different reactions were observed, the host ranges of the isolates under study were similar to each other and resembled those of N-host-type isolates of TYFRV from pepper, potato, and soybean (Golnaraghi 2009; Golnaraghi et al. 2008, 2013; Pourrahim

Fig. 1. Field symptoms observed on peanut leaves infected with Tomato yellow fruit ring virus (TYFRV): **A**, chlorotic ring spots, mosaic, necrotic patches, and chlorosis; **B**, chlorotic ring spots and mottling; and **C**, mosaic and chlorosis. **D**, Symptoms induced on peanut leaves by the mechanical inoculation of TYFRV under greenhouse conditions.

et al. 2012b). The host reactions of three peanut isolates from different locations are shown in Table 2.

Serological relationships. ELISA assays were performed to evaluate the reactions of the peanut isolates TY-PeG14, TY-PeG28, TY-PeG53, TY-PeM55, and TY-PeMz61 with different tospovirus-specific antibodies. These isolates strongly reacted with TYFRV antibodies. In contrast, the isolates did not have significant reactions with GRSV, INSV, IYSV, TCSV, TSWV, and WSMoV antibodies. The results were similar to those obtained earlier for TYFRV (Golnaraghi 2009; Winter et al. 2002, 2006).

Nucleotide sequences. The N gene sequences of the five TYFRV isolates were 825 nucleotides long, and were deposited in the GenBank database and received the accession numbers MF574030 to MF574034. BLAST search results confirmed that these sequences encompassed the coding region for nucleocapsid protein, the entire N open reading frame (ORF). This ORF in all sequences started with an AUG and terminated with a UAA stop codon. The deduced N protein sequences contained 274 amino acid residues with molecular weights of 29.97 to 30.04 kDa. The data obtained were similar to those of other TYFRV isolates studied (Golnaraghi 2009; Pourrahim et al. 2012b; Winter et al. 2002). Sequences from these five isolates were found to match sequences from the virus, and alignments generated by BLAST searches yielded identity scores of 99% at nucleotide and amino acid sequence levels.

Recombination analyses and phylogenetic relationships. The nucleotide sequences of the N gene of the five TYFRV isolates from peanut were assessed for evidence of recombination using RDP 4.39. No evidence of clear or tentative recombination events (P value $< 1 \times 10^{-6}$ and $1 \times 10^{-3} > P$ value $> 1 \times 10^{-6}$, respectively) were observed in the N gene sequences of the peanut isolates and the other isolates studied. Therefore, all 49 nonrecombinant sequences were applied for phylogenetic analyses. ML and NJ algorithms were used to study the relationships of these isolates and one isolate of PolRSV (the outgroup); the ML tree is shown in Figure 2. The trees obtained from both methods categorized these sequences into three major groups: IRN-1 and IRN-2 (Pourrahim et al. 2012b) and a new phylogenetic group named POL, with high bootstrap values. The 36 Iranian isolates studied were clustered in the IRN-1 and IRN-2 groups, while the 12 isolates from Poland were placed in the POL group. The only isolate from Kenya was clustered in group IRN-1. The seven virus isolates from ornamental plants were all placed in group IRN-1. The virus isolates from tomato were placed in the groups IRN-1 and POL. Although the potato and soybean isolates were found in both IRN-1 and IRN-2, most of them (10 of 14 and 2 of 3 isolates, respectively) were clustered in group IRN-2. The two pepper isolates and the five peanut isolates studied in this work were located in group IRN-2. All host types (or strains) N and S were placed in group IRN-2, while the host types A, Al, Cl, L, T, and TS fell into group IRN-1. The Iranian TYRV isolates analyzed in this work were clustered together with TYFRV isolates in the IRN-1 and IRN-2 groups.

Multiple sequence alignment showed high amino acid sequence similarities between the peanut isolates of TYFRV and the other isolates, and between TYFRV and TYRV isolates studied as well. In contrast to the presence of numerous conserved amino acid sequences, especially among the isolates belonging to the same phylogenetic group, a number of amino acid changes were observed among the isolates belonging to different groups. The results showed that some of them were genogroup-specific amino acid substitutions, including A_{115,209,249}, E₂₀₅, G₂₀₅, I₇₈, K₁₁₇, N_{10,75,131,257}, P₁₃₉, Q_{140,244}, S_{115,205,266,267}, and T_{29,43,115,168,207,230,233,249,268} (Fig. 3).

RT-PCR using genogroup-specific primers. The TYf and TYr primers were used to study the possible presence of group IRN-1 isolates of TYFRV in the peanut samples collected. Finally, the expected DNA (Golnaraghi et al. 2007b) did not produce in RT-PCR of 26 peanut leaf samples tested.

Similarity and diversity. The mean nucleotide distance between isolates within a group was 0.0197, 0.0156, and 0.0045 for groups IRN-1, IRN-2, and POL, respectively, whereas the net mean distance between two groups ranged from 0.1031 to 0.1661. Therefore, the phylogenetic clustering of the isolates into three groups was reasonable.

The mean distances of the isolates in each group with respect to PolRSV sequence used as the outgroup were 0.2127 to 0.2612 and 0.1389 to 0.1776 based on nucleotide and amino acid sequences, respectively (Table 3). Some correlations were found between genetic variation, the hosts, and geographical regions where these isolates were collected. The mean nucleotide distance between the Iranian isolates (0.0882) was higher than that of the Polish isolates (0.0045), and was 0.0976 when the net between-group mean distance was calculated. The mean nucleotide distances within virus isolates from potato, soybean, or tomato isolates were higher (0.0759 to 0.1094) than those for isolates from ornamentals, peanut, or pepper (0.0161 to 0.0204). Moreover, the mean nucleotide distance between TYFRV and TYRV isolates in the same group was very low (< 0.0067 and < 0.0029 based on nucleotide and amino acid sequences, respectively). We also estimated d_n and d_s differences as well as d_n/d_s ratios. Based on the results, d_n was always smaller than d_s , showing the presence of selection against amino acid substitutions (i.e., “negative selection”). However, the parameters differed significantly in different phylogenetic groups: the highest d_n/d_s ratio was for the POL group and the lowest for group IRN-1. The d_n/d_s ratio for all isolates was 0.0790 (Table 4). This ratio was also calculated to find the possible differences in different parts of the N gene of different virus isolates. Our results showed some differences in the d_n/d_s ratio for different parts of the N gene of the virus isolates belonging to different genogroups (Table 5).

Genetic differentiation and gene flow. Population differentiation analyses were done using different statistical tests (Hudson 2000) to evaluate whether the virus isolates, different for their geographical origin or phylogenetic group, are genetically differentiated populations. These comparisons indicated that the populations from Iran and Poland and those belonging to different genogroups (i.e., IRN-1, IRN-2, and POL) were partially differentiated populations, which was in agreement with phylogenetic analyses (Fig. 2). The results were supported by P values (< 0.001) obtained for the all of the statistical tests applied (K_S^* , Z , and Snn). Moreover, the absolute values obtained for F_{ST} were larger than 0.33 (> 0.67) in all of these comparisons, suggesting the infrequent gene flow of the virus populations between these regions or groups (Librado and Rozas 2009). These tests also were used to evaluate whether the isolates are genetically differentiated populations, taking into consideration of their geographical origin (Iran and Poland) and original host plants (ornamentals, pepper, peanut, potato, soybean, and tomato). The results showed that the Polish isolates from tomato were partially genetically differentiated populations, which was in agreement with phylogenetic analyses (Fig. 2). Our findings also indicated that there has been frequent gene flow between peanut, potato, and pepper isolates of the virus in Iran ($F_{ST} < 0.33$) (Librado and Rozas 2009). However, K_S^* , Z , and Snn were not significant in some comparisons, which may be attributed to the lack of statistical power (Table 6).

Levels and patterns of intraspecific polymorphism. The patterns of nucleotide polymorphism of the TYFRV populations were evaluated using statistical tests Tajima's D , Fu and Li's D^* , and F^* to differentiate between demographic expansion and contraction. We expected the three statistical tests to produce negative values resulting from background selection, genetic hitchhiking, or demographic expansion. Therefore, such values indicated a low frequency of polymorphism in the population and also pointed out that demographic forces were acting on the population (Hey and Harris 1999; Tajima 1989; Tsompana et al. 2005). We also expected the statistical tests to generate positive values resulting from balancing selection and a reduction in the size of the population. Our results indicated significant negative values for group POL by using all three statistical tests utilized in this study, supporting the idea that there had been a demographic expansion for this group but not for IRN-1 and IRN-2. The haplotype diversity in all three groups analyzed was high and had a value of 0.936 to 1.000 (Table 7).

Discussion

In the current study, the occurrence of a new tospovirus disease on peanut in Iran was described for the first time. The causal agent was identified as TYFRV based on biological, serological, and molecular

properties. The virus induced chlorotic spots, chlorosis, and necrosis and, sometimes, a remarkable growth reduction in the affected plants, especially in early infections. The symptoms are similar to those produced by other tospoviruses infecting peanut in Iran (Golnaraghi et al. 2001, 2002a). Such severe symptoms also have been reported

in association with TYFRV infections of other hosts in the country (Golnaraghi et al. 2007a; Pourrahim et al. 2001). The tospovirus was found in seven of eight fields surveyed, showing the possible presence of a very high inoculum reservoir (host plants) and the prevalence of efficient thrips vectors in these areas. Similar results have

Fig. 2. Maximum-likelihood (ML) phylogenetic tree constructed from nucleoprotein (N) gene sequences of 49 isolates of Tomato yellow fruit ring virus (TYFRV). Bootstrap values obtained in ML and neighbor-joining (NJ) methods are respectively shown at each node; only values of more than 50 are shown. The corresponding sequence of one isolate of Polygonum ringspot virus (PolRSV) was used as the outgroup. For each sequence, the isolate name, country of origin, original host plant, year of isolation, host infecting type, and accession number in the GenBank are listed. "TY" and "TR" appearing in the name of each isolate represent TYFRV and Tomato yellow ring virus (TYRV) isolates, respectively.

been obtained for TYFRV infections of other crops from different regions in Iran (Ghotbi et al. 2005; Golnaraghi et al. 2007a,b). A wide range of plant hosts for TYFRV or its vectors is present in the two provinces surveyed (personal observations). We also found samples with positive reactions with WSMoV antibodies, indicating the presence of GBNV (Golnaraghi et al. 2002a) or other serologically cross-reacting tospoviruses (DSMZ 2017) in Iranian peanut crops. Interestingly, some samples showing tospovirus-like symptoms did not react with the antibodies tested; the possible role of other viruses (e.g., tospoviruses) in these diseases should be investigated in the future. In this study, we could not detect TSWV and INSV; however, their potential impacts on peanut production in Iran should be further studied because they can infect a wide range of host plants in different

regions of the country (Ghotbi et al. 2005; Moini and Izadpanah 2001; Pourrahim et al. 2012a).

Recombination has an important role in variation and evolution of different plant viruses (Prasanna and Rai 2007; Yasaka et al. 2017), including tospoviruses (Lian et al. 2013; Ramesh and Pappu 2016). The results obtained in this work showed that the N genes of the TYFRV isolates from peanut and the other hosts were nonrecombinant. However, the occurrence of recombination events in other parts of the virus genome remains to be answered. Recombination analyses of the genome of a wide range of TYFRV isolates are necessary in future studies. We also could not find the host type L or group IRN-1 isolates of the virus in peanut plants. However, previous studies have shown the susceptibility of peanut to infection by such isolates under greenhouse

Fig. 3. Amino acid sequence alignment of nucleoprotein genes obtained from 12 isolates of Tomato yellow fruit ring virus (TYFRV) belonging to three phylogenetic groups (i.e., IRN-1, IRN-2, and POL). Four isolates from each group were used. Genogroup-specific amino acid substitutions are shown in black rectangles. “TY” and “TR” appearing in the name of each isolate represent TYFRV and Tomato yellow ring virus (TYRV) isolates, respectively.

Table 3. Within- and net between-group nucleotide and amino acid mean distances in the nucleoprotein gene region for subpopulations of Tomato yellow fruit ring virus isolates

Phylogenetic group ^a	Mean distance (standard error)			
	IRN-1	IRN-2	POL	Outgroup
Nucleotide ^b				
IRN-1 (<i>n</i> = 18)	0.01968 (0.00267)	0.13809 (0.01424)	0.16614 (0.01574)	0.24708 (0.01907)
IRN-2 (<i>n</i> = 19)	...	0.01563 (0.00313)	0.10313 (0.01142)	0.21272 (0.01857)
POL (<i>n</i> = 12)	...	...	0.00453 (0.00108)	0.26120 (0.02176)
Amino acid ^c				
IRN-1 (<i>n</i> = 18)	0.01105 (0.00315)	0.06772 (0.01527)	0.07720 (0.01684)	0.13889 (0.02395)
IRN-2 (<i>n</i> = 19)	...	0.01440 (0.00442)	0.04744 (0.01307)	0.16368 (0.02523)
POL (<i>n</i> = 12)	...	...	0.00772 (0.00237)	0.17758 (0.02671)

^a Number of sequences is shown in parentheses (*n*).

^b Nucleotide sequence distances were assessed using Tamura three-parameter model in Mega 6.0 (Tamura et al. 2013).

^c Amino acid sequence distances were assessed using Jones-Taylor-Thornton model in Mega 6.0 (Tamura et al. 2013).

conditions (Golnaraghi et al. 2007a,b; Pourrahim et al. 2012b). Considering the presence of L host type and group IRN-1 isolates in the same region (Golnaraghi et al. 2007b; Hassani-Mehraban et al. 2006), we expect the infection of peanut plants with similar isolates in the future. The coincidence of different virus isolates and hosts in the same location may also facilitate recombination and reassortment events between the virus isolates and emerging new virus strains, which is of great importance epidemiologically; similar situations have been observed for other tospoviruses (Lian et al. 2013; Ramesh and Pappu 2016; Tentchev et al. 2011).

Our phylogenetic analyses of TYFRV isolates by the NJ and ML methods indicate three geographical subpopulations: two from Iran (i.e., IRN-1 and IRN-2) (Pourrahim et al. 2012b) and one from Poland (POL) (Fig. 2). Phylogenetic grouping of tospovirus isolates based on their geographical origin has been reported previously (Tsompana et al. 2005). We also found some correlations between the original hosts and phylogenetic grouping of the isolates. For example, all peanut and pepper and most potato and soybean isolates of the virus, but none of the ornamental or tomato isolates, were clustered in group IRN-2. The phylogenetic grouping of the isolates in IRN-1 and IRN-2 was closely similar to their grouping based on the host infecting types, as reported previously (Pourrahim et al. 2012b); however, we do not have enough information about the host types of the Polish isolates. The nucleotide and amino acid distances between isolates in the IRN-2a subgroup (Fig. 2) were very low (data not shown), as reported previously (Pourrahim et al. 2012b); some of the isolates (from peanut, potato, and soybean) were obtained from geographically distant locations. This may have resulted from the transmission of TYFRV through plant propagative materials (Golnaraghi et al. 2007a). Further studies seem necessary to understand more about the possible role of such a transmission in the spread of TYFRV in different hosts. This study also showed some genogroup-specific amino acid substitutions that can be used to design genogroup-specific primer pairs for fast and accurate detection of different populations of the virus, which can improve our future quarantine programs.

Several studies have been performed on the genetic structure of different plant viruses; for example, begomoviruses (Briddon and Stanley 2006), potyviruses (Yasaka et al. 2017), and tospoviruses (Tentchev et al. 2011; Tsompana et al. 2005). We calculated and compared d_n/d_s ratios of the N gene of TYFRV isolates to evaluate the extent of translational selection (acting at the amino acid sequence of a protein). Using this measure, it was found that there was a strong selection against change (i.e., negative selection) operating on all three TYFRV genogroups and different parts of their N genes (Tables 4 and 5). The ratios were remarkably different among the groups and different parts of their N genes; this possibly seems to indicate that limitations in different genetic groups are different. However, we do not know whether such differences reflect variations of biological properties or geography. These findings are in agreement with those that found in the majority of viruses infecting plants and animals (Domingo et al. 2001; García-Arenal et al. 2001), and tospoviruses as well (Tentchev et al. 2011; Tsompana et al. 2005). We also found a great haplotype diversity together with a small genetic diversity, especially for the POL group (Table 7), which seems to show a recent increase in the population size preceded by a genetic bottleneck, i.e., a founder effect. Similar evidence has been found for tospoviruses and other multipartite genome viruses (Lin et al. 2004; Tentchev et al. 2011; Tsompana et al. 2005). However, the low diversity and close evolutionary relationship among isolates in the POL group also may have resulted from selection by the host plant and the geographical location; all of the group isolates were collected from tomato plants in Kujawsko-Pomorskie (Poland) in a 2-year period, similar to those reported earlier (Lin et al. 2004). The origin of POL group isolates also remains to be answered in future studies.

Our results also indicate partial genetic differentiation between the phylogenetic, geographical, and host groups of the virus isolates. Moreover, the results seem to suggest that there has been frequent gene flow between some populations of TYFRV in Iran (Table 6). However, more studies are necessary to confirm gene flow between the virus populations. These findings, together with the results of the phylogenetic analyses, seem to indicate that both host and geographical

Table 4. Nucleotide diversities in the nucleoprotein gene region for subpopulations of Tomato yellow fruit ring virus isolates^a

Nucleotide diversity	All isolates (<i>n</i> = 49)	Phylogenetic group		
		IRN-1 (<i>n</i> = 18)	IRN-2 (<i>n</i> = 19)	POL (<i>n</i> = 12)
d_n	0.02894 (0.00532)	0.00508 (0.00140)	0.00691 (0.00203)	0.00356 (0.00112)
d_s	0.36624 (0.03770)	0.05302 (0.00910)	0.04185 (0.01203)	0.00674 (0.00234)
d_n/d_s	0.07902	0.09581	0.16511	0.52819

^a Nonsynonymous (d_n) and synonymous (d_s) differences were calculated using Pamilo-Bianchi-Li method in Mega 6.0 (Tamura et al. 2013); *n* = number of sequences and data in parentheses represent standard error.

Table 5. Nucleotide diversities in different regions of nucleoprotein (N) gene for subpopulations of Tomato yellow fruit ring virus isolates^a

N gene region, diversity	All isolates (<i>n</i> = 48)	Phylogenetic group		
		IRN-1 (<i>n</i> = 17)	IRN-2 (<i>n</i> = 19)	POL (<i>n</i> = 12)
Nt 1-273				
d	0.08291 (0.01229)	0.01237 (0.00344)	0.01251 (0.00434)	0.00308 (0.00141)
d_n	0.01941 (0.00600)	0.00518 (0.00236)	0.00592 (0.00253)	0.00215 (0.00123)
d_s	0.32046 (0.06966)	0.02583 (0.00952)	0.03347 (0.01848)	0.00456 (0.00325)
d_n/d_s	0.06057	0.20054	0.17688	0.47149
Nt 274-546				
d	0.11034 (0.01439)	0.02029 (0.00446)	0.01648 (0.00542)	0.00544 (0.00190)
d_n	0.02919 (0.00879)	0.00537 (0.00263)	0.01164 (0.00513)	0.00463 (0.00237)
d_s	0.37859 (0.06948)	0.05264 (0.01445)	0.02466 (0.01178)	0.00681 (0.00424)
d_n/d_s	0.07710	0.10201	0.47202	0.67988
Nt 547-823				
d	0.12049 (0.01520)	0.02971 (0.00664)	0.02032 (0.00605)	0.00408 (0.00187)
d_n	0.04015 (0.01220)	0.00332 (0.00158)	0.00171 (0.00122)	0.00319 (0.00192)
d_s	0.41041 (0.06982)	0.09620 (0.02537)	0.07573 (0.02731)	0.00675 (0.00408)
d_n/d_s	0.09783	0.03451	0.02258	0.47259

^a Nonsynonymous (d_n) and synonymous (d_s) differences were calculated using Pamilo-Bianchi-Li method in Mega 6.0 (Tamura et al. 2013); *n* = number of sequences and data in parentheses represent standard error. The Kenyan isolate TR-Loitoktok was not used in this analysis.

origin may play important roles in the evolution of this virus; similar results have been obtained for other RNA viruses (Yasaka et al. 2017). Also, this tospovirus may have possibly emerged in the mid-Eurasian region of Iran, because the highest genetic diversity of a pathogen typically occurs in its centers of origin (Jones 2005). It is worth noting that, in genetic population studies, we used different population sizes

(number of sequences) obtained from different numbers of hosts in different geographical scales and in different time spans (isolate collection dates); these factors might have possibly affected the results presented here. Therefore, studies of more isolates of TYFRV from different geographical locations are necessary to confirm these hypotheses and to know more about different populations of the virus.

Table 6. Gene flow and genetic differentiation of Tomato yellow fruit ring virus isolates, considering their phylogenetic group, original host plants, and geographical origins^a

Country, phylogenetic group ^c	Parameter ^b				
	<i>Ks</i> * (<i>P</i> value)	<i>Z</i> (<i>P</i> value)	<i>Snn</i> (<i>P</i> value)	<i>F_{ST}</i>	<i>Nm</i>
Iran vs. Poland					
Iran (<i>n</i> = 36) vs. Poland (<i>n</i> = 12)	3.04399 (0.0000)***	399.99138 (0.0000)***	1.00000 (0.00000)***	0.67488	0.12
Groups					
IRN-1 (<i>n</i> = 18) vs. IRN-2 (<i>n</i> = 19)	2.38323 (0.0000)***	161.78716 (0.0000)***	1.00000 (0.00000)***	0.87156	0.04
IRN-1 (<i>n</i> = 18) vs. POL (<i>n</i> = 12)	2.15672 (0.0000)***	101.18901 (0.0000)***	1.00000 (0.00000)***	0.92178	0.02
IRN-2 (<i>n</i> = 18) vs. POL (<i>n</i> = 12)	1.89462 (0.0000)***	112.87298 (0.0000)***	1.00000 (0.00000)***	0.90280	0.03
Hosts and origins					
Iran (peanut, <i>n</i> = 5 vs. Poland (tomato, <i>n</i> = 12)	1.68754 (0.0000)***	40.48112 (0.0000)***	1.00000 (0.00000)***	0.88282	0.03
Iran (peanut, <i>n</i> = 5 vs. Iran (potato, <i>n</i> = 14)	3.03406 (0.5110)	85.35868 (0.4960)	0.59649 (0.4980)	0.13050	1.67
Iran (peanut, <i>n</i> = 5) vs. Iran (tomato, <i>n</i> = 5)	2.76223 (0.0040)**	9.50000 (0.0040)**	1.00000 (0.0100)*	0.83320	0.05
Iran (peanut, <i>n</i> = 5) vs. Iran (soybean, <i>n</i> = 3)	2.92879 (0.4540)	13.82083 (0.4760)	0.27083 (0.9380)	-0.06433	-4.14
Iran, (peanut, <i>n</i> = 5) vs. Iran (pepper, <i>n</i> = 2)	2.54886 (0.6590)	11.25000 (0.9090)	0.71429 (0.1570)	-0.19643	-1.52
Iran (peanut, <i>n</i> = 5) vs. Iran (ornamentals, <i>n</i> = 7)	2.66488 (0.0000)***	15.11220 (0.0000)***	1.00000 (0.0030)**	0.85414	0.04
Poland (tomato, <i>n</i> = 12) vs. Iran (potato, <i>n</i> = 14)	2.37071 (0.0000)***	105.15303 (0.0000)***	1.00000 (0.00000)***	0.69135	0.11
Poland (tomato, <i>n</i> = 12) vs. Iran (tomato, <i>n</i> = 5)	1.78601 (0.0000)***	41.26923 (0.0000)***	1.00000 (0.00000)***	0.90221	0.03
Poland (tomato, <i>n</i> = 12) vs. Iran (soybean, <i>n</i> = 3)	1.66909 (0.0000)***	37.09091 (0.0020)**	1.00000 (0.0020)**	0.57673	0.18
Poland (tomato, <i>n</i> = 12) vs. Iran (pepper, <i>n</i> = 2)	1.42914 (0.0000)***	32.50000 (0.0100)*	1.00000 (0.0100)*	0.90247	0.03
Poland (tomato, <i>n</i> = 12) vs. Iran (ornamentals, <i>n</i> = 7)	1.86426 (0.0000)***	46.98268 (0.0000)***	1.00000 (0.00000)***	0.91938	0.02
Iran (potato, <i>n</i> = 14) vs. Iran (tomato, <i>n</i> = 5)	3.11941 (0.0140)*	71.19319 (0.0130)*	0.63158 (0.3550)	0.54231	0.21
Iran (potato, <i>n</i> = 14) vs. Iran (soybean, <i>n</i> = 3)	3.22560 (0.9720)	70.01479 (0.9230)	0.56471 (0.9780)	-0.27075	-1.17
Iran (potato, <i>n</i> = 14) vs. Iran (pepper, <i>n</i> = 2)	3.15536 (0.5070)	61.40659 (0.6520)	0.87500 (0.1950)	0.16982	1.22
Iran (potato, <i>n</i> = 14) vs. Iran (ornamentals, <i>n</i> = 7)	3.03157 (0.0040)**	81.21967 (0.0040)**	0.71429 (0.0590)	0.57124	0.19
Iran (tomato, <i>n</i> = 5) vs. Iran (soybean, <i>n</i> = 3)	3.24884 (0.1020)	10.88333 (0.1020)	0.50000 (0.4160)	0.36199	0.44
Iran (tomato, <i>n</i> = 5) vs. Iran (pepper, <i>n</i> = 2)	2.97560 (0.0190)*	5.40000 (0.0380)*	1.00000 (0.0930)	0.84884	0.04
Iran (tomato, <i>n</i> = 5) vs. Iran (ornamentals, <i>n</i> = 7)	2.82491 (0.7050)	33.68899 (0.7050)	0.25000 (0.9050)	-0.06833	-3.91
Iran (soybean, <i>n</i> = 3) vs. Iran (pepper, <i>n</i> = 2)	4.06856 (1.0000)	6.50000 (1.0000)	0.20000 (0.9010)	-0.04115	-6.33
Iran (soybean, <i>n</i> = 3) vs. Iran (ornamentals, <i>n</i> = 7)	2.95683 (0.0780)	16.88095 (0.0610)	0.80000 (0.1170)	0.39717	0.38
Iran (pepper, <i>n</i> = 2) vs. Iran (ornamentals, <i>n</i> = 7)	2.73449 (0.0380)*	10.73810 (0.0380)*	1.00000 (0.0380)*	0.86969	0.04

^a Genetic differentiation and the gene flow level between populations were assessed by three statistical tests as well as *F_{ST}* in DnaSP version 5.10.01 (Librado and Rozas 2009).

^b *Ks** and *Z* are the sequence-based statistics (Hudson 2000); *Snn* is the nearest-neighbor statistic; *F_{ST}* is the interpopulation component of genetic variation of the standardized variance in allele frequencies across populations; *N* is the population size of each subpopulation; and *m* is the migration fraction per generation. *P* values were determined by permutation tests with 1,000 replicates (* indicates 0.01 < *P* < 0.05, ** indicates 0.001 < *P* < 0.01, and *** indicates *P* < 0.001).

^c Isolates from Iran, Poland, and Kenya were used. Comparisons are between Iranian and Polish populations (Iran vs. Poland), between populations of different phylogenetic groups (Groups), and between populations from different hosts and geographical origins (Hosts and origins). The number (*n*) of sequences for each country is indicated.

Table 7. Neutrality tests and haplotype analysis of each population of Tomato yellow fruit ring virus^a

Phylogenetic group ^b	Tajima's <i>D</i>	Fu & Li's <i>D</i> *	Fu & Li's <i>F</i> *	Number of haplotypes	Haplotype diversity ^c
IRN-1 (<i>n</i> = 18)	-0.96204	-1.29004	-1.38680	16	0.980 (0.00081)
IRN-2 (<i>n</i> = 19)	1.19818	0.41695	0.75122	14	0.936 (0.00223)
POL (<i>n</i> = 12)	-1.86578* ^e	-2.48039**	-2.64027**	12	1.000 (0.00116)

^a Tajima's *D* (Tajima 1989), Fu and Li's *D** and *F** (Fu and Li 1993) are statistical tests of neutrality; number of haplotypes and haplotype diversity were calculated by DnaSP version 5.10.01 (Librado and Rozas 2009). *P* values: * and ** indicate *P* < 0.05 and 0.02, respectively.

^b Number of sequences in parentheses (*n*).

^c Numbers in parentheses = variance of haplotype diversity.

The results of this study, together with previous studies (Beikzadeh et al. 2012; Ghotbi et al. 2005; Golnaraghi et al. 2007b; Hassani-Mehraban et al. 2005, 2006; Rasoulpour and Izadpanah 2007; Winter et al. 2002, 2006), also unequivocally indicate that TYFRV and TYRV are isolates of the same virus on the basis of their biological, serological, and molecular properties; we suggest TYFRV (or ToVV) for the name of this tospovirus because it was proposed and used for the first time to describe the virus (Winter et al. 2002) (AJ493270 and AJ493271); thus, we used “TYFRV” to address this tospovirus in almost all parts of this article.

The discovery of chlorotic ring spots and chlorosis disease on peanut caused by TYFRV adds another disease to the list of virus diseases of peanut. The present work indicates the genetic structure of different populations of the virus, and illustrates that a better understanding of the genetic structure and biological composition of TYFRV populations is important to design and develop better control strategies.

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