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Research Article

Incidence and distribution of *Tobacco streak virus* isolated from parsley *Petroselinum sativum*

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Abstract: The purpose of this study was to determine the distribution of *Tobacco streak virus* (TSV) in the vegetable fields of the Markazi, Qom, Lorestan and Hamadan provinces. This study was performed in 2017, and a total of 475 samples of parsley plants were collected. Using the specific antibody of the virus, the TSV infection of these samples was investigated by the immunosorbent assay through the double antibody sandwich ELISA (DAS-ELISA) method. The results of this study indicated that the TSV infection of parsley samples in Qom, Markazi, Hamedan and Lorestan provinces were 14, 18.8, 15.4 and 20.1%, respectively. Also, the phylogenetic analysis of nucleotide and amino acid sequences of the coat protein of these isolates showed that Iranian and Indian isolates could be clustered along with each other. The phylogenetic tree obtained based on nucleotide and amino acid sequences of the coat protein gene, showed that the isolates were divided into two and three clusters, respectively. Iranian isolates were clustered along with global TSV isolates and other Ilarviruses formed a separate cluster. This is the first report of TSV genetic diversity in Iran, and also the first report of TSV infection in the vegetable fields of Qom, Markazi, Hamedan and Lorestan provinces.

Keyword: Parsley, Ilarvirus, ELISA, RT-PCR

Introduction

Parsley *Petroselinum sativum* Hoffm. is a biennial plant, which belongs to the Apiaceae family and is used in the food, pharmaceutical, perfumery and cosmetics industries (Lopez *et al*., 1999). The plant is widely cultivated in Asian countries, and contains vitamins A, B, C and various minerals like iron and is widely used in foods of many regions (Yanardağ *et al*., 2003; Zargari, 2004).

Tobacco streak virus (TSV) belongs to the genus Ilarvirus, Bromoviridae family and is quasi-isometric plant virus with a diameter of 30 nm. The genome of the virus is RNA-positive, single-stranded, and triplicate, similar to other members of the Bromoviridae. The entire genome has 7941 nucleotides. RNA1, RNA2, RNA3 and sgRNA4 have 3491, 3050, 2205 and 850 nucleotides, respectively. RNA No. 1 encodes an RNA dependent RNA polymerase (RdRp) protein. RNA4 is actually an sgRNA derived from the negative RNA-3 strand. Also, RNA1 contains 3491 nucleotides and has an ORF that encodes a protein with 1094 amino acids (protein 1a). An untranslated region (UTR) at the 5' and 3' end contain 37 and 169 nucleotides, respectively. RNA2 contains 3050 nucleotides and two ORFs. Larger ORF range from nucleotide 42 to 2444 encodes a protein with 800 amino acids (protein 2a). The host rang of the virus is wide, and infects many species in more than 30 dicot and monocot plants families. The TSV virus was for the first time isolated in the United States in 1936 from

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tobacco (Johnson, 1936). In addition, the virus has also been reported in sunflower, pepper, cotton, cowpea, peanut, various types of mung bean, zucchini, soybean, okra, hollyhock, *Hibiscus cannabinus*, *Calendula officinalis* and *Parthenium hysterophorus* (Ravi *et al*., 2001; Reddy *et al*., 2002; Greber *et al.,* 1991).

In Australia, the virus was first identified in 1971 in tobacco, strawberries, dahlia (Tzanetakis *et al*., 2003). Sharman *et al.* (2011) isolated TSV from sunflower, cotton, pea, and mung bean, and also isolated it from *P. hysterophorus* in 2009. In the USA, the virus has been isolated from hollyhock, strawberries and American Cranberry cultivars (Jones *et al*., 2001). In Italy, Bellardi *et al*. (2006) reported TSV from the boxwood. In Iran, TSV infections have been reported from soybean fields by Rahimian *et al*. (1995) in Golestan province and also by Golnaraghi *et al*. (2002) in Mazandaran, Lorestan, Ardabil and Khuzestan provinces. Khateri *et al*. (2006) isolated the virus from tobacco in Mazandaran province. TSV virus was first isolated from sunflowers by Hosseini *et al*. (2006). Motamedi *et al*. (2013) determined the TSV infection of sunflower fields in Tehran, Isfahan, West Azarbaijan, Hamadan, Markazi and Qom provinces to be 23.85%, and investigated the molecular weight of the viral coat protein (CP), experimental host range, phylogenetic analysis with the CP amino acid sequences of a TSV isolate collected from Isfahan province. The TSV virus is easily transmitted mechanically as well as through cutting and grafting (Almeida *et al*., 2005). However, the seed-borne virus has been reported in various plants including *Parthenium* sp., *Dautura steramonium*, *Chenopodium quinoa*, soybeans, beans, *Gomphrena globosa*, tomato, tobacco (*Nicotiana clevelandii*) and mung bean (Kaiser *et al*., 1991).

Prasada Rao *et al*. (2003) collected the thrips near the flowers of TSV infected plants, to investigate the role of thrips in transmission of the TSV virus. The results demonstrated that thrips carrying infected pollens transmitted the virus to healthy plants at the rate of 40%. Also, the most important TSV vectors were reported to be *Thrips tabaci* and *Frankliniella occidentalis* (Prasada Rao *et al*., 2003).

Up to now, there are no reports of viral diseases of parsley in Iran. Globally, viruses such as *Potato virus Y*, *Tomato spotted wilt virus*, *Cucumber mosaic virus*, and the *Tobacco ring spot virus* have been reported for this plant (Loebenstein and Lecoq, 2012). Recently parsley severe stunt – associated virus (PSSaV), a novel nanovirus, member of the family Nanoviridae has been reported from Germany (Vetten *et al.,* 2019):

Detecting and determining the distribution of viral agents in different hosts underlies the selection of an appropriate method to control the damage caused by these agents. In addition, according to TSV reports from different parts of Iran (Golnaraghi *et al*., 2004; Rakhshandehroo *et al*., 2005; Moini, 2007), it is necessary to control this virus and also to investigate the genetic diversity of Iranian isolates. No information is currently available about molecular variation among Iranian TSV isolates; the aim of this study was to investigate the distribution of TSV isolate from parsley and sequencing of its coat protein gene.

Materials and Methods

Sample collection

From spring to autumn of 2017, the vegetable fields of the Qom, Markazi, Lorestan and Hamadan provinces were observed. A total of 475 samples were collected based on viral symptoms including; yellowing, deformation, chlorotic or necrotic lesions, mottling of the leaves, and stunting. Young leaves from some symptomatic plants were collected. Accordingly, 117 samples were collected from Markazi province, 121 from Qom province, 123 from Lorestan province and 114 from Hamadan province (Table 1).

Table 1 Percentage of TSV infection in 2017 in the surveyed provinces.

Province	No. of samples		Infection (%)
	Studied	Infected	
Qom	121	17	14.0
Markazi	117	22	18.8
Lorestan	123	19	15.4
Hamedan	114	23	20.1
Total	475	81	17.0

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Enzyme-linked immunosorbent assay (DAS-ELISA)

Analysis of the collected samples infected by the *Tobacco streak virus* was performed using the specific antibody (AS-906) prepared by DSMZ (Brunswick-Germany) by applying DAS-ELISA (Double Antibody Sandwich ELISA) according to the manufacturer's instructions. Each sample extraction was randomly placed into two wells as replicates. The rate of color change was evaluated in the substrate material of 4-para-nitrophenyl phosphate (Merck Co., Germany) at 405 nm using ELISA reader model Beckman AD340 USA, every 15 minutes up to 90 minutes after the addition of the substrate material. The evaluation was performed using the equation R= x + 3 SD, where x is the mean absorbance of negative sample, SD is the standard deviation of wells and R stands for the infection threshold.

Mechanical inoculation

Nicotiana benthamiana (benth), *N. tabacum*. cv. Samsun (cultivated tobacco), *Chenopodium album* subsp. *amaranthicolor* (goosefoot), *Vigna unguiculata* (cowpea), and *Cucumis sativum* (cucumber) were inoculated by the virus isolates. Sap was prepared from infected parsley leaves with phosphate buffer (pH 7) and 2% polyvinyl pyrrolidone was used as an additive, and then sap was rubbed onto leaves using carborundum powder.

RNA isolation, RT-PCR, cloning, and sequencing

Extraction of total RNA was performed using the RNeasy plant Mini kit (Kit Qiagen, Germany) to enhance the quality of the extracted RNA. At first stage, 0.1 g of plant tissue was prepared, and grounded by the liquid nitrogen, after that extraction was conducted according to the Kit instructions. At the final stage, the extracted total RNA was dissolved in sterile distilled water and placed on the 1% agar gel to control the quality.

The TSV CP RNA3 primer pair (5'- TCG ACT CTA GAA ACT AGT CTT GAT TCA CCA GAA ATC TTC-3' and 5'- AGG TAG CAG AG ATA TAA CAA TGA ATA CTT TGA TCC AAG G-3') was used for the multiplication of the CP region. The reverse transcriptase reaction was performed in a final volume of 10 µl. Initially, 15 micrograms of RNA with 20 picomole of primer was poured into PCR tubes, and then treated in a thermocycler for 5 min at 70 °C. After that, a mixture of two microliters of the 5X reaction buffer, 1 mM of deoxy nucleotide triphosphate (dNTPs), 20 units of ribonuclease inhibitor enzyme, 50 units of M-MuLV RT enzyme and DEPC-treated water (Vivantis, Malaysia) were added to each of the tubes, so that the final reaction volume was 10 µl. The cDNA production reaction was performed in a thermocycler for four minutes at 37 °C, for 60 minutes at 42 °C, and finally for 10 minutes at 70 °C (Eppendorf, Germany).

PCR reaction with the final volume of 20 µl as the leader included 2.5 µL of reverse transcriptase reaction product, 10 picomole of each primer, 2 mM of $MgCl_2$, 2 µl of reaction buffer with 10-fold concentration (including 500 mM KCl and Tris-HCl (PH 8.4)), 1.5 mM of dNTPs, and 1.25 units of Taq Polymerase enzyme (Vivantis, Malaysia) in the thermocycler (Eppendorf, Germany). The temperature cycles used in this step included an initial denaturation cycle for two minutes at 95 °C, 35 denaturation cycles for one minute at 95 °C, annealing for 30s at 55 °C and finally, the extension step for five minutes at 72 °C. The polymerase chain reaction products were isolated from 1% agarose gel. Four PCR products were cloned for 2 hours at room temperature after gel separation and purification by gel extraction kit (Denazist Asia, Iran) to be bonded to the pTZ57R/T plasmid according to the manufacturer's instructions.

Samples were placed at the temperature of 70 °C for 10 min, in order to deactivate the ligase enzyme, and then, they were transformed into *Escherichia coli* DH5α competent cells by electric shock. Afterwards, one or two ml of LB-Amp culture solution was added and placed for one hour at 37 °C. Then, the above mentioned solution was cultured on the plates containing LB-Amp-Xgal-IPTG (1ml of ampicillin 100 mg/ml in water, 1ml of IPTG 47mg/ml in water and 1ml of X-Gal 40 mg/ml).

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Plates were kept at 37 °C overnight to grow bacterial colonies. After purification of white colonies and culturing in LB-Amp medium, plasmid was extracted using the plasmid DNA isolation kit. Then, for the digestion, four microliters of recombinant plasmid with appropriate cut enzymes, KpnI and XbaI, were placed for 1 to 1.5 h at 37 °C according to the manufacturer's instructions. Finally, the samples were analyzed on 1% agarose gel, and the clones were sent to Macrogen Company (South Korea) for nucleotide sequencing.

Phylogenetic and genetic analysis

The obtained nucleotide sequences were edited by Vector NTI advance 11.5 software (Invitron, USA). The sequences of the studied isolates were then aligned with the sequences deposited in the GenBank using CLUSTAL W method by MEGA7 software (Kumar *et al*., 2016). Accordingly, the similarity of sequences was then calculated by SDT (Sequence Demarcation Tools) software (Muhire *et al*., 2014). MEGA7 software was used to investigate the phylogenetic relationships and to determine the selective pressure on TSV coat protein.

Results

TSV detection and distribution

Based on ELISA, infection of parsley samples obtained from Qom, Markazi, Hamedan and Lorestan provinces were 14, 18.8, 15.4 and 20.1%, respectively. Infection of samples collected from vegetables fields in the studied provinces is presented in Table 1.

Mechanical inoculation

Symptoms observed 14 days after inoculation included: Mosaic on *N. benthamiana*, mottle on *N. tabacum.* cv. *samsun*, chlorosis on *C. amaranticolor*, necrosis on *Vigna unguiculata*, and mosaic on *C. sativum* (Fig. 1). The presence of TSV in these plants was reconfirmed by ELISA.

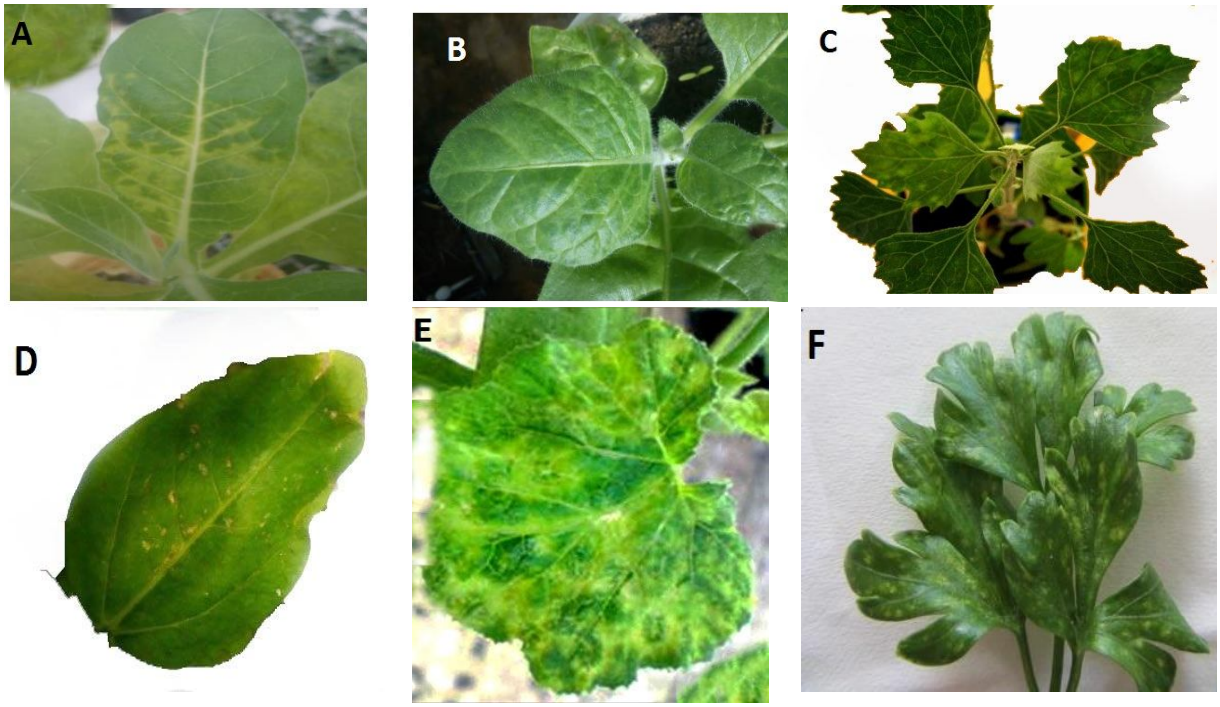

Figure 1 Mosaic on *Nicotiana benthamiana* (A), mottle on *Nicotiana tabacum*. cv. Samsun (B), chlorosis on *Chenopodium amaranticolor* (C), necrosis on *Vigna unguiculata* (D), and mosaic on *Cucumis sativum* (E) in greenhouse environment. Mosaic and chlorosis on parsley (F).

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Molecular characterization of TSV isolates

The expected 747-bp amplicon was amplified using specific primer pair TSV CP RNA3 in RT-PCR reaction. The sequences of four TSV isolates were deposited in the GenBank with accession numbers from MK576107 to MK576110 (Table 2).

The phylogenetic analysis of the nucleotide sequence was performed using Neighbor joining method along with other isolates in GenBank with MEGA7 software, and showed that the isolates were categorized into two clusters (Fig. 2).

Table 2 List TSV isolates deposited in the GenBank database and the accession number for each, together with the origin of the viral isolate, accession number and source.

Accession number	Virus	Host	Isolate	Country
MK576107	TSV	Parsley	Prs1	Iran
MK576108	TSV	Parsley	Prs2	Iran
MK576109	TSV	Parsley	Prs3	Iran
MK576110	TSV	Parsley	Prs4	Iran
KT923144	CLRV	Citrus limon	-	USA
NC_003546	CLRV		-	
EU650677	CVV	Sour orange	AM2	Italy
AB745627	AV-2	Asparagus	-	Australia
AB745629	AV-2	Asparagus	-	Mexico
HG328277	ApMV	-	-	Poland
FN435317	ApMV	Apple	-	India
AM408908	PNRSV	Peach	-	India
AJ133199	PNRSV	Apricot	-	Italy
KP256520	TSV	Soybean	OK	USA
FJ561301	TSV	pumpkin	-	India
JX463339	TSV	Crownbeard	2334	Australia
HQ130448	TSV	Ruharb	pGem2	USA
JX463336	TSV	Sunflower	1973	Australia
KX394691	TSV	Cotton	TSVAV1	India
HM131488	TSV	Groundnut		India
KX397347	TSV	Cotton	TSVMH1	-
AY505082	TSV	Cotton	-	India
AY505081	TSV	Cotton	-	India
AF515824	TSV	Cotton	-	India
AY940155	TSV	Cotton	-	India
FJ608537	TSV	Watermelon		India
KC996727	TSV	Jasmine	-	India
KF264470	TSV	Sunflower	CPKAR	India
KU242586	TSV	Cotton	TSVTS2	-
KU242585	TSV	Cotton	TSVTS1	-
KU242582	TSV	Cotton	TSVTN1	-
KU242587	TSV	Cotton	TSVTS3	-
KU242584	TSV	Cotton	TSVTP1	-
JX294487	TSV	onion	-	India
KU509214	TSV	Cotton	TSVSG2	India
JF340383	SNV	Sunflower	-	India
KU242583	TSV	Cotton	TSVTN2	-
KJ825822	TSV	Soybean	CPCBE3	India
KF264467	TSV	Okra	CPCBE1	India
AF515825	TSV	Sunn-hemp	-	India
X95284	RTSV		-	-

ApMV: Apple mosaic virus, AV-2: Asparagus virus 2, CLRV: Citrus leaf rugose virus, CVV: Citrus variegation virus, PNRSV: Prunus necrotic ringspot virus, RTSV: Rice tungro spherical virus, SND: Sunflower necrosis disease, TSV: Tobacco streak virus.

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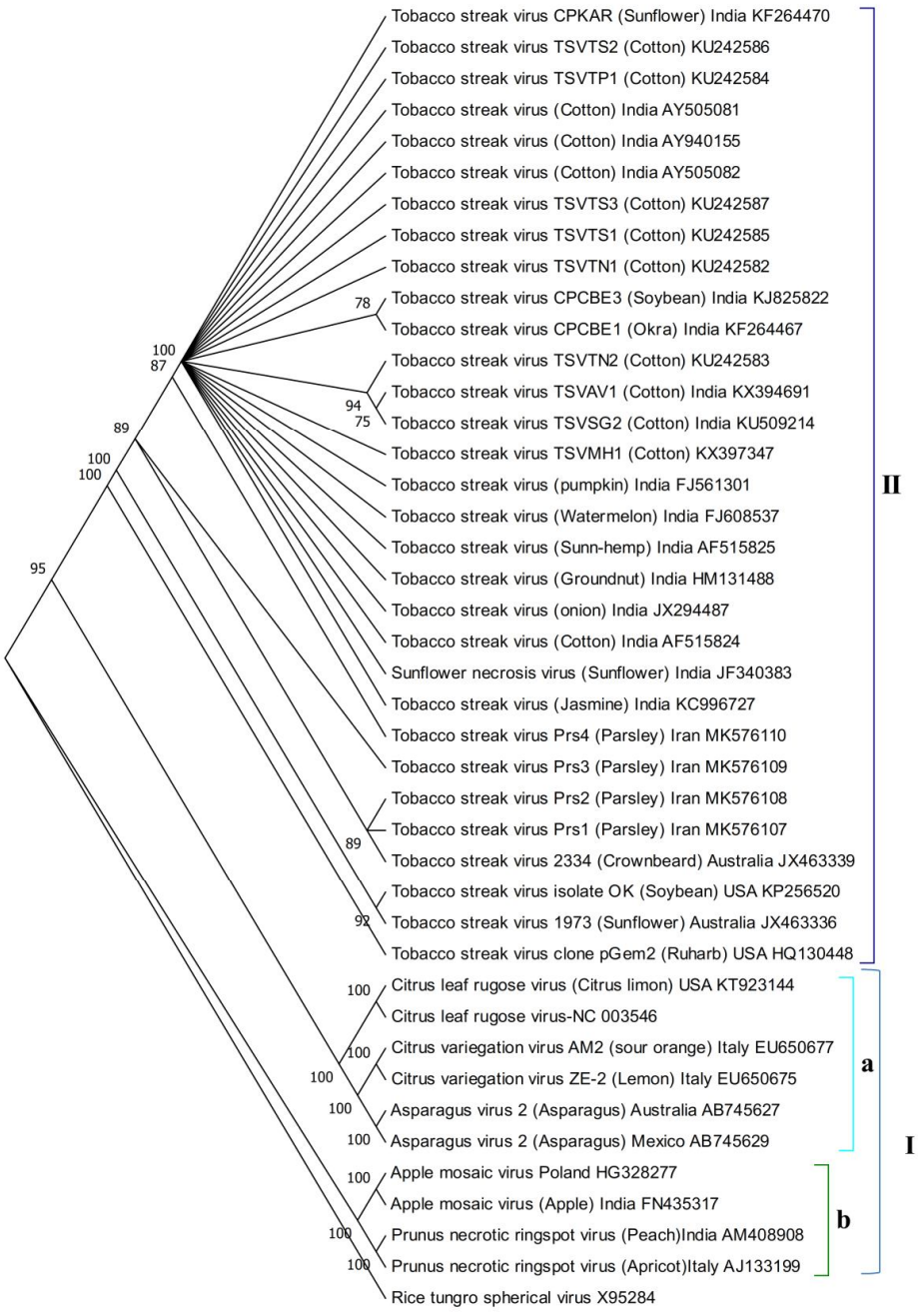

Figure 2 Phylogenetic tree based on CP gene nucleotide sequence of the studied Iranian TSV isolates along with other isolates in GenBank. The tree was constructed by MEGA 7.0 using neighbor joining method with 1000 bootstrap replicates and was rooted with *Rice tungro spherical virus* (accession number X95284).

The TSV isolated from different hosts around the world were placed in Cluster 2, and other members of the genus Ilarovirus were placed in Cluster 1. The members of the second group were subdivided into two sub clusters as a and b, indicating differences between species. Accordingly, Iranian isolates were in a same group with other TSV isolates in cluster 1 (Fig. 2).

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The phylogenetic tree drawn based on amino acid sequence of the virus coat protein was similar to the phylogenetic tree based on the nucleotide sequence, however with the difference that *Apple mosaic virus* (APMV) and *Prunus necrotic ring spot virus* (PNRSV) were classified into the same group as Cluster 2. Other viruses, belonging to the Ilarovirus group, were classified into Cluster 2 and the TSV isolates were in Cluster 3. Meanwhile, TSV isolates from crown beard (Australia), sunflower (Australia), soybean (USA) and common hop (USA) formed a sub cluster due to their differences from other isolates. (Fig. 3).

These results showed little differences among Iranian, US and Australian TSV isolates. Other members of the genus Ilarvirus were separately classified into one cluster (Fig. 3). The phylogenetic analysis showed that the coat protein gene sequence has been preserved among all isolates.

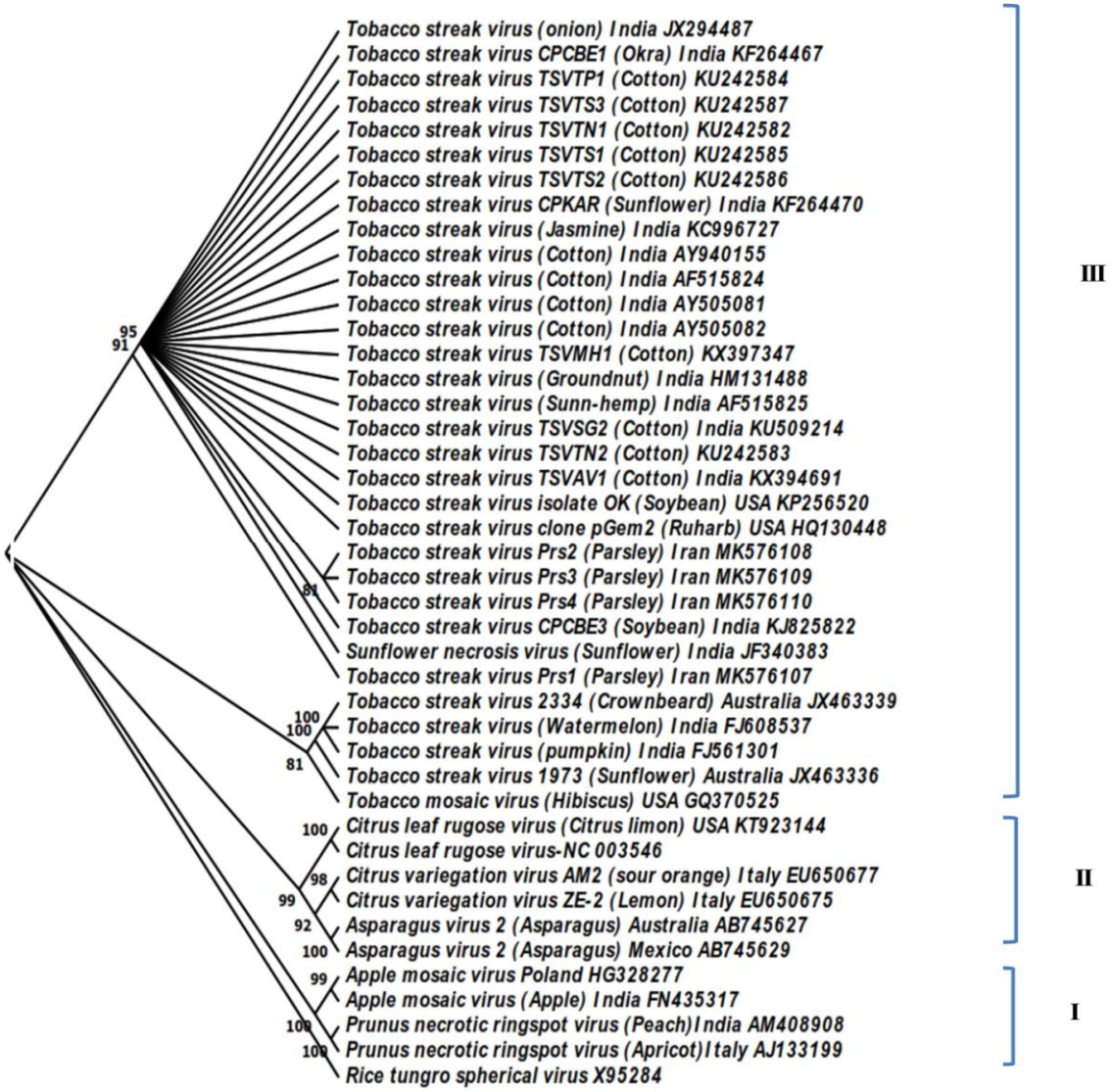

Figure 3 Phylogenetic tree based on of the CP gene amino acids of the studied Iranian TSV isolates along with other isolates in GenBank. The tree was constructed by MEGA 7.0 using neighbor joining method with 1000 bootstrap replicates and was rooted with *Rice tungro spherical virus* (accession number X95284).

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Using SDTv software, the similarity matrix indicated that there were 99.4-100% and 97.6-100% similarity at the level of nucleic acids and amino acids among Iranian isolates, respectively (Fig. 4 and 5). Sequence similarity at the nucleotide level between Iranian isolates and other TSV isolates was more than 90%. The nucleotide sequence similarity of Iranian isolates with TSV isolates from crownbeard (Australia), sunflower (Australia), soybean (US) and common hops (US) ranged from 78 to 83%. Sequence similarity in amino acid levels between Iranian isolates and other TSV isolates was more than 80%.

The sequence similarity of amino acids of Iranian isolates with the TSV isolates from crownbeard (Australia), sunflower (Australia), soybean (US) and common hops (US) was from 70 to 75%.

Determination of selective pressure in TSV coat protein

Estimation of Tajimas's D factor and dN/dS ratio (dN: 0.019 and dS: 0.170) using MEGA7 software showed that dN/dS ratio in TSV coating protein was less than one (0.122). Also, result of the Tajimas's D test was negative (-2.2), confirming negative selection in this area.

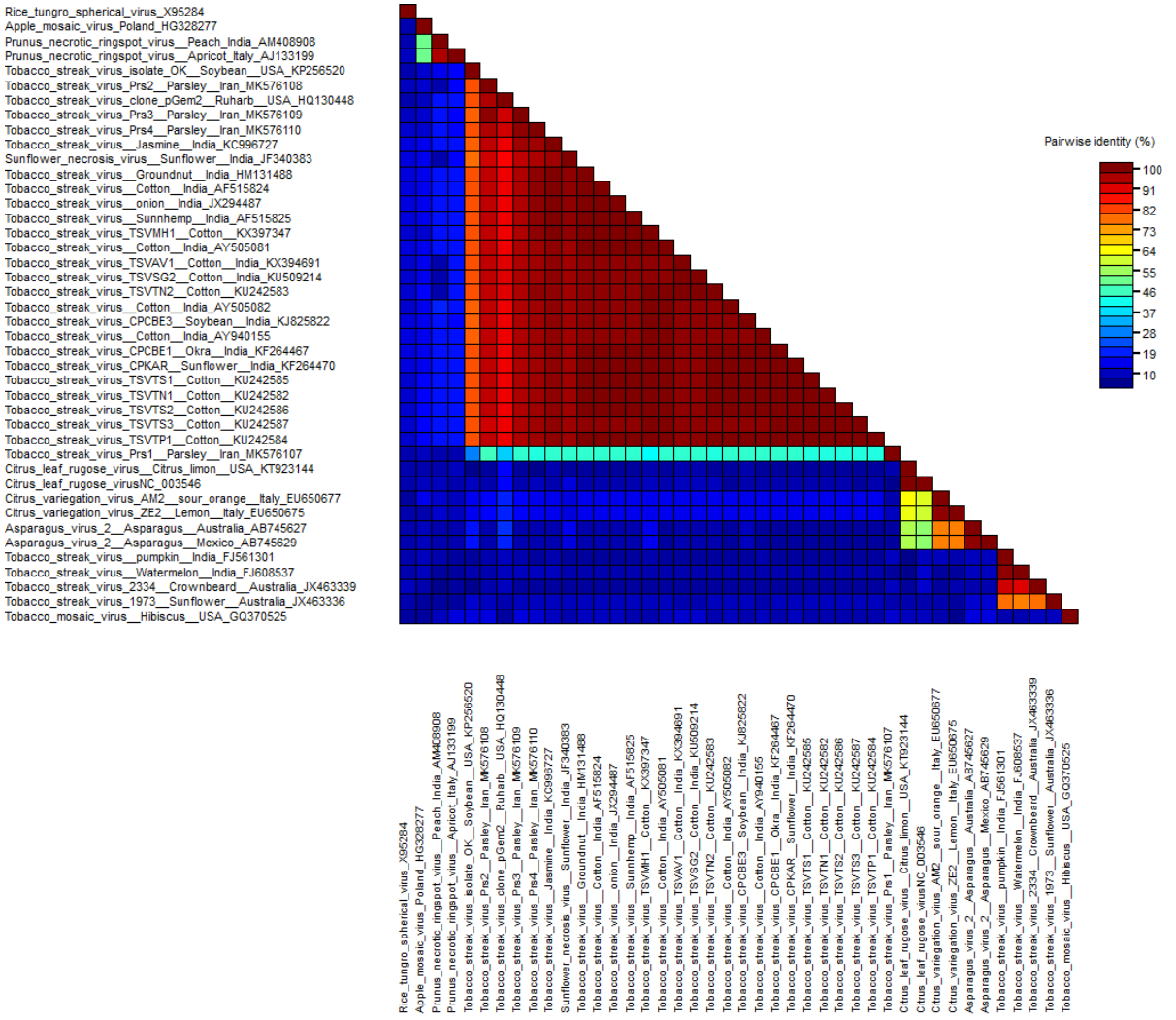

Figure 4 Matrix of pairwise equivalence percentage between amino acids of the coat protein gene of Iranian TSV and other isolates retrieved from the GeneBank.

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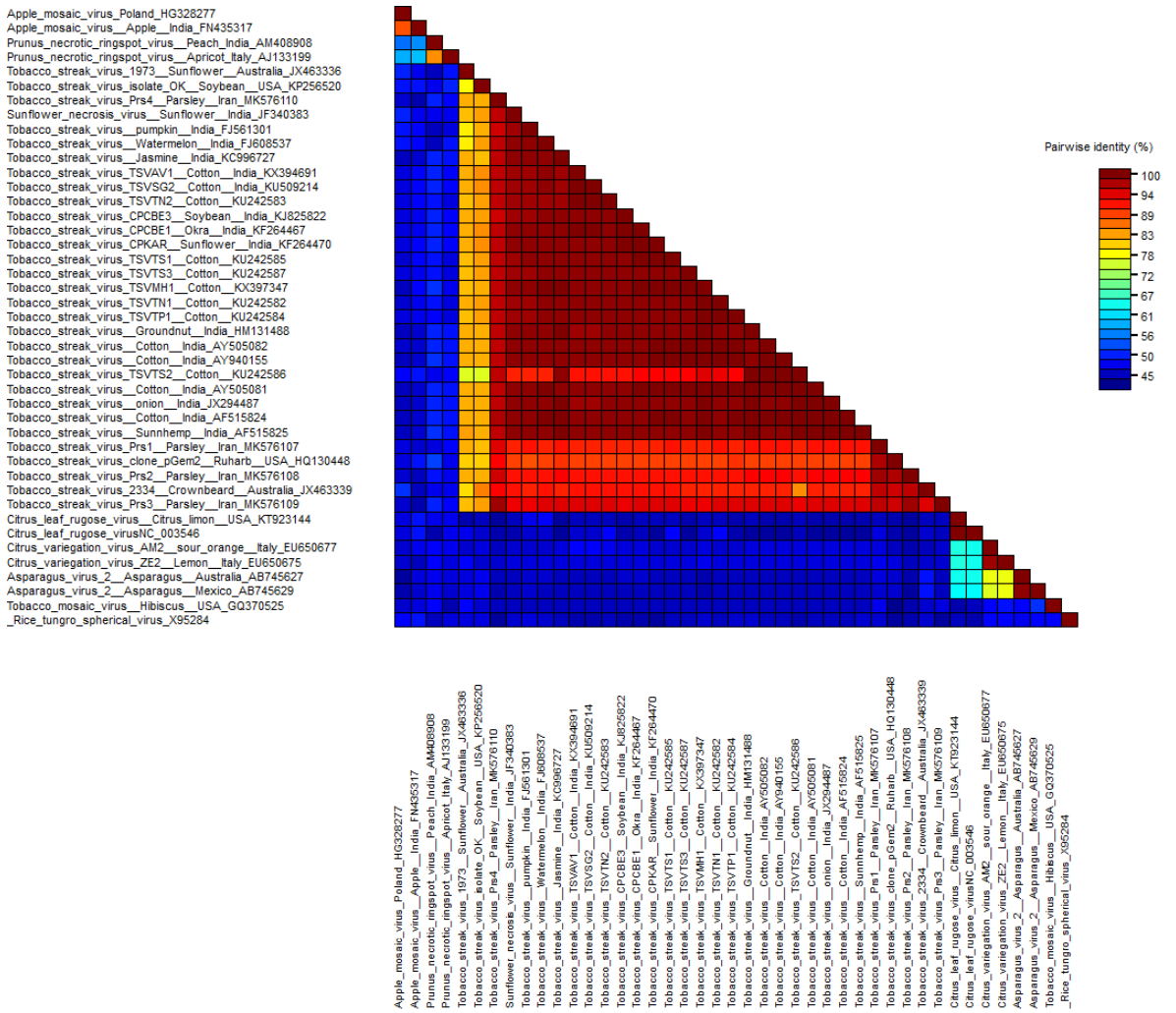

Figure 5 Matrix of pairwise equivalence percentage between nucleotides of the coat protein gene of Iranian TSV and other isolates retrieved from the GeneBank.

Discussion

The exchange of vegetable seeds with annual seeding of crops by the farmers, in addition to the widespread distribution of thrips as the TSV vector has resulted in the spread of TSV virus in many parts of the country. The extensive spread of the virus has provided the background for its evolution to infect a large number of hosts of different plant families in various climatic conditions (Jailani *et al.,* 2019). Our results showed incidence of TSV in the vegetable fields of the Qom, Markazi, Lorestan and Hamadan provinces, of 17%. Up to now, this is the first report of genetic diversity of TSV in Iran. Results of sampling and immunosorbent assay demonstrated that Hamadan province had the highest infection rate of TSV in parsley (20.1%) and Qom province had the lowest rate (14%). The rate of infection with the virus was 18.8% and 15.4% in Markazi and Lorestan provinces, respectively. Environmental factors such as minimum temperature, relative humidity, and leaf moisture play an important role in the occurrence of TSV. Low temperatures, high relative humidity, and higher leaf moisture are suitable for the spread and prevalence of TSV (Elmer, 2001; Vinodkumar *et al*., 2017).

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Researchers have reported that TSV causes severe necrosis at temperatures from 25 to 30°C under greenhouse conditions (Greber *et al*., 1991). On the other hand, planting date has a significant effect on the frequency and prevalence of this virus (Shirshikar, 2003). As indicated in the results, the highest incidence and frequency of TSV was in Hamadan province, and the lowest in Qom. Hamadan region has a more moderate weather conditions in comparison with Qom, because Qom has a hot desert climate. Therefore, considering that parsley is cultivated in Qom province during the hot months of June, July and August, the prevalence of TSV is lower, while the temperature range of cultivating parsley in Hamadan province is suitable for distribution and prevalence of this virus. However, the low prevalence of TSV in Lorestan province is significant, because its climate is similar to that of Hamadan province, the low infection rate in Lorestan province might be due to population of *Thrips tabacci* Linderman and *Frankliniella occidentalis* Pergande (Kaiser *et al*., 1991), cultured accession of parsley, the presence of weeds such as *Dautura stramonium* and *Chenopodium quinoa* (was observed during sampling) because TSV is seed-borne in these plants (Sdoodee and Teakle, 1987), and also the effects of neighboring fields such as soybeans, tomatoes and mung beans (Tzanetakis *et al*., 2003). The large number of a vector population such as thrips due to the infected pollens, probably affects the prevalence of TSV in healthy plants (Prasada Rao *et al.,* 2003).

Strong winds in the growing season cause widespread dispersion of pollens from infected plants, which, increase the rate of virus spread and it shows the critical role of pollens in transmission of TSV virus, if it is going to be dispersed by vector thrips (Sharman *et al*., 2011).

Although TSV has been reported in many countries around the world and is an important economic virus, its complete genome sequence has been only reported for seven isolates from Australia, United States, and India. Studies have demonstrated that the TSV coat protein gene isolated from different hosts such as other Ilarviruses is highly preserved (Vinodkumar *et al*., 2017; Jailani *et al*., 2019). Therefore, in this study, the phylogenetic analysis of TSV isolates was performed based on the coat protein coding region.

In the phylogenetic tree of Iranian isolates and the isolates from different crops in other parts of the world, two and three separate clusters were formed based on the nucleic acid and amino acid sequences, respectively, (Figs. 2 and 3). This finding is consistent with previous study (Rao *et al*., 2003).

All TSV isolates were placed in Cluster 2, based on the nucleotide sequence. The Iranian isolates were placed near to the Indian isolates with the similarity rate of 92%. Two Iranian isolates, Prs 1 and Prs2, are placed in one sub cluster, both of which have been isolated from Markazi and Lorestan provinces. This indicates that probably due to the exchange of agricultural products and seeds between these two provinces, the two isolates are more closely related. The other two isolates, Prs3 and Prs4, were isolated from the Hamadan and Qom provinces, respectively. In terms of nucleic acid sequence, the Iranian isolates have the highest affinity (96%) with the Indian isolates from the sunflower, 94% from soybean and 92% affinity with those from onions. The results indicated that the sequence of coat proteins of Iranian and Indian isolates were not similar in two amino acids, however Iranian isolates differed from American and Australian isolates in 72 nucleotides and 20 amino acids. Changes in some of the amino acids in the gene coding region require further study to determine their effects on virus biology. Based on the phylogenetic tree of the nucleotide sequence, the isolates of cluster 2 have on average 92% similarity with each other, and cluster 1 and cluster 2 have an approximately 82% similarity. Evolutionary genomic analysis revealed the conserved nature of TSV encoded CP gene infecting cotton, mungbean, sunflower and sun hemp, however a variable region has been identified

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(Bhat *et al*., 2002). The conserved nature of CP gene in TSV was also confirmed by Bheemathatti *et al*. (2010) in sunflower, gherkin and pumpkin. Sivaprasad *et al*. (2013) revealed that, 15 isolates of TSV infecting various hosts including, groundnut, sunflower, onion, blackgram, greengram, jute, marygold, calotropis, pumpkin, watermelon and kenaf plants in India had highly conserved coat protein genes. TSV affects an increasingly wide range of crop species but the CP of isolates from different hosts and locations appears highly conserved, displaying >95% identity among the aa of the CP (Pallas e al., 2013). Other subgroup 1 Ilarviruses (*Bacopa chlorosis virus* (BCRV), *Parietaria mottle virus* (PMoV) and *Strawberry necrotic shock virus* (SNSV)) share <80% identity with the CP of TSV (Galipienso *et al*., 2008).

Phylogenetic studies of the Indian isolates also showed that geographical differences were more important in the grouping of these isolates than the host (Rageshwari *et al*., 2017). The similarity of nucleotide sequence of Iranian isolates with TSV isolates of Australia and the US was 78 to 83%, therefore the Iranian isolates in this study are more similar to Asian isolates from India. Studies on the genus Ilarovirus and TSV isolates have shown that because of the widespread distribution of the virus due to the export of agricultural products to different parts of the world, virus isolates may not be distinguished by geographic region, so that US isolates could be placed in one cluster along with the Australian and Asian isolates which is inconsistent with the previous report from Vinodkumar *et al.* (2017) who studied genetic diversity of seven TSV isolates by sequencing the CP region and aligning a matrix of 900 bp with data of other TSV isolates from GenBank. In viruses whose genome is a single-stranded RNA, its recombination is likely to be more than other viruses. This is unavoidable in the evolutionary process of viruses and results in the creation of new isolates in a region, and thereby infection of new hosts. The amino acid composition is conserved at positions 62, 73, 127 and 180 as alanine^{62}, valine^{73}, phenylalanine^{127}, serine^{180}, respectively, which, in Iranian isolates, are the same as those of other countries (Senthilraja *et al*., 2018). A considerable proportion of the arginine and/or lysine residues that occur in the CP of ilarviruses are found in the N-terminal region of the molecule (Pallas *et al*., 2013). The amino-terminal peptides containing this basic motif are sufficient to bind to the 3'-nontranslated region (3'-NTR) of its own RNA (Ansel-McKinney *et al*., 1996). Mutational and comparative analysis of the N-terminal CP sequences of TSV have led to the proposal of an RNA-binding consensus sequence (Q/K/R-P/N-T-X-R-S-R/Q-Q/N/S-W/F-A). In this sequence, a single arginine (R) is the only residue that is essential and responsible for the specific binding of either those peptides corresponding to this motif or full-length CPs to a terminal fragment of 3'NTR RNA (Swanson *et al*., 1998)

The overall dN/dS ratio for the CP gene was less than 1.00 which most of the mutations did not alter the amino acid, indicating negative or purifying selection, and these results were predictable according to the roles of the coat protein. Therefore, it could be concluded that the *CP* gene is under a high selection pressure (dN/dS = 0.122).

Low values of dN/dS have been reported in different genomic regions for the other members of the family Bromoviridae (Revathy and Bhat 2017; Nouri *et al*., 2014; De Silva *et al*., 2002).

Detection of viral agents in different hosts makes the selection of suitable strategy for their control more feasible. One of the most important ways to control plant viruses is the use of resistant cultivars. Before investigating the relative resistance of cultivars or transgenic plants to control the virus, it is necessary to study the genetic diversity of Iranian virus isolates, because determining the genetic diversity in a viral group and understanding the mechanisms and factors affecting diversity and variability are important for determining and applying resistance genes.

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Declaration of conflicting interests

The Authors states that there is no conflict of interest.

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بررسی وقوع و پراکنش ویروس رگه‌ای توتون جدا شده از گیاه جعفری *Petroselinum sativum*

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چکیده: هدف از انجام این پژوهش تعیین پراکنش ویروس رگه‌ای توتون (TSV) در سبزی کاری‌های استان‌های قم مرکزی، لرستان و همدان بود. در این پژوهش در سال ۱۳۹۶، ۴۷۵ نمونه گیاه جعفری جمع‌آوری شد.آلودگی این نمونه‌ها به TSV، با استفاده از آزمون سرولوژیکی به روش ساندویچ دو طرفه الیزا (Double Antibody Sandwich-ELISA, DAS-ELISA) و با استفاده از پادتن اختصاصی ویروس، مورد بررسی قرار گرفت. نتایج این پژوهش نشان داد که آلودگی نمونه‌های گیاه جعفری از استان‌های قم، مرکزی، همدان و لرستان به TSV، به‌ترتیب ۱۴، ۱۸/۸، ۱۵/۴ و ۲۰/۱ درصد کل نمونه‌ها است. آنالیز توالی نوکلئوتیدی و اسید آمینه پروتئین پوششی این جدایه‌ها نشان داد که جدایه ایرانی و جدایه هندی با هم در یک گروه قرار می‌گیرند. درخت فیلوژنتیکی ترسیم شده براساس توالی نوکلئوتیدی ناحیه پروتئین پوششی و توالی اسید آمینه، نشان داد که جدایه‌ها به‌ترتیب در دو و سه گروه قرار می‌گیرند. جدایه‌های ایرانی به‌همراه جدایه‌های TSV دنیا در یک گروه قرار گرفتند و سایر ایلارویروس‌ها گروه جداگانه‌ای تشکیل دادند. این اولین گزارش از بررسی تنوع ژنتیکی TSV در ایران و اولین گزارش از وقوع آلودگی مزارع سبزیجات استان‌های قم، مرکزی، همدان و لرستان به TSV می‌باشد.

واژگان کلیدی: گیاه جعفری، ایلارویروس، الایزا، RT-PCR

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