J Genet Resour 2024; 10(1): 98-108 *Homepage: http://sc.journals.umz.ac.ir/*

RESEARCH ARTICLE *DOI: 10.22080/jgr.2024.27079.1389*

Molecular Detection and Determination of Phylogenetic Position of *Watermelon Chlorotic Stunt Virus* Khouzestan Isolate Based on Coat Protein Gene

Samira Pakbaz*, Zahra Jelavi, Ehsan Hasanvand and Hossein Mirzaei Najafgholi

Department of Plant Protection, Faculty of Agriculture, Lorestan University, Khoramabad, Iran

ARTICLE INFO

Article history:
Received 18 August 2023
Accepted 30 September 2023
Available online 17 October 2023

Keywords:
Begomovirus
Iran
Sequencing
ssDNA
WmCSV.

Corresponding authors:
✉ S. Pakbaz
pakbaz.s@lu.ac.ir

p-ISSN 2423-4257
e-ISSN 2588-2589

ABSTRACT

Watermelon chlorotic stunt virus (WmCSV) is a destructive *Begomovirus* from the family of *Geminiviridae* with a bipartite genome including similar-sized circular, single-stranded DNA (DNA-A and DNA-B) which has recently developed dangerously in many countries in the Middle East. WmCSV is transmitted by the whitefly *Bemisia tabaci* in a persistent and circulative manner. In order to detect this virus, in the summer of 2023, samples of watermelon with symptoms of yellowing and severe leaf curl, leathery and blistering of the young leaves surface, chlorotic spots, and dwarf were collected from watermelon fields in the north of Khouzestan province (Dezful, Shoush, and Andimeshk). PCR was performed using two primer pairs of *Begomovirus* related to AV1 and also AV2 genomic region. The sequencing results confirmed WmCSV infection in the samples. In the phylogenetic tree drawn based on the coat protein gene (AV1), Khouzestan isolates were placed in a group next to other Iranian isolates and they had most similarities to Noorabad isolate from Fars province. Also, results of two by two comparisons of nucleotide sequences of the AV1 genomic region using SDT v1.2 software showed the sequences of three Khouzestan isolates are highly similar (99.40%) and had the most identity (99.60%) with Noorabad Mamassani isolate from Fars province and the greatest genetic distance with Saudi Arabia isolates (95.80%) and Lebanon isolate (96.50%). This is the first report of WmCSV occurrence in Khouzestan province's watermelon fields. WmCSV is rapidly advancing on their host crops in Iran, especially in the southern regions of the country.

Please cite this paper as: Pakbaz, S., Jelavi, Z., Hasanvand, E., & Mirzaei Najafgholi, H. (2024). Molecular detection and determination of phylogenetic position of *Watermelon chlorotic stunt virus* Khouzestan isolate based on coat protein gene. *Journal of Genetic Resources*, *10*(1), 98-108. doi: 10.22080/jgr.2024.27079.1389

Introduction

Virus diseases of *Cucurbitaceae* cause annual quantitative and qualitative economic damages to these products (Radouane *et al*., 2021). So far, many viruses and viroids have been reported that can infect one or a few species of cucurbits naturally or in the laboratory. *Watermelon chlorotic stunt virus* (WmCSV) is one of the important viruses that causes damage to *Cucurbitaceae* (Bananej *et al*., 2009). WmCSV was first identified with symptoms such as yellowing of veins, severe plant dwarfism and leaf chlorosis on watermelon in Yemen and infected 90-100% of watermelon fields (Czosnek *et al*., 2021). This disease was present in every region where watermelon was planted regularly and caused a severe reduction in the yield. In some areas of Saudi Arabia, WmCSV destroyed all watermelon crops (Al-Saleh *et al*., 2014). WmCSV has been identified in watermelon and muskmelon in eastern and central Sudan. Then, this virus was detected in the Palestine region (Mohammed et al., 2014) and Jordan (Al-Musa et al., 2011) as well as in southern Lebanon in the cultivation areas of cucurbits, including cucumbers, melons, watermelons, and pumpkins. WmCSV was reported from *Cucurbita moschata*

in Oman in 2012 (Khan *et al*., 2012). It was also reported on *Cucurbitaceae* in Mexico (Domínguez-Durán *et al*., 2018). In recent years, WmCSV has been a serious and significant threat to Cucurbitaceae in the Middle East, Arabian Peninsula, and North Africa (Shafiq *et al*., 2021).

WmCSV belongs to *Begomovirus* genus and *Geminiviridae* family and has a bipartite genome including similar-sized circular, single-stranded DNA (DNA-A and DNA-B) (Hanley-Bowdoin *et al*., 2013; Hasanvand and Pakbaz, 2022). The DNA-A of bipartite *Begomoviruses* encodes two genes in virion-sense (AV1 and AV2), while four genes are encoded in the complementary-sense orientation (AC1, AC2, AC3, and AC4) (Rosario et al., 2016; Gomathi *et al*., 2023). The genome in *Begomovirus* is encapsidated in twinned icosahedrons. WmCSV is transmitted by the whitefly *Bemisia tabaci* in a persistent and circulative manner (Czosnek *et al*., 2021, Breves *et al*., 2023).

So far in Iran, WmCSV has been reported from the provinces of Bushehr, Kerman, Hormozgan, Sistan & Baluchistan, Golestan (Bananej, 2016), Fars and Gilan (Esmaeili *et al*., 2015). This virus has a wide spread in the south and southeast of Iran due to the suitable climate for the activity of the vector. Also, the source of the virus is maintained for subsequent crops by transmitting the disease to the weeds next to the fields. Considering that Khouzestan province is one of the major areas for growing *Cucurbitaceae* including watermelon, and in recent years there have been suspected signs of viral infections with the presence of whiteflies in watermelon fields, the purpose of this research is to detect of *Watermelon chlorotic stunt virus* in watermelon fields of the north of Khuzestan province and determine the phylogenetic position of its isolates.

Materials and Methods

Sample collection and DNA extraction

In the spring and summer of 2023, 40 leaf samples were taken from suspected plants with symptoms such as dwarf, vein clearing, mottling and chlorotic spots between the veins, severe leaf curl from watermelon fields in the northern cities of Khouzestan province (Dezful, Shoush and Andimeshk). The samples were transferred to the laboratory on ice and used for molecular tests. A part of each sample was transferred to a -70$^{\circ C}$ freezer for long-term storage. DNA extraction was done from leaf samples using the CTAB-PVPP method (Zhang *et al*., 1998) and stored at -20$^{\circ C}$.

Polymerase chain reaction

The Polymerase chain reaction was first performed using a pair of degenerate primers (Bc and PCRv181) belonging to whitefly-transmissible Begomoviruses on DNA-A (Rojas *et al*., 1993; Czosnek *et al*., 2021) (Table 1).

Table 1. Primers used in this study

Primer Name	Nucleotide Sequence (5'-3')	Gene region	Size Product
Begomo. PCRv181	5'TAATATTACCGGWTGGCC3'	AV2	**550bp**
Begomo. Bc	5'TGGACYTTRCAWGGBCCTTCACA3'		
WmCSV-700A-F	5'AGCCCCTACATGAGCCGTGCT3'	AV1	**650bp**
WmCSV-1334A-R	5'ACACAGGATTCGATGCGTGTG3'		

This reaction was carried out in a final volume of 25µl, including 12.5µl of 2x PCR BioTaq Mix Red kit (PCR Biosystem company), 10pmol of forward and reverse primers, and 100ng of DNA. To amplify the expected fragment, the thermal program was done as follows: an initial denaturation at 94°C for 3min and then 35 cycles, including denaturation at 94°C for 35s, annealing at 55°C for 35s, and extension at 72°C for 35s, and finally the final extension step at 72$^{\circ C}$ for 7min. Sterile distilled water was used instead of DNA as a negative control in one reaction. Safe DNA stain solution (Pishgam Company) was used to stain the PCR products, and to observe the amplified products and evaluate their quantity and quality, 1% agarose gel electrophoresis was used in the presence of a 100bp standard molecular marker (Pishgam Company) and finally the results were observed with gel document.

Nucleotide sequencing to detect viral species

One representative of amplified PCR products using degenerate primers was selected based on quality and sent to Codon company for purification and sequencing. To determine the species of viral isolate, the nucleotide sequence of this sample was compared with other related sequences in the gene bank using the nucleotide Blast tool in NCBI.

PCR using species-specific primers

After evaluating the results obtained from determining nucleotide sequencing based on Bc and PCRv181 primers and determining the virus species, PCR was performed using specific primers of WmCSV (Esmaeili *et al*., 2015) to amplify the coat protein (CP) gene (Table 1). The concentration and values of the PCR, the commercial kit, and the thermal program were almost similar to the reaction performed for amplification using degenerate primers, except for the annealing temperature of specific primers, which was 58˚C.

Determination of phylogeny position

Amplified products were sent to Codon company for purification and sequencing. Finally, the obtained nucleotide sequences were first evaluated using the nucleotide Blast tool in NCBI. The ends of the sequences were edited using BioEdit software. The final sequences were used to draw the phylogeny tree. To determine the phylogeny position of the viral species were used several nucleotide sequences related to the studied genomic regions belonging to different hosts and countries, which were previously registered in the gene bank. For this purpose, multiple sequence alignment was done using the ClustalW tool in MEGA11 software, and then a phylogeny tree was drawn with the neighbor-joining method and based on 1000 repetitions in bootstrap evaluation. Also, the identity analysis of the nucleotide sequence of the studied genomic regions between the sequenced isolates and the gene bank was done using SDT v1.2 software (Muhire *et al*., 2014).

Results and Discussion

Field survey

In visiting the watermelon fields of Khouzestan province including Dezful, Shoush, and Andimeshk cities, symptoms such as severe mosaic and chlorosis, blistering of the leaf surface, small leaves and wavy leaf margins, fruit deformity, and plant dwarfing were observed (Fig. 1), which were similar to the previous reports of symptoms of this virus (Bananej *et al*., 2009). In the previous research, infected plants with WmCSV showed symptoms such as yellow veins, mottling, and severe stunting of young leaves in Palestine (Mohammed *et al*., 2014). Also, *Cucurbita maxima* plants infected with WmCSV showed yellowing, curling, and stunting symptoms in Oman (Shafiq *et al*., 2021). The variety of symptoms can be caused by the difference in the type of cultivars, so some watermelon cultivars are more tolerant to this virus and also, different melon cultivars have different resistance to WmCSV (Yousif *et al*., 2007).

Fig. 1. Symptoms of WmCSV in watermelon samples collected from fields in Khouzestan province: a) severe mosaic and chlorosis; b) yellowish, leathery, and blistering of the young leaves surface and plant dwarfing; c) reduction of fruit growth; d) reduced leaf size and wavy leaf margins

Detection of WmCSV by PCR

In a PCR test using degenerate primers of Begomoviruses transmissible with whiteflies (Bc and PCRv181), a 550bp fragment was amplified which belonged to the 3' end part of the intergenic region and about 200 base pairs from the 5' end of the pre-coat protein gene (AV2) (Fig. 2A), and indicated the definitive infection of the samples to viral species of the genus *Begomovirus*. In a study, in order to detect

Tomato yellow leaf curl virus (TYLCV) in the herbaceous hosts in the southern regions of Kerman province, PCR was performed using the degeneration primers Bc and PCRv181. These primers were able to amplify a fragment of 550 base pairs in the infectious samples (Salari *et al*., 2015). Also, the other researchers used these primers to detect TYLCV in Khorasan Razavi (Gharouni *et al*., 2014) and Lorestan provinces (Rostami *et al*., 2023) and ToLCPMV in Kouzestan province (Jelavi *et al*., 2022). In another study, in order to investigate the phylogenetic relationship of WmCSV isolates in *Cucurbitaceace* in Sistan & Baluchestan province, WmCSV was identified using specific primers Gem-CP-V-5' and Gem-CP-V-3' belonging to *Begomovirus* and a fragment of 550bp was amplified in suspected watermelon samples (Jafari *et al*., 2020).

An amplified fragment of Shoush isolate was purified and sequenced by the Sanger method at Codon Company. Evaluation of the sequence using the Nucleotide Blast tool (nBlast) in the gene bank revealed that the studied isolate based on the nucleotide sequence of the AV2 genomic region belonged to *Watermelon chlorotic stunt virus* (WmCSV). This sequence had 96-99% nucleotide identity with other sequences available and registered from this region in NCBI, and the most and least similarity was related to the isolate recorded from Noorabad city of Mamsani, Fars province (KT272767.1) and the isolate of Saudi Arabia (KT943457.1), respectively based on nBlast tool analysis. Accession number of this isolate is available in Table 2. After confirming the virus species, the specific primers of WmCSV species were used to perform the PCR test. These primers were able to amplify a fragment with a length of about 650bp including the coat protein gene (AV1) of WmCSV (Fig. 2B).

Table 2. Gene bank accession numbers related to WmCSV isolates sequenced in this study.

Location	Genomic fragment	Gene region	Accession number	Host
Shoush	DNA-A	AV1	OP954295	*C. lanatus*
Dezful	DNA-A	AV1	OP967001	*C. lanatus*
Andimeshk	DNA-A	AV1	OP926974	*C. lanatus*
Shoush	DNA-A	AV2	OP926973	*C. lanatus*

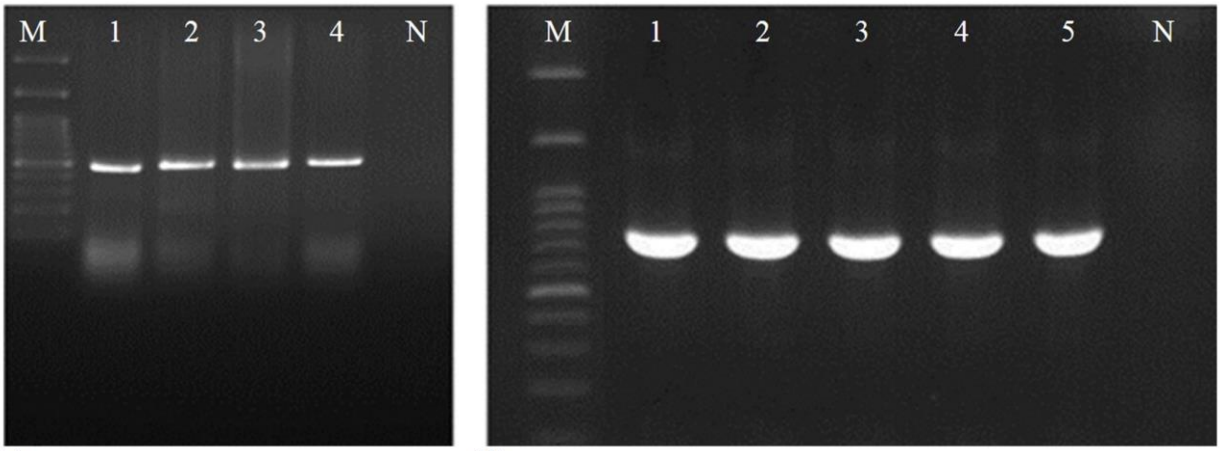

Fig. 2. Electrophoresis pattern of PCR products on 1% agarose gel: A) Amplification of a 550bp fragment using degenerate primers of *Begomoviruses* (BC and PCRv181); B) Amplification of a 650bp fragment using specific WmCSV primers. M= Molecular marker (100bp); N= Negative control.

Out of 40 samples collected from watermelon fields in three cities, 24 infected samples were identified (9 out of 23 samples collected belonging to Dezful, 8 to Andimeshk, and 7 to Shoush). The results of this research were consistent with those of Esmaili et al. In their research, the infection of 14 species from 12 genera and 9 families of weeds in different

regions in the south and southeast of Iran was confirmed by PCR test, and a 650bp fragment was amplified (Esmaeili *et al*., 2015).

WmCSV has been reported in watermelon fields of Fars province, including Lar, Estehban, Firuzabad, Khonj, Mamsani, and Darab (Esmaeili *et al.*, 2015) and also in watermelon and melon fields of Sistan and Baluchistan province (Jafari *et al*., 2020). In a study, the sensitivity of different cultivars that were mostly cultivated by farmers in the southern regions of Iran was investigated. In this study, WmCSV species-specific primers were able to amplify fragments of the expected size. The cultivars Ps, Proseed, WLNOOL-20046, and Charlee showed the least sensitivity to infection with this virus (Esmaeili *et al*., 2015).

Most species in *Cucurbitaceae*, such as cucumber, melon, and pumpkin, and some species of Solanaceae, such as tomato, are sensitive to WmCSV. On the other hand, experimental plants such as *Nicotiana benthamiana* and *N. glutinousa* are also hosts of this virus in most southern regions of Iran with tropical climates (Bananej, 2016). *Lagenaria siceraria*, *Vigna radiate*, *Datura stramonium*, *Amaranthus viridis* have been identified as hosts of this virus (Ahmad *et al*., 2018).

Determining the phylogenetic position

After grouping the amplified products based on quality and geographic region, one sample from each city was sequenced. Then, the received sequences were evaluated on the NCBI site and the nBlast tool confirmed that the investigated isolates belong to WmCSV based on the AV1 gene nucleotide sequence and can be phylogenetically investigated with the other registered isolates in the gene bank. On average, the studied isolates had 95.28-98.28 percent nucleotide similarity with other sequences registered from this region in NCBI. The results obtained from the comparison of the sequences in the gene bank based on the AV1 gene showed the highest percentage of similarity at the nucleotide level between the Khouzestan isolates and the isolate recorded from Noorabad Mamssani in Fars province. The accession numbers of isolates studied in this research are listed in Table 2.

In order to determine the relationships and phylogenetic position, multiple sequence alignment of Khouzestan province isolates, along with several isolates of WmCSV available in the gene bank, was done. These sequences were related to different hosts and geographical regions, including Iran and other countries (Tables 3 and 4). These analyses were performed using the ClustalW tool in BioEdit software, and a phylogeny tree was drawn based on both of the studied genomic regions using MEGA11.

In the phylogeny tree drawn based on nucleotide sequences of the AV2 genomic region, the studied isolates were placed in two groups, I and II (Fig. 3). Group I included two subgroups. The Shoush isolate had the most similarity with the Noorabad city isolate from Fars province, on watermelon host with accession number (KT272767.1), and was placed in subgroup I in a separate sub-branch next to this isolate. In this subgroup, isolates related to Jordan, Lebanon, Palestine region, West Bank, Mexico, Sudan, and Germany were placed in separate branches.

Table 3. Accession numbers and characteristics of WmCSV isolates related to the AV2 genomic region available in the gene bank used in phylogeny analysis.

Ac Num*	Host	Country	Ac Num*	Host	Country
KT272767.1	*C. lanatus*	Mamassani, Fars, Iran	KJ854914.1	*C. lanatus*	West Bank
KT272773.1	*C. pepo*	Jiroft, Kerman, Iran	KJ854915.1	*C. lanatus*	West Bank
KT272771.1	*C. lanatus*	Khash, Sistan, Iran	KC462552.1; EF201809.1	*C. lanatus*	Palestine
KY825715.1	*B. rapa var. rapa L.*	Bagher-Abad, Kerman, Iran	JX131283.1	*S. arvensis*	Jordan
KT272769.1	*C. lanatus*	Khonj, Fars, Iran	HM368371.1	*C. melo*	Lebanon
KT272765.1	*C. lanatus*	Darab, Fars, Iran	EU561237.1	*C. lanatus*	Jordan
JN618983.1	*Cucurbita*	Oman	OK058529.1	*C. lanatus*	Germany
JN618982.1	*Cucurbita*	Oman	KY488568.1	*D. innoxia*	Sudan
JN618981.1	*Cucurbita*	Oman	KJ939448.1; KJ958911.1	*C. lanatus*	Saudi Arabia
KY124280.1	*C.lanatus*	Mexico	KJ958912.1	*C. pepo*	Saudi Arabia
MW588390.1	*Opuntia auberi*	Mexico	KC677628.1	*C. maxima*	Sudan

*Ac Num= Accession number

Table 4. Accession numbers and characteristics of WmCSV isolates related to the AV1 gene available in the gene bank used in phylogeny analysis.

Ac Num*	Host	Country	Ac Num*	Host	Country
KT272767.1	*C. lanatus*	Mamassani, Fars, Iran	MH244454.1	*C. lanatus*	Chabahar, Iran:
JX480487.1	*H. szovitsii*	Minab, Kerman, Iran	MH220206.1	*C. melo*	Zabol, Iran
KT272769.1	*C. lanatus*	Khonj, Fars, Iran	JN618983.1	*Cucurbita*	Oman
KT272765.1	*C. lanatus*	Darab, Fars, Iran	JN618982.1	*Cucurbita*	Oman
JX480480.1	*S. aegyptiaca*	Minab, Hormozgan, Iran	JN618981.1	*Cucurbita*	Oman
JX480479.1	*C. hierosolymitana*	Jiroft, Kerman, Iran	KJ854919.1	*C. lanatus*	West Bank
KY825715.1	*B. rapa var. rapa.*	Bagher-Abad, Kerman, Iran	KJ854918.1	*C. lanatus*	West Bank
KT272771.1	*C. lanatus*	Khash, Sistan, Iran	MW588390.1	*O. auberi*	Mexico
MH244451.1	*C. lanatus*	Konarak, Iran	JX131283.1	*S. arvensis*	Jordan
MH244455.1	*C. lanatus*	Hamun, Iran	EU561237.1	*C. lanatus*	Jordan
MH244461.1	*C. lanatus*	Iranshahr, Iran	EF201809.1	-	Palestine region
MH244450.1	*C. melo var. cantalupo*	Sarbaz, Iran	HM368371.1	*C. melo*	Lebanon
MH244459.1	*C. lanatus*	Zahedan, Iran	KJ958911.1	*C. lanatus*	Saudi Arabia
MH244457.1	*C. lanatus*	Saravan, Iran	KC876037.1	*C. lanatus*	Saudi Arabia
MH244447.1	*C. melo var. cantalupo*	Nikshahr, Iran	-	-	-

*Ac Num= Accession number

The Iranian isolates related to Jiroft (KT272773.1) on *Cucurbita pepo* and Bagherabad (KY825715.1) on *Brassica rapa* detected from Kerman province were classified in subgroup II. Saudi Arabia isolates (KJ939448.1), (KJ958911.1), and (KJ958912.1) were included in subgroup I of group II along with isolates from Khash city of Sistan and Baluchestan province (KT272771.1). Iranian isolates from Fars province (Darab: KT272765.1) and (Khonj: KT272769.1) and also Oman isolates (JN618983.1), (JN618982.1) and (JN618981.1) were classified in subgroup II.

Also, the results of the two by two comparison of nucleotide sequences of the AV2 genomic region using SDT v1.2 software confirmed the obtained results in the phylogeny tree, and the Shoush isolate had the most identity with the isolate from Noorabad Mamassani city, Fars province (KT272767.1) and the percentage of their nucleotide similarity was 99.10%. Also, this isolate had the greatest genetic distance with Saudi Arabia isolates, especially KJ958911.1, with 95.90% similarity. Jordan isolates had very little genetic distance from the Palestine, West Bank, Lebanon, and Mexican isolates in this matrix, and among the Iranian isolates, the Kerman, Jiroft isolate (KT272773.1) had the most genetic similarity with those isolates (Fig. 5).

Based on the nucleotide sequence of the AV1 gene, the investigated isolates were placed in two groups, I and II, in the phylogeny tree. Group I contains two subgroups I and II (Fig. 4). All three isolates of Khouzestan province (Dezful, Shoush, and Andimeshk), along with the Noorabad Mamassani isolate of Fars province (KT272767.1), were classified in a separate sub-branch in subgroup I. In addition, Sistan & Baluchestan isolates include Nikshahr (MH244447.1), Chabahar (MH244454.1), Saravan (MH244457.1), Konarak (MH244451.1), Zabol (MH220206.1) and Sarbaz (MH244450.1) and also Saudi Arabia and Oman isolates were placed in separate sub-branches in subgroup I. The other Iranian isolates, such as Kerman, Fars, Hormozgan, and Hamun, were placed in subgroup II. Isolates from Jordan, West Bank, Lebanon, and Mexico were classified in group II.

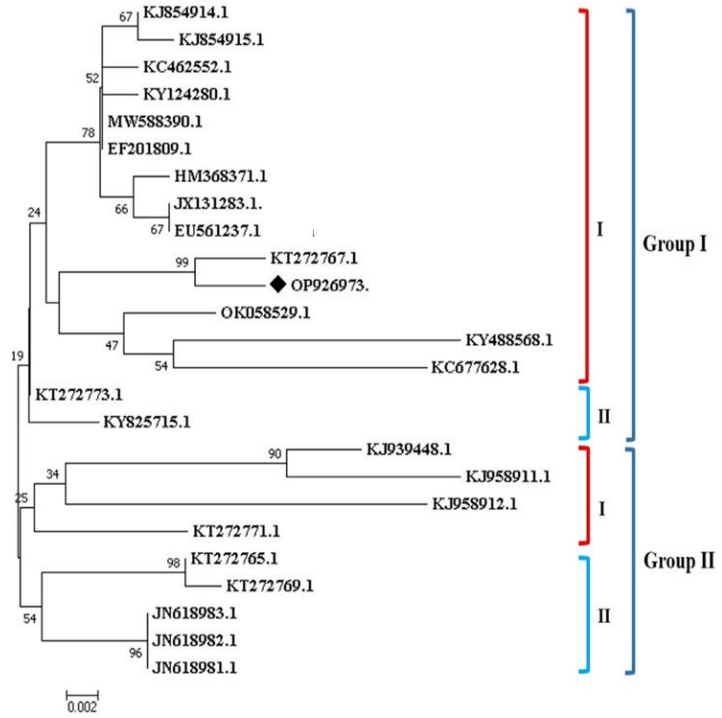

Fig. 3. The phylogeny tree was drawn based on the nucleotide sequence of AV2 genomic region of WmCSV-Shoush isolate and some isolates from the gene bank. Tree was drawn using MEGA11 software by neighbor-joining method and 1000 repetitions in evaluation. The isolates sequenced in this study are marked with in the tree.

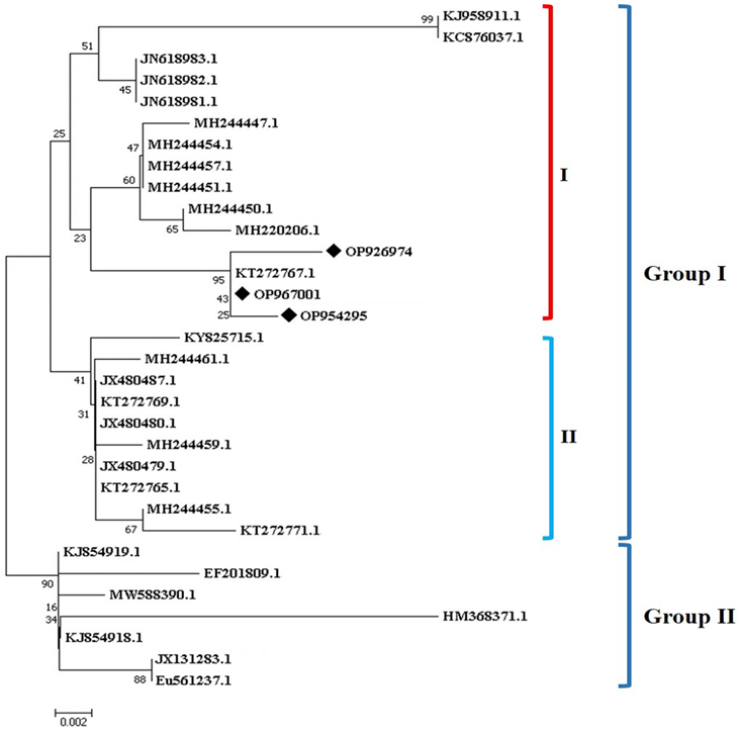

Fig. 4. The phylogeny tree drawn based on the nucleotide sequence of the AV1 genomic region of WmCSV-Khouzestan province isolates and some isolates in the gene bank. The tree was drawn using MEGA11 software by neighbor-joining method and 1000 repetitions in evaluation. The isolates sequenced in this study are marked with in the tree.

Also, the results of the two by two comparison of nucleotide sequences of the AV1 genomic region using SDT v1.2 software showed that the sequences of the three Khouzestan viral isolates are highly similar and, on average, the percentage of their nucleotide similarity is 99.40%. According to the drawn comparison matrix, Khouzestan isolates had the most identity (99.60%) with Noorabad Mamassani isolate from Fars province, and they had the greatest genetic distance with Saudi Arabia isolates (95.80%), and Lebanon isolates (96.50%) which are shown in Figure 6.

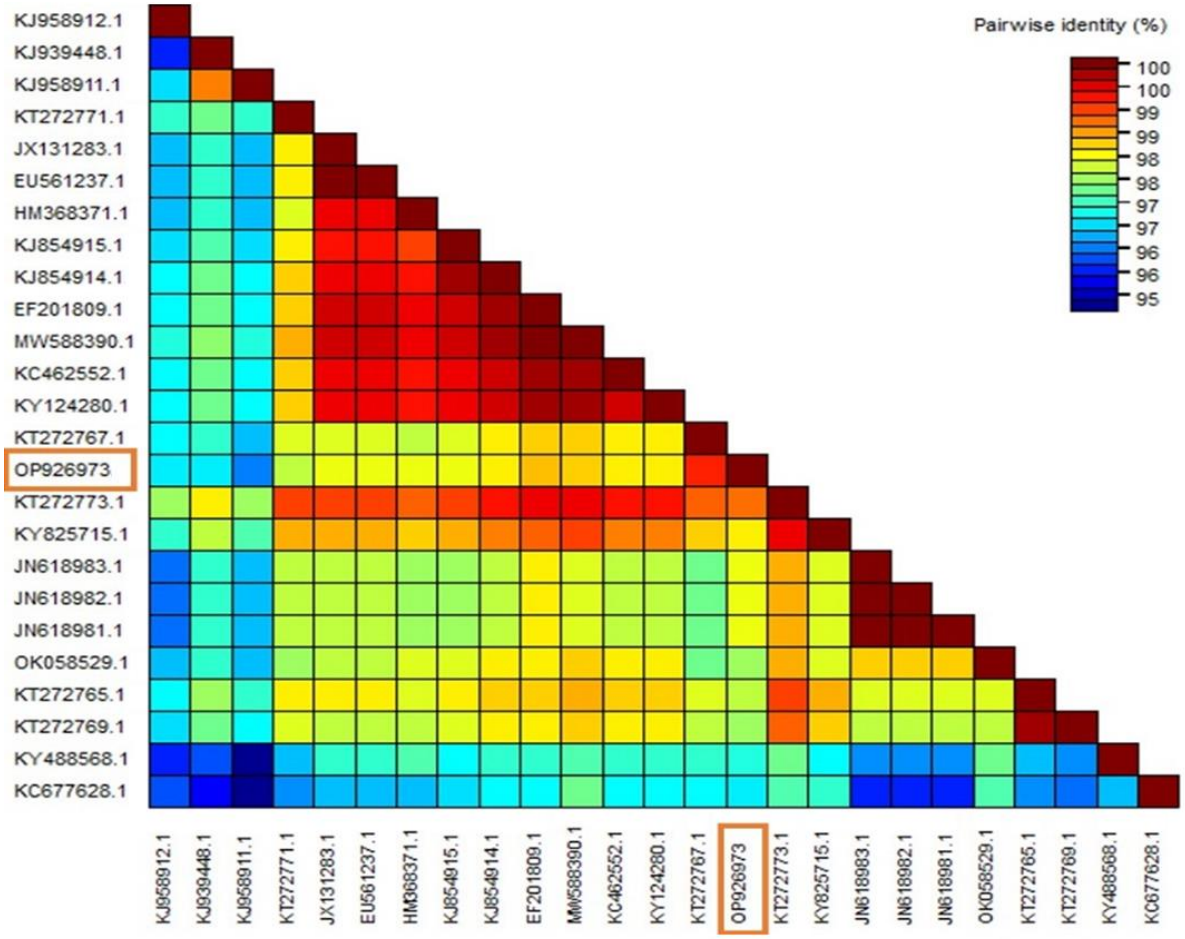

Fig. 5. Pair-by-pair comparison matrix of nucleotide sequence of the AV2 genomic region of WmCSV Khouzestan isolate with 24 selected isolates from the gene bank using SDT v1.2 software.

The studies have shown that, in the phylogenetic analysis of complete nucleotide sequences of DNA-A and DNA-B fragments of different WmCSV isolates, Iranian isolates are separated in a cluster which is supported by a high bootstrap value. Similar results have been obtained by comparing the partial nucleotide sequences of Iranian isolates and other countries of the world (Mohammed *et al*., 2014), as if all the Iranian isolates were placed in separate sub-clusters in Figure 4 in the present research; however, it still observed genetic diversity among them. In this phylogeny tree, Iranian isolates, including Khouzestan and Sistan & Baluchistan, are next to Oman and Saudi Arabia isolates in subgroup I, and Khouzestan isolates are very similar to Sistan & Baluchistan isolates. In the last research, the analysis of the sequences of this virus has shown that the isolates from Iran, Oman, and Saudi Arabia belong to the same group. While Palestine region, Jordan, Lebanon, and Palestine are placed in the same group. Also, isolates from Iran and Saudi Arabia have more diversity than other regions (Jafari *et*

al., 2020). The close relationship between isolates from Sistan & Baluchistan provinces and isolates from Oman and Saudi Arabia was consistent with the research of Jafari *et al*. (2020). In fact, despite the location of the sea, the Oman and Saudi Arabia isolates and Iranian isolates are still transferred between these countries. The virus is probably transmitted between these countries through infected whiteflies (Jafari *et al*., 2020).

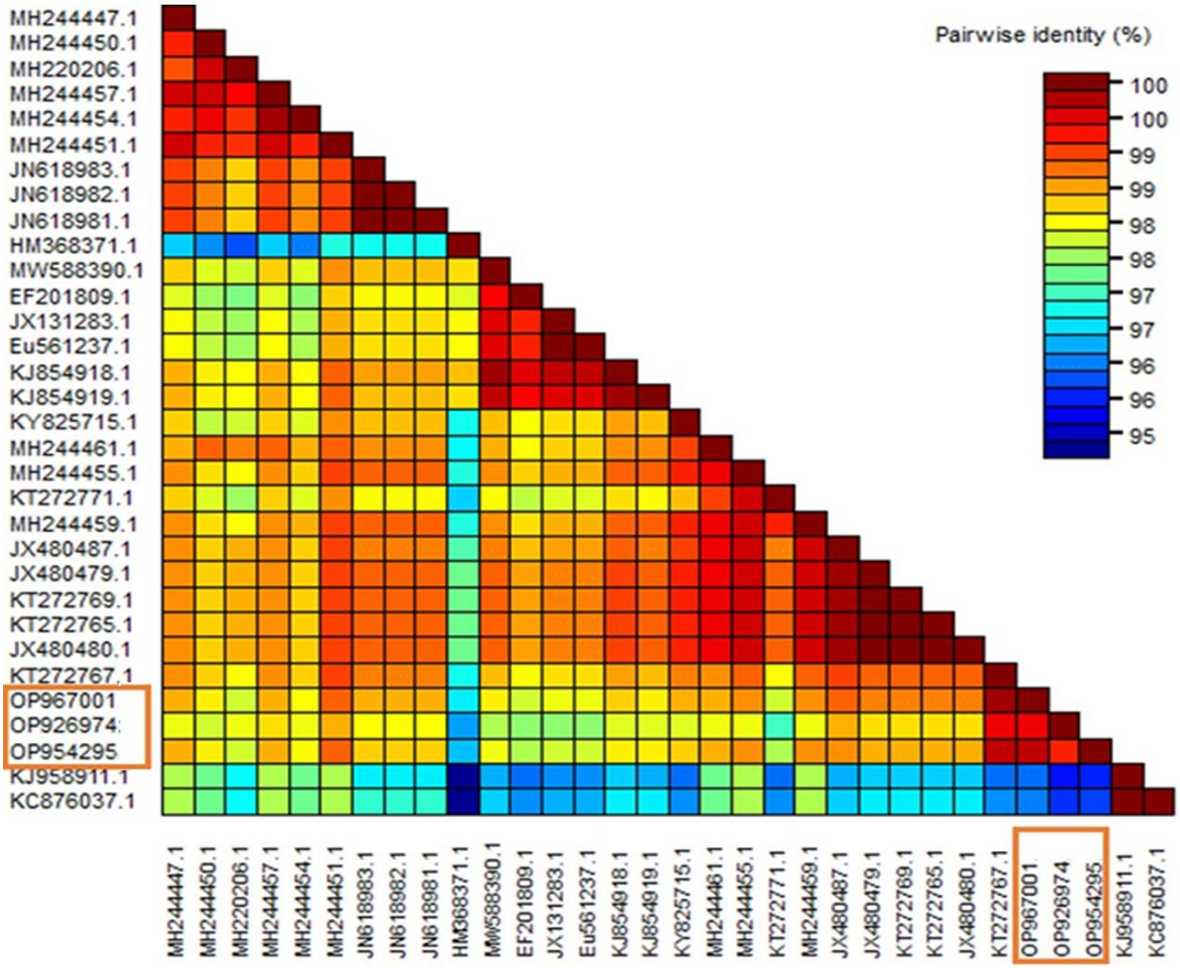

Fig. 6. Pair-by-pair comparison matrix of nucleotide sequence of the AV1 genomic region of WmCSV Khouzestan isolate with 29 selected isolates from the gene bank using SDT v1.2 software.

Conclusion

In this study, WmCSV was detected and reported in watermelon fields of Khouzestan province for the first time. Molecular tests using specific primers showed that more than 50% of the studied samples were infected with this virus. Based on the nucleotide sequence of WmCSV DNA-A, especially the AV1 gene, three investigated isolates of Khouzestan province (Dezful, Shoush, and Andimeshk) along with the Noorabad Mamassani isolate of Fars province (KT272767.1) were classified in a separate sub-branch in subgroup I, in the phylogeny tree. Also, Sistan & Baluchistan isolates include Nikshahr, Chabahar, Saravan, Konarak, Zabol, and Sarbaz, and Saudi Arabia and Oman isolates were placed in separate sub-branches in subgroup I. Most of the nucleotide sequences belonging to WmCSV registered in the gene bank are related to the Middle East region. Warm weather, favorable conditions for the activity, and a high population of *Begomovirus*-carrying whiteflies have caused the transmission and spread of this virus in the Middle East region, including the southern regions of Iran. The high population of whitefly, co-infection of

WmCSV and *Tomato leaf curl Palampur virus* and other viruses that infect watermelons, as well as mismanagement caused that currently, in the watermelon fields in the north of Khouzestan province, including Dezful, Shoush and Andimeshk, farmers have stopped planting watermelon seeds in these areas and other crops have replaced. Therefore, in addition to reducing the use of insecticides due to the development of resistance, the use of virus-resistant cultivars is strongly recommended. It is necessary to evaluate the resistance of different watermelon cultivars to this virus and to find and use the most resistant cultivar or a cultivar with less sensitivity for planting in the case of this virus. Also, it is suggested to produce resistant transgenic plants through genetic engineering and gene silencing to resist WmCSV.

Conflicts of Interest

The authors declare no conflict of interest.

References

Ahmad, M. H., Shakeel, M. T., Al-Shahwan, I. M., Al-Saleh, M. A., & Amer, M. A. (2018). Insights into the incidence of watermelon chlorotic stunt virus causing yellowing disease of watermelon in western and southwestern regions of Saudi Arabia. *The Plant Pathology Journal*, 34(5), 426. https://doi.org/10.5423/PPJ.OA.04.2018.0059

Al-Musa, A., Anfoka, G., Al-Abdulat, A., Misbeh, S., Haj Ahmed, F., & Otri, I. (2011). Watermelon chlorotic stunt virus (WmCSV): a serious disease threatening watermelon production in Jordan. *Virus Genes*, 43, 79-89. https://doi.org/10.1007/s11262-011-0594-8

Al-Saleh, M. A., Ahmad, M. H., Al-Shahwan, I. M., Brown, J. K., & Idris, A. M. (2014). First report of watermelon chlorotic stunt virus infecting watermelon in Saudi Arabia. *Plant Disease*, 98(10), 1451. https://doi.org/10.1094/PDIS-06-14-0583-PDN

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., Vahdat, A., & Hosseini-Salekdeh, G. (2009). Begomoviruses associated with yellow leaf curl disease of tomato in Iran. *Journal of Phytopathology*, 157(4), 243-247. https://doi.org/10.1111/j.1439-0434.2008.01479.x

Breves, S. S., Silva, F. A., Euclydes, N. C., Saia, T. F., Jean-Baptiste, J., Andrade Neto, E. R., & Fontes, E. P. (2023). Begomovirus-host interactions: viral proteins orchestrating intra and intercellular transport of viral DNA while suppressing host defense mechanisms. *Viruses*, 15(7), 1593. https://doi.org/10.3390/v15071593

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. *In Plant Virus-Host Interaction* 231-266. https://doi.org/10.1016/B978-0-12-821629-3.00001-4

Domínguez-Durán, G., Rodríguez-Negrete, E. A., Morales-Aguilar, J. J., Camacho-Beltrán, E., Romero-Romero, J. L., Rivera-Acosta, M. A., ... & Méndez-Lozano, J. (2018). Molecular and biological characterization of Watermelon chlorotic stunt virus (WmCSV): an Eastern Hemisphere begomovirus introduced in the Western Hemisphere. *Crop Protection*, 103, 51-55. https://doi.org/10.1016/j.cropro.2017.09.006

Esmaeili, M., Heydarnejad, J., Massumi, H., & Varsani, A. (2015). Analysis of watermelon chlorotic stunt virus and tomato leaf curl Palampur virus mixed and pseudo-recombination infections. *Virus Genes*, 51, 408-416. https://doi.org/10.1007/s11262-015-1250-5

Gomathi Devi, R., Jothika, C., Sankari, A., Lakshmi, S., Malathi, V. G., & Renukadevi, P. (2023). Seed transmission of Begomoviruses: a potential threat for bitter gourd cultivation. *Plants*, 12(6), 1396. https://doi.org/10.3390/plants12061396

Hanley-Bowdoin, L., Bejarano, E. R., Robertson, D., & Mansoor, S. (2013). Geminiviruses: masters at redirecting and reprogramming plant processes. *Nature Reviews Microbiology*, 11(11), 777-788. https://doi.org/10.1038/nrmicro3117

Hasanvand, E., & Pakbaz, S. (2022). Genome Packaging in Plant Viruses. *Genetic Engineering and Biosafety Journal*, 10(2), 311-320. http://gebsj.ir/article-1-417-en.html

Jafari, M., Sadeghi, Z., Nasrollahnezhad, S., & Shahrokhi, A. R. (2020). Phylogenetic

relation of Watermelon chlorotic stunt virus (WmCSV) isolates of Sistan and Baluchestan province by sequencing of coat protein gene. *Journal of Iranian Plant Protection Research*, 34(3), 325-336. https://doi.org/10.22067/jpp.v34i3.85959

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. http://gebsj.ir/article-1-419-en.html

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

Khan, A. J., Akhtar, S., Briddon, R. W., Ammara, U., Al-Matrooshi, A. M., & Mansoor, S. (2012). Complete nucleotide sequence of Watermelon chlorotic stunt virus originating from Oman. *Viruses*, 4(7), 1169-1181. https://doi.org/10.3390/v4071169

Mohammed, H. S., Zicca, S., Manglli, A., Mohamed, M. E., El Siddig, M. A., Tomassoli, L., & El Hussein, A. A. (2014). Identification and phylogenetic analysis of common pumpkin viruses in Sudan. *Journal of Plant Pathology*, 96(1), 77-84. https://doi.org/10.4454/JPP.V96I1.005

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

Radouane, N., Ezrari, S., Belabess, Z., Tahiri, A., Tahzima, R., Massart, S., ... & Lahlali, R. (2021). Viruses of cucurbit crops: Current status in the Mediterranean region. *Phytopathologia Mediterranea*, 60(3). https://doi.org/https://doi.org/10.36253/phyto-12340

Rojas, M. R., Gilbertson, R. L., Russell, D. R., & Maxwell, D. P. (1993). Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. *Plant Disease*, 77(4), 340-347. https://doi.org/10.1094/PD-77-0340

Rosario, K., Marr, C., Varsani, A., Kraberger, S., Stainton, D., Moriones, E., ... & Breitbart, M. (2016). Begomovirus-associated satellite DNA diversity captured through vector-enabled metagenomic (VEM) surveys using whiteflies (Aleyrodidae). *Viruses*, 8(2), 36. https://doi.org/10.3390/v8020036

Rostami, F., Pakbaz, S., & Darvishnia, M. (2023). The first report of Tomato Yellow Leaf Curl Virus (TYLCV) occurrence in tomato greenhouses of Lorestan province. *Journal of Genetic Resources*, 20-28. https://doi.org/10.22080/jgr.2024.26347.1378

Salari, K., Hosseini, A., & Heydarnejad, J. (2015). Molecular Identification of Weed hosts of Tomato yellow leaf curl virus in southeast of Kerman Province. *Journal of Iranian Plant Protection Research*, 29(3), 427-436. https://doi.org/10.22067/jpp.v29i3.37374

Shafiq, M., Sattar, M. N., Shahid, M. S., Al-Sadi, A. M., & Briddon, R. W. (2021). Interaction of watermelon chlorotic stunt virus with satellites. *Australasian Plant Pathology*, 50, 117-128. https://doi.org/10.1007/s13313-020-00757-x

Yousif, M.T., Kheyr-Pour, A., Gronenborn, B., Pitrat, M., & Dogimont, C. (2007). Sources of resistance to Watermelon chlorotic stunt virus in melon. *Plant Breeding*, 126(4), 422-427. https://doi.org/10.1111/j.1439-0523.2007.01366.x

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