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The First Report of *Tomato Yellow Leaf Curl Virus* (TYLCV) Occurrence in Tomato Greenhouses of Lorestan Province

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ABSTRACT

Tomato yellow leaf curl virus (TYLCV) is one of the most important tomatoes (*Solanum lycopersicum* L.) viral diseases in tropical, subtropical, and temperate regions of the world. The amount of damage caused by this virus is severe and may reach 100%. Its natural vector is *Bemisia tabaci*. In order to detect and investigate the genetic diversity of TYLCV, suspected leaf samples were collected from tomato greenhouses in Khoramabad city. In the molecular evaluations, a 550bp fragment was amplified using the specific degenerate primer pair of Begomoviruses transmitted by whiteflies, which indicates TYLCV infection in the investigated samples, and overall, 18 infected tomato samples were detected out of 30 samples suspected of TYLCV. Also, the comparison of the nucleotide sequences of Khoramabad isolates (Kh1 and Kh2) in NCBI confirmed the detection of TYLCV in the studied region. The determined nucleotide sequences showed 94.79-97.63% and 90.5-95.69% nucleotide and amino acid identities with other available sequences of TYLCV in NCBI, respectively. Pair-by-pair comparison matrix of nucleotide sequences of two Khoramabad isolates with 15 selected isolates from the gene bank using SDT v1.2 software showed the sequences of the two studied viral isolates are highly similar and the percentage of their nucleotide similarity is 95.60%. Also, Kh1 isolate had the most similarity (95.40%) with the Kuwait isolate and the Kh2 isolate showed the highest similarity (97.60%) with the Iraq isolate. These results were identical to what was seen in the drawn phylogeny tree using Mega11. This was the first report of widespread occurrence of TYLCV in tomato greenhouses of Lorestan province.

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Introduction

Solanum lycopersicum L. belongs to the *Solanaceae* family and is one of the most important agricultural products in Iran. According to the latest statistics of the Ministry of Agriculture in 2022, the under-cultivation area of tomatoes was equal to 143 thousand hectares. The total area of greenhouse in 2021 is 9800 hectares of which about 7% belongs to tomatoes. Greenhouse tomato has the second rank in terms of the area under greenhouse cultivation and also the amount of production among vegetable and summer varieties (Ahmadi *et al.,* 2020).

Among pathogenic agents, the role of viruses in reducing tomato yield is very important and among them, the viruses responsible for tomato yellow leaf curl disease (TYLCD) which include several viruses belonging to different species are very important as a limiting factor in tropical and subtropical regions of the world, so that farmers are forced to replace tomato cultivation with other crops (Prasad *et al*., 2020). Due to the serious damage and the wide spread of the disease, one of the members of this group named *Tomato yellow leaf curl virus* (TYLCV) has been introduced as one of the ten most important plant viruses (third rank) in the world (Bananej, 2016). TYLCV is a species in the genus *Begomovirus* and the family *Geminiviridae* and has a single-stranded DNA genome with twin particles and is transmitted by the whitefly *Bemisia tabaci* in a

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persistent, circulative, and non-propagative manner. TYLCV has a widespread distribution in Mediterranean, Middle Eastern, and African countries, including Cyprus, Israel, Ivory Coast, Jordan, Lebanon, Senegal, Egypt, and Tunisia (H El-Sappah *et al*., 2022). This disease has been observed in Israel and the Jordan Valley since the early 1960s and has currently economic importance in many countries (Prasad *et al*., 2020).

This virus has also been reported from the central and southern provinces of Iran and it is relatively widespread in tomato fields and greenhouses of Sistan and Baluchistan, Kerman, Yazd, Razavi Khorasan, Hormozgan, Fars, and Khouzestan in Iran, however, its host range is not limited to tomato (Ramazani *et al*., 2022). TYLCV spreads systemically in the plant and causes cytological changes in the phloem. Symptoms of contamination include curling, yellowing and cupping of leaves, dwarfism, lack of flowering, and reduced crop production. The damage of this disease sometimes includes the entire product (Patil and Fauquet, 2021).

The genome of *Begomoviruses* is monopartite or bipartite. TYLCV is a single-segmented *Begomovirus* that has six ORFs, two of which are located on the viral strand (V1 and V2) and four on the complementary strand (C1 to C4), the ORFs C1, C2, and C3 are relatively similar to each other are overlapped and C4 is completely covered by C1. Single-segmented *Begomoviruses* lack two genes in the B segment (Gharouni *et al*., 2014, Hasanvand and Pakbaz, 2022). Czosnek et al. (2021) have investigated the biological, molecular, epidemiological, and management characteristics of TYLCV and have shown that this viral disease is spreading rapidly throughout the world. In recent years, the damage caused by this virus in tomatoes has been associated with the global spread of the whitefly *B. tabaci* biotype B. Compared to other biotypes, this biotype has a wider host range and superior reproductive ability, and its feeding behavior is associated with more aggression (Czosnek *et al*., 2021).

The occurrence of recombination among different isolates and races of TYLCV is the main cause of high genetic diversity in this virus (García-Andrés *et al*., 2007). The results obtained from the phylogenetic studies indicated that the Iranian virus isolate (TYLCV-IR, Acc. No. Aj132711) is the result of recombination in the intergenic region among the Iranian isolate (*Tomato leaf curl Iran virus*, Acc. No. AY29792) (Tabein and Behjatnia, 2022) and the Israeli isolate of the virus (TYLCV-Mid, Acc. No. X76319) (Bananej *et al*., 2004). Considering the spread of this important and damaging virus in most parts of the country especially the neighboring cities of Lorestan province, the purpose of this study is to detect TYLCV in the greenhouses of Khoramabad city which are involved in high whitefly populations.

Materials and Methods

Sampling and DNA extraction

In visiting the greenhouses of Khoramabad city in 2022, sampling was done from plants with virus symptoms such as chlorotic spots between the veins, blistering and twisting and cup-shaped leaves, small leaves, and plant dwarfism. Thirty samples were transported to the laboratory under controlled temperature conditions and utilized for molecular evaluations. A part of each sample was transferred to a -80 °C freezer for long-term storage. For molecular evaluations, firstly, the total DNA of the plant was extracted. DNA extraction was performed using CTAB buffer (Zhang *et al*., 1998).

Detection of TYLCV

Polymerase chain reaction (PCR) was used to determine the contamination of samples with TYLCV. For this purpose, a pair of degenerate primers PCRv181 and Bc (Table 1) specific for whitefly-transmissible *Begomoviruses* were used (Rojas, 1993; Deng *et al*., 1994). These primers can amplify the 3' end part of the intergenic region and about 200 base pairs from the 5' end of the coat protein gene.

The PCR was performed in a final volume of 25 microliters, including 12.5 microliters of 2x PCR Bio Taq Mix Red kit from PCR Bio-system company, one microliter of each forward and reverse primers (10pmol), and 100ng of total DNA. The thermal program required for amplification of the desired fragment included 94$^{℃}$ for three minutes as an initial denaturing followed by 35 cycles of denaturing step at 94$^{℃}$ for 30 seconds, an annealing step at 56$^{℃}$ for 40 seconds, an extension step in 72$^{℃}$ for 35 seconds

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and a final extension for 10 minutes at 72℃ to complete the polymerization. In the negative control sample, sterile distilled water was used instead of DNA.

Safe DNA stain solution of Pishgam company was used to stain the PCR products and also, in order to observe the amplified products and evaluate their quantity and quality, 1% agarose gel electrophoresis was used in the presence of the 100bp standard molecular marker of Pishgam company to determine the size of the amplified fragments and the results were observed using gel document.

Table 1. Oligonucleotide primers used in this research

Primer name	Size	Sequences (5'→3')	Fragment size
PCRv 181	18	TAATATTACCGGWTGGCC	550 bp
Primer Bc	23	TGGACYTTRCAWGGBCCTTCACA	
PAL1v1978	26	CTGCAGGCCCACATYGTCTTYCCNGT	1300 bp
PAR1c496	23	CTGCAGGGCTTYCTRTACATRGG	

Unspecified bases with letters other than A, T, C and G in the primer sequences are as follows: Y= C/T, W= A/T, R= A/G, K= G/T, B= C/G/T

Then, the PCR products that were amplified using the degenerate primers of the genus *Begomovirus* and their quantity and quality were evaluated using Nano drop and electrophoresis, were grouped based on the quality of the PCR product and sent to Codon company of Iran for purification and sequencing in order to determine the virus species. A comparison of obtained nucleotide sequences with other sequences in the gene bank was done using Nucleotide Blast tool (nBlast) in NCBI. The amplified region was including the end part of the intergenic region and about 200bp from the 5' end of the TYLCV coat protein gene.

Also, in order to amplify and access a larger area of the virus genome length in infected samples, a specific degenerate primer pair named PAL1v1978 and PAR1c496 were used (Table 1) (Rojas, 1993), which had been designed to amplify a 1300bp fragment in the infected samples including the Rep protein gene, the intergenic region (the stem-loop and the conserved TAATATTAC nucleotide sequence) and part of the coat protein gene in the TYLCV genome (Samretwanich *et al.*, 2000). The annealing temperature of these primers in the thermal program of the thermocycler was considered to be 56 ℃.

Sequences and phylogeny position analysis

The obtained sequences from Codon Company were first evaluated using Nucleotide Blast tool in NCBI and confirmed the virus species. After editing the 3' and 5' ends of the nucleotide sequences using Chromas and BioEdit software, the final sequences were used to draw a phylogeny tree. Several nucleotide sequences related to the same genomic region of TYLCV that were registered in the gene bank were used to determine the phylogeny position of the virus isolates detected from Khoramabad city. For this purpose, multiple sequence alignment was done using ClustalW tool in Mega11 software and then the phylogeny tree was drawn by Neighbor-joining method and based on 1000 replications in bootstrap evaluation. Also, the similarity analysis of the nucleotide sequence of the studied genomic region between the sequenced isolates and the gene bank was carried out using SDT v1.2 software (Muhire *et al.*, 2014).

Greenhouse studies and virus transmission

Since TYLCV does not have mechanical transmission, one isolate was selected from the plants in which the presence of the virus had been confirmed by PCR and transplanted on healthy tomato plants. These plants were kept in the greenhouse under suitable temperature and light conditions for the appearance of symptoms and the availability of viral sources.

Results and Discussion

Sampling and greenhouse observations

During the visit and sampling from different stages of tomato growth in greenhouses of Khoramabad city, a wide range of TYLCV symptoms in the infected samples was observed, including severe dwarfism of tomato plants, severe leaf spotting especially in the upper leaves, yellowness, twisting of the leaf margins

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and cup-shaped leaves, wrinkling, blistering and deformity of the leaf (Fig. 1). In some cases, the flowers fell after the infection and it caused a sharp reduction in the amount of fruit or its size. This variety of symptoms was similar to previous reports (Gharouni *et al*., 2014). In another research, the scientists reported that tomato plants that were infected naturally, showed severe stunting, marginal chlorosis, upward or downward leaf curling and flower abortion, and stem upright and yellowing (Haq *et al*., 2018).

The variety of observed symptoms can be caused by the difference in the type of planted cultivars, the difference in the age of infection, and also the difference in the infectious viral isolates (Fazeli *et al*., 2009). However, only observing the symptoms is not reliable to diagnose the infection definitely and the mentioned symptoms may appear as a result of infection caused by other viruses and pathogens or environmental stresses. Therefore, definitive diagnosis of the disease is possible through the transmission of the pathogen to healthy plants (via vector insects or grafting) and serological and molecular tests (Ramazani *et al*., 2022). In addition, host weeds can act as an important source for maintaining this virus. Weeds naturally play an important role in early-season infections and their biology and epidemics. Fazeli and colleagues have introduced two weeds *Chrozophora tinctoria* and *Herniaria* sp. as the hosts of TYLCV in the southern regions of Iran (Fazeli *et al.,* 2009).

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Fig. 1. Observed symptoms in tomato plants infected with TYLCV in the greenhouses of Khoramabad city: a) yellowing, b) stunting and severely reduced leaf size especially in the upper leaves, c) blistering and deformation, d) cup-shaped leaves and leaf curl.

Transmission of TYLCV

Virus symptoms were observed on tomato plants about 14-21 days after grafting. The symptoms of infection included dwarfism, leaf curl, yellowing, green veins in the yellow text of the leaf (Fig. 2). Although this virus can be easily transmitted through grafting, this method is not important in natural infections and is only used in research.

Fig. 2. The results of grafting in the greenhouse: a) Grafting on the first day, b) Appearance of TYLCV symptoms on transplanted tomato including yellowing, leaf curling and small leaves after 14 days.

Detection of TYLCV

The results of the PCR using PCRv181 and Bc degenerate primers confirmed the definitive infection of 18 tomato samples to TYLCV in greenhouses of Khoramabad city. These primers were able to amplify a fragment of 550bp, while this fragment was not amplified in the negative control sample (Fig. 3a).

The amplified fragment includes the 3' end sequence of the intergenic region and about 200 base pairs from the 5' region of the coat protein gene. Also, these degenerate primers were able to reproduce the expected fragment in tomato

samples suspected of viral infection. Additional studies including sequencing and the use of species-specific primers led to the confirmation of TYLCV infection in Khorasan province and also *Tomato leaf curl Palampur virus* (ToLCPMV) in watermelon fields in Khuzestan province (Gharouni *et al*., 2014; Jelavi *et al*., 2022). Also, PAL1v1978 and PAR1c496 primers were able to amplify a 1300bp fragment related to the sequence of Replicase gene, the intergenic region and the partial coat protein gene (Samretwanich *et al*., 2000) in TYLCV infected samples, while no fragment was amplified in the negative control sample (Fig. 3b).

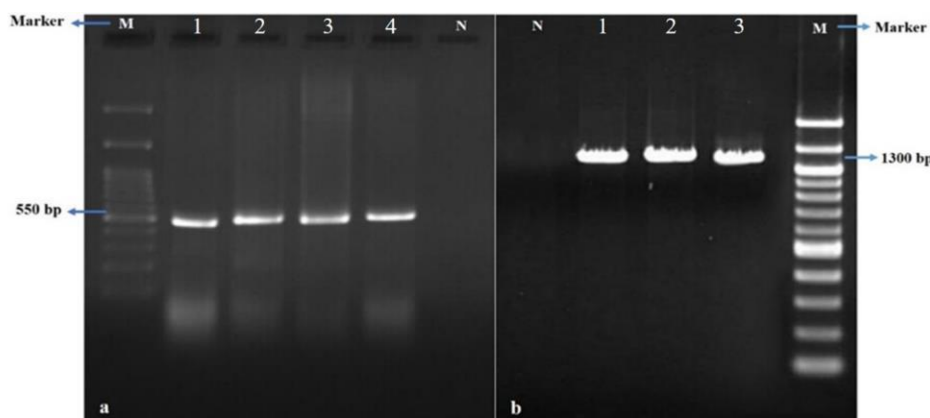

Fig. 3. Electrophoresis pattern of amplified fragments in 1% agarose gel: a) Amplified fragments using degenerate primers PCRv181 and Bc in the range of 550bp (columns 1,2,3 and 4); b) Amplified fragments using degenerate primers PAL1v1978 and PAR1c496 with a size of 1300bp (columns 1,2 and 3); M: DNA marker 100-3000bp, N: negative control.

Obtained nucleotide sequences from Codon Company after editing the 3' and 5' ends nucleotide sequences were recorded in NCBI database by Bankit. The accession numbers of the isolates studied in present study are OR711559 and OR711560 for isolates name of TYLCV.IR:Kh1 and TYLCV.IR:Kh2, respectively.

In this research, the sequence of the 3' end part of the intergenic region and 200 nucleotides from the 5' end of the coat protein gene were used to analyze and draw the phylogeny tree. In order to determine the phylogenetic relationships and position, several sequences of TYLCV isolates in the studied gene region and related to different hosts and geographical regions, including Iran and other countries, were extracted from NCBI (Table 2) and compared with Khoramabad TYLCV isolates. Also, *Maize streak virus* (MSV) (HQ693474.1) was selected as outgroup to draw the phylogeny tree (Fig. 4). The comparison between isolates of Khoramabad showed that there is not significant difference between the isolates of this city in the investigated area and their similarity is very obvious. Based on the results of nucleotide blast in NCBI, Kh1 and Kh2 Khorramabad isolates showed the most similarity at the level of nucleotide sequence with Iraq (KY657553.1) and Kuwait (KJ830841.1) isolates with 99% similarity. These results were identical to what was seen in the phylogeny tree (Fig. 4).

In order to determine the relationship of TYLCV isolates, three very specific regions of the viral genome including intergenic region, coat protein and Replicase gene are used to compare the isolates (Czosnek and Laterrot, 1997). For this purpose, 200 nucleotides at the end of the 5' of the coat protein gene can also be used. The phylogenetic analyses in TYLCV based on those three regions are very similar, but the comparison based on the 200 nucleotides of the 5' end of the coat protein gene differentiates the isolates better (Brown *et al*., 2001; Gharouni *et al*., 2014).

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Table 2. Accession numbers and characteristics of TYLCV isolates available in gene bank used in phylogeny analyses.

Isolate	Accession number	Host	Country
Nas1	KY657553.1	*Solanum lycopersicum*	Iraq
KISR-2	KJ830841.1	*S. lycopersicum*	Kuwait
Tom96	LN680631.1	*S. lycopersicum*	Oman
MG20	MG970362.1	*Phaseolus vulgaris*	Oman
KB-18	HE819239.1	*Capsicum sp*	Oman
NAS-61	MT800839.1	*Eggplant*	Pakistan
NAS-69	MT800822.1	*Cucurbita pepo* (zucchini)	Pakistan
m54073	MW427155.1	*S. lycopersicum*	Pakistan
IR:Jir:44J:MD:19	OQ319130.1	*Dracocephalum moldavica*	Iran: Jiroft
IR:Jir:Soja19	OQ319127.1	*Glycine max*	Iran: Jiroft
IR/Jir/88J/Sun/19	OR427292.1	*Helianthus annuus*	Iran: Jiroft
IR:Ho33:06	GU076443.1	*S. lycopersicum*	Iran: Minab
MAKAL61	MN432877.1	*S. lycopersicum*	Iran: Fars
63-Birjand	JQ928346.1	*S. lycopersicum*	Iran: Birjand
AZ24-3	MN397779.1	*S. lycopersicum*	Saudi Arabia

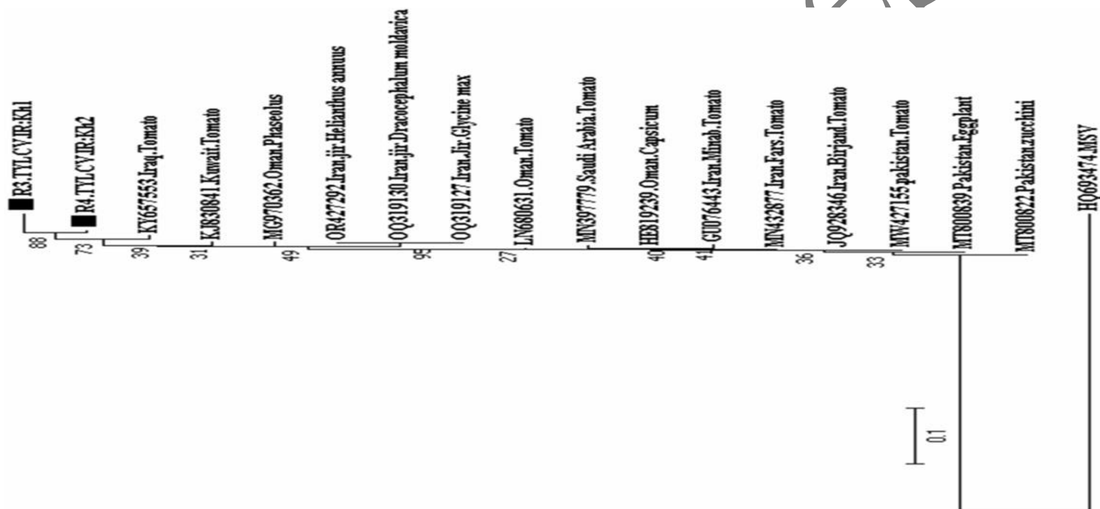

Fig. 4. Phylogeny tree generated by Mega11 software with Neighbor-joining method (Bt×1000) based on the amplified region using PCRv181/Bc primer pair in TYLCV isolates sequenced in the present study and some isolates available in the Gene Bank. The MSV was used as an outgroup. The isolates sequenced in this study are marked as ■ in the tree.

Also, the results of the two-by-two comparison of nucleotide sequences of the studied genomic region using SDT v1.2 software showed that the sequences of the two studied viral isolates are very similar and the percentage of their nucleotide similarity is 95.60% (Fig. 5). According to the result comparison matrix, Kh1 isolate had the most similarity (95.40%) with Kuwait isolates and it was 93.90-94.60% similar with the other. The Kh2 isolate showed the greatest similarity (97.60%) with the Iraq isolate. Also, it showed 90.40-96.30% similarity with the other isolates. The genetic distance between all the Iranian isolates is well known in this matrix.

Presently, the percentage of infection in some areas infected with TYLCV in Iran is so high that leads to a reduction of more than 90% of the product. In these areas, due to the high prevalence of TYLCV infection and the high population of the vector, farmers carry out delayed cultivation. Since this virus has no mechanical transmission, it is transmitted through seeds and soil, and its transmission depends on the vector *B. tabaci* (Bananej, 2016). So, one of the most important reasons for the spread of this virus towards the northern regions of Iran in recent years can be attributed to the earth's warming followed by the expansion of the

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warm weather towards higher latitudes, which is favorable for the activity of vector.

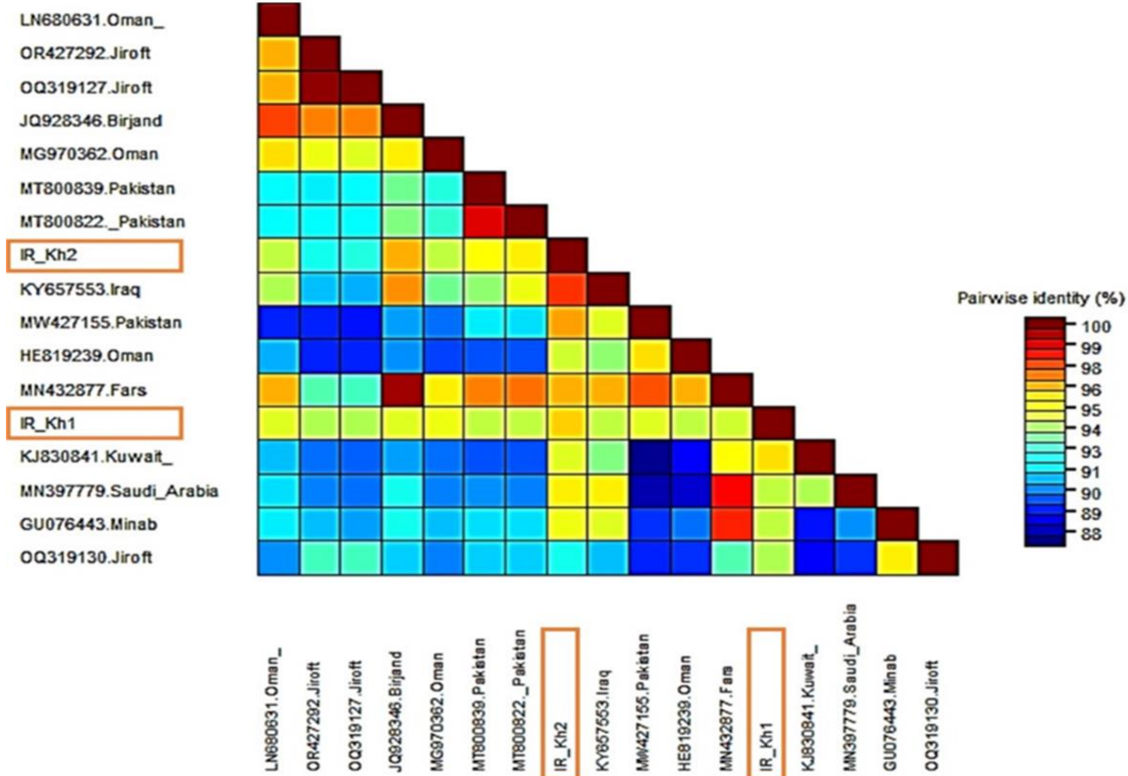

Fig. 5. Pair-by-pair comparison matrix of nucleotide sequences of the studied genomic region of two Khoramabad TYLCV isolates with 15 selected isolates from the gene bank using SDT v1.2 software.

Recently, TYLCV disease has spread in most tomato growing areas in the southern, central and northern regions of Iran (fields and greenhouses) and also large populations of *B. tabaci* in many regions of the country have appeared as a result of changes in weather conditions in recent years. In addition, due to the high rate of recombination among the members of the genus *Begomovirus*, the emergence of new strains can be expected, though there are reports about this.

Conclusion

In present study, the observed symptoms variation in the infected tomatoes can be attributed to the difference in the type of planted cultivars, the difference in the age of infection and also the difference in the infectious isolate type. In addition, weeds in the fields are important sources for maintaining this virus and passing the winter. Weeds naturally play an important role in early season infections and their biology and epidemics (Fazeli *et al*., 2009). According to the mentioned cases and also the policy of developing greenhouse crops in the country, if the control methods presented are not followed, more severe damage is predicted for tomato cultivation in the country. Preventing the production and transmission of infected tomato seedlings is of high priority and the issuance of health certificates by relevant institutions is strongly recommended. Considering the freshness of tomatoes and also the rapid emergence of resistance of vector insects to chemical pesticides, the use of low-risk pesticides with suitable and effective quality is also very important. In many countries of the world by observing the mentioned cases, significant successes have been achieved in preventing the spread of TYLCV disease to other places and reducing the amount of damage (Prasad *et al*., 2020). The results of this research show that nowadays, TYLCV is not limited to the northern and southern provinces of the country and has spread to the central regions of the country as well, so the high percentage of infection to TYLCV in Khoramabad city was reported for the first time in this research and

based on the matrix result, there was no significant similarity between the Iranian isolates. This virus has a great genetic diversity in Iran and determining the existence of new recombinant strains in Iran that requires further studies of Iranian isolates, though the report of five TYLCV strains in Iran, suggested Iran is a potential region for genetic diversity of populations of TYLCV (Tabein and Behjatnia, 2022).

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Conflicts of interest

Authors declare that there is no conflict of interests regarding the publication of this article.

References

Ahmadi, K., Qolizadeh, H., Ebadzadeh, H., Hosseinpour, R., Abdshah, H., Kazemian, A., & Rafiee, M. (2020). Crop Agri. *Publications of the Ministry of Agricultural Jihad*, 86pp. (In Persian).

Bananej, K. (2016). Introduction and management of tomato yellow leaf curl disease. *Applied Entomology and Phytopathology*, *84*(1), 157-174. https://doi.org/10.22092/jaep.2016.106535.

Bananej, K., Kheyr-Pour, A., Hosseini Salekdeh, G., & Ahoonmanesh, A. (2004). Complete nucleotide sequence of Iranian *Tomato yellow leaf curl virus* isolate: further evidence for natural recombination amongst begomoviruses. *Archives of Virology*, *149*, 1435-1443. https://doi.org/10.1007/s00705-004-0308-9.

Brown, J. K., Idris, A. M., Torres-Jerez, I., Banks, G. K., & Wyatt, S. D. (2001). The core region of the coat protein gene is highly useful for establishing the provisional identification and classification of begomoviruses. *Archives of Virology*, *146*, 1581-1598. https://doi.org/10.1007/s007050170080.

Czosnek, H., & Laterrot, H. (1997). A worldwide survey of *Tomato yellow leaf curl viruses*. *Archives of Virology*, 142, 1391-1406. https://doi.org/10.1007/s007050050168.

Czosnek, H., Gorovits, R., & Ghanim, M. (2021). Factors controlling the fate of *Tomato yellow leaf curl virus* (TYLCV) in its vector, the whitefly vector *Bemisia tabaci*. *Plant Virus-Host Interaction*. Academic Press. https://doi.org/10.1016/B978-0-12-821629 3.00001-4.

Deng, D., McGrath, P. F., Robinson, D. J., & Harrison, B. D. (1994). Detection and differentiation of whitefly-transmitted geminiviruses in plants and vector insects by the polymerase chain reaction with degenerate primers. *Annals of Applied Biology*, *125*(2), 327-336. https://doi.org/10.1111/j.1744-7348.1994.tb04973.x.

Fazeli, R., Heydarnejad, J., Massumi, H., Shaabanian, M., & Varsani, A. (2009). Genetic diversity and distribution of tomato-infecting begomoviruses in Iran. *Virus Genes*, *38*, 311-319. https://doi.org/10.1007/s11262-008-0310-5.

García-Andrés, S., Accotto, G. P., Navas-Castillo, J., & Moriones, E. (2007). Founder effect, plant host, and recombination shape the emergent population of begomoviruses that cause the tomato yellow leaf curl disease in the Mediterranean basin. *Virology*, *359*(2), 302-312. https://doi.org/10.1016/j.virol.2006.09.030.

Gharouni Kardani, S., Jafarpour, B., Mehrvar, M., & Tarighi, S. (2014). Identification and Sequencing of Coat Protein Gene of TYLCV Isolates from Khorasan Razavi Southern and Northern Khorasan Provinces. *Journal of Iranian Plant Protection Research*, *27*(4), 427-433. http://dx.doi.org/10.22067/jpp.v27i4.29879.

H El-Sappah, A., Qi, S., A Soaud, S., Huang, Q., M Saleh, A., AS Abourehab, M., ... & Li, J. (2022). Natural resistance of tomato plants to *Tomato yellow leaf curl virus*. *Frontiers in plant science*, *13*, 1081549. https://doi.org/10.3389/fpls.2022.1081549.

Haq, G., Arif, M., Ali, A., & Inaaullah, M. (2018). *Tomato yellow leaf curl virus* in tomato crop of Khyber Pakhtunkhwa province: virus and vector prevalence and transmission properties. *Sarhad Journal of Agriculture*, *34*(3), 500-508. http://dx.doi.org/10.17582/journal.sja/2018/34.3.500.508.

Hasanvand, E., & Pakbaz, S. (2022). Genome Packaging in Plant Viruses. *Genetic*

Article in Press

Engineering and Biosafety Journal, *10*(2), 311-320. https://dorl.net/dor/20.1001.1.25885073.1400.10.2.12.4.

Jelavi, Z., Pakbaz, S., Gharouni Kardani, S., & Darvishnia M. (2022). The first report of *Tomato leaf curl Palampur virus* (ToLCPMV) from the watermelon fields in the north of Khouzestan province. *Genetic Engineering and Biosafety Journal*, *11*(1), 72-84. https://dorl.net/dor/20.1001.1.25885073.1401.11.1.8.5.

Muhire, B. M., Varsani, A., & Martin, D. P. (2014). SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. *PloS One*, *9*(9), e108277. https://doi.org/10.1371/journal.pone.0108277.

Patil, B. L., & Fauquet, C. M. (2021). Ecology of plant infecting viruses, with special reference to geminiviruses. *Studies in Viral Ecology*, 183-229. https://doi.org/10.1002/9781119608370.ch6.

Prasad, A., Sharma, N., Hari-Gowthem, G., Muthamilarasan, M., & Prasad, M. (2020). *Tomato yellow leaf curl virus*: impact, challenges, and management. *Trends in Plant Science*, *25*(9), 897-911. https://doi.org/10.1016/j.tplants.2020.03.015.

Ramazani, M. A., Ayazpour, K., Niazmand, A. R., & Najafipour, G. (2022). Detection of begomoviruses of *Solanaceae* crops in southern Iran. *Indian Phytopathology*, *75*(4), 1137-1142. https://doi.org/10.1007/s42360-022-00545-1.

Rojas, M. R. (1993). Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. *Plant Disease, 77*(4), 340. https://doi.org/10.1094/PD-77-0340.

Samretwanich, K., Chiemsombat, P., Kittipakorn, K., & Ikegami, M. (2000). *Tomato Leaf Curl Geminivirus* associated with Cucumber yellow leaf disease in Thailand. *World Journal of Microbiology and Biotechnology*, *16*, 401-403. https://doi.org/10.1023/A:1008962402329.

Tabein, S., & Behjatnia, S. A. (2023). Status of geminiviruses in Iran, incredible plant pathogens. *Iran Agricultural Research*, *41*(2), 1-23. https://doi.org/10.22099/IAR.2023.45048.1516.

Zhang, Y., Uyemoto, J. K., & Kirkpatrick, B. C. (1998). A small-scale procedure for extracting nucleic acids from woody plants infected with various phytopathogens for PCR assay. *Journal of Virological Methods*, *71*(1), 45-50. https://doi.org/10.1016/s0166-0934(97)00190-0.

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