J Genet Resour2021;7(1): 106-115 *Homepage: http://sc.journals.umz.ac.ir/*

RESEARCH ARTICLE *DOI: 10.22080/jgr.2021.20641.1232*

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Detection and Molecular Characterization of Two Potyvirus Species on Cucurbits from Northwestern of Iran

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ARTICLE INFO

Article history:
Received 29 August 2020
Accepted 02 November 2020
Available online 17 November 2020

Keywords:
Detection
Universal primer
WMV
ZYMV

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

ABSTRACT

Cucurbit crops belonging to the family Cucurbitaceae are one the most important horticultural crops worldwide and cultivated widely in Iran. To aim detection and phylogenetic analysis of potyvirus members, during 2015-2016, 215 leaf samples from cucumber, melon, watermelon, and squash symptomatic with deforming and reduction in leaf size, blistering, mild and severe mosaic, fruit deformation, and stunting were collected from 10 major cucurbits-growing locations in West Azerbaijan province. Following mechanical inoculation of *Cucurbita pepo* as an indicator plant with symptomatic cucurbit crop extracts, a variety of symptoms developed on the plants. Then, total RNA extracted from 70 symptomatic leaf samples, and partial RNA-dependent RNA polymerase (NIb) (350 bp) was amplified using universal primer pairs (NIb2F/NIb3R) by reverse transcription-polymerase chain reaction (RT-PCR). The infection by potyviruses in 38.75% of the samples was confirmed based on the result. The five PCR products belonging to different hosts at the expected size (350bp) were sequenced. One sample (Ir-Na: MH491979) was determined to be infected by the Watermelon mosaic virus (WMV) whereas four samples (Ir-Ma:MH491979, Ir-Ng:MH491980**,** Ir-Pi: MH491981, and Ir-Ur: MH491982) infected by *Zucchini yellow mosaic virus* (ZYMV). In the phylogenetic analysis based on Maximum-likelihood, three ZYMV isolates (Ir-Ng, Ir-Pi, and Ir-Ur) were placed in subgroup I whereas one isolate (Ir-Ma) was placed in subgroup II belonging to group A. Also, WMV isolate (Ir- Na) was placed in CL phylogroup.

Please cite this paper as: Morteza F, Nematollahi S, Koolivand D. 2021. Detection and molecular characterization of two Potyvirus species on cucurbits from Northwestern of Iran. *J Genet Resour* 7(1): 106-115. doi: *10.22080/jgr.2021.20641.1232*

Introduction

The major cultivated cucurbit species such as melon (*Cucumis melo* L.), cucumber (*C. sativus* L.), squash (*Cucurbita* sp.), and a watermelon (*Citrullus lanatus* L.), which belong to the family Cucurbitaceae are the most economically important vegetable crops worldwide (Schaefer and Renner, 2010). Cucurbits are major vegetable crops in Iran ranking first in economic value, second in yield and third in acreage (Bananej and Vahdat, 2008). Because of the favorable climate, West Azerbaijan province is one of the largest areas for cucurbit production in Iran (Maghamnia *et al*., 2018). Among approximately 35 viruses reported from cucurbits *Zucchini yellow mosaic virus* (ZYMV) and *Watermelon mosaic virus* (WMV), members of the genus *Potyvirus* in the family *Potyviridae,* are devastating pathogens with a worldwide distribution and wide host range (Lecoq *et al*., 2001; Sharifi *et al*., 2008).

Following the first report of ZYMV from squash in Iran, this virus has been reported from different vegetable-producing regions and has become the limiting factor for the production of cucurbits crops during growth seasons leading to losses of up to 80–100% (Hosseini *et al*., 2007). ZYMV has a wide host range comprising 11 plant families including Amaranthaceae,

Papilionaceae, Ranunculaceae, Scrophulariaceae, Asteraceae, Cucurbitaceae, Solanaceae, and Umbelliferae (Desbiez and Lecoq, 1997). WMV first described by Webb and Scott (1965) from Italy and detected in several plant species, commonly cucurbits in Iran (Massumi *et al*., 2007; Sharifi *et al*., 2008). WMV infects more than 170 species in over 27 families of dicotyledonous plants including vegetables, ornamentals, and grains. Also, some natural weed hosts can serve as WMV sources (Mayo 1995).

ZYMV and WMV are both single-stranded positive-sense RNA viruses that are transmitted in a non-persistent manner by several aphid species. Also, ZYMV can transmit vertically via pollen (Harth *et al*., 2017). It has been proved that ZYMV can be transmitted via seeds in some plant species such as zucchini, buttercup squash, holl-less seeded oil pumpkin, and cucurbit (Fletcher *et al*., 2000; Tobias and Kovacs, 2001). The rate of this type of transmission is low; however, it plays an important role in the virus epidemic because a few infected plants can lead to heavy virus infection (Tobias and Tulipan, 2001).

According to several studies conducted in Iran, ZYMV, WMV, and *Cucumber mosaic virus* (CMV) is the major viral diseases of cucurbits (Massumi *et al*., 2007; Sharifi *et al*., 2008). General symptoms induced by ZYMV on cucurbits are mosaic, leaf yellow mottle, stunting of the entire plant, and occasionally leaf and fruit deformities (Hosseini *et al*., 2007). Cucurbit plants infected with WMV show an array of symptoms such as mild and severe mosaic, mottling, leaf deformation, and discoloration (Shoeybi *et al*., 2009).

During 2015-2016, viral symptoms similar to those caused by ZYMV and WMV were observed in cultivated cucurbits crops including cucumber, melon, squash, and watermelon located in West Azerbaijan. Lack of information about the type of viruses damaging filed-grown cucurbit crops is an obstacle for planning sustainable strategy. The objective of this survey was the determination of geographical origins of the potyvirus species especially from the locations where no detailed studies have already been done and to partially characterize the isolates infecting heavily cucurbit plants in West Azerbaijan based on partial NIb nucleotide sequences.

Materials and Methods

Sampling

During spring and summer 2015-2016, a total of 215 leaves from cucumber (*C. sativus*), melon (*C. melo*), watermelon (*Citrullus lanatus*), and squash (*Cucurbita* sp.) were collected from 10 major cucurbit-growing locations in West Azerbaijan (Table 1). Each sample was collected from an individual plant. The samples were randomly selected among the plants showing virus-like symptoms such as mosaic, mottling, discoloration, stunting, leaves, and fruit deformities. Samples were preserved on calcium chloride ($CaCl_2$) granules at 4 °C and then stored at -20 °C for RNA extraction.

Virus isolates and mechanical inoculation of plants

Individual pumpkins (*Cucurbito pepo*) were used as indicator plants. Crude sap (1 g of 70 fresh leaf samples ground in 10 ml of 0.01 M potassium phosphate buffer, pH 7.4, containing active charcoal) from symptomatic cucurbit crop leaf extracts including cucumber, melon, watermelon, and squash were mechanically inoculated on the test plant. The pumpkin plants were pre-dusted with carborundum (600 mesh) at the two-leaf stage and then kept in a glasshouse for symptoms development. To analysis the different symptoms, inoculated plants were visited from five days post-inoculation (5dpi) up to 15 days post-inoculation (15dpi) and the symptoms of each plant were recorded. The infection by viruses was tested in RT-PCR after 15dpi from the inoculated plants.

Amplification of partial NIb region by RT-PCR

Seventy RNA samples were extracted from the field samples using ‘Method 4' of Rowhani *et al*. (1993). The extracts were then used in RT-PCR protocol to amplify genomic partial NIb regions of infected potyvirus samples using a pair of *Potyvirus* universal primers including forward (Nib2F) and reverse (Nib3R) primers (Table 2). Briefly, the first-strand cDNA was synthesized using total RNA as template in 20 µl reaction mixture containing 5 µl RNA, 4 µl RT-PCR

buffer 5x, 1 µl dNTPs (10 mM), 1 µl DTT (100 mM), 0.5 µl RNase inhibitor enzymes (100 mM) and 1 µl (100 pM) of the reverse primer and 7 µl sterile distilled water. The RT mix was incubated at 72 ºC for 3 min (for enzyme inactivation) and immediately incubated on ice for 3 min and 0.5 µl MMLV reverse transcriptase (200 U/ µl) was added to each reaction. The reactions were incubated at 42 ºC for an hour in a thermocycler (Palm cycler, CG1-960, Cobett research, Australia).

Initial denaturation was performed at 94 ºC for 2 min, followed by 35 cycles at 94 ºC for 45s, 45 ºC for 45s, 72 ºC for 2 min, and a final elongation step at 72 ºC for 10 min. PCR products were analyzed on 1% agarose gel at 80 V with 1x TBE. The gel was stained in ethidium bromide solution (1 mg/ml) for 15 min and visualized with gel documentation (White/Ultraviolet transilluminator-UVP-UK).

Table 1. Location and the number of collected samples.

Geographical location	Cucurbit crop	No. of samples	Observed symptoms [a]
Piranshahr (Kohne lajan)	Squash	9	DL, LR
	Cucumber	9	SM, VC
	Melon	7	BL
	Watermelon	7	M, MT
Piranshahr (Bardeh Qel)	Squash	6	M
	Cucumber	6	SM
	Melon	8	DL, BL
	Watermelon	7	M, DL
Nalus	Squash	5	FL, LR
	Melon	4	DL
	Watermelon	9	M, MT
Naghadeh (Qarna)	Squash	6	FL, ST
	Cucumber	5	M, SM
Naghadeh (Tazeghale)	Squash	11	DL,BL,M
Mohamadyar	Squash	8	DL, M
Mahabad (Darlak)	Squash	9	FL, BL
	Cucumber	8	VC
	Melon	7	MT, DL
Bukan (Dash Band)	Squash	10	DL, BL, FL, M
	Watermelon	9	M, MT, DL
Moiandoab (Hasan Abad)	Squash	8	M
	Cucumber	8	VC
	Melon	9	MT
	Watermelon	7	DL
Urmia	Squash	6	FL
	Cucumber	10	M, SM
	Melon	11	MT, BL
	Watermelon	6	DL
Total		215	27

[a] DL: Deformation of leaves, SM: Severe mosaic, M: Mosaic, VC: Vein-clearing, BL: Blistering, MT: Mottling, FL: Filiform Leaves, LF: Leaf-rolling of leaves, ST: Stunting.

Table 2. Characteristics of primer pairs used for this study to amplify partial Nib region of potyvirus isolates.

Primer	Sequence	Tm (° C)	Position	Amplicon size (bp)	Amplification region	Reference
Nib2F	5'- GTITGYGTIGAYGAYTTYAAYAA -3'	45	7619-7641	350	Partial NIb	Zheng *et al*., 2010
Nib3R	5'- TCIACIACIGTIGAIGGYTGNCC -3'	45	7945-7968			

Sequencing and phylogenetic analysis

A DNA band of the expected size (350 bp) amplified from two squash samples from Piranshahr and Naghadeh, one watermelon from Nalus, one melon from Mahabad, and one cucumber from Urmia were extracted from agarose gel by Vivantis nucleic acid extraction kit (Malaysia) and sequenced in both directions by Bioneer Inc. (South Korea). After primer trimming, assembling of the contigs was performed by DNA STAR Lasergene (SEQMAN and EDITSEQ, version 10). Phylogenetic analysis was performed to determine the relationships between potyvirus isolates sequenced in this study with the counterpart sequences available in the GenBank (Tables 3 and 4) by MEGA 6 using maximum-likelihood (ML) procedures (Tamura *et al*., 2013). To this aim, sequences retrieved from NCBI and new sequences, aligned in MEGA 6 by ClustalW. All sequences were edited based on manufacturing to obtain the best alignment. Then, the best DNA model was selected in MEGA 6 software to generate the phylogenetic tree. As mentioned above, the phylogenetic tree generated by Maximum-likelihood with 1000 replicates bootstrap in Jukes-Cantor method with Gamma distribution. Two isolates including ZYMV and *Yambean mosaic virus* (YBMV) with the accession numbers KP710870 and JN190431, respectively, were included as outgroups for each phylogenetic tree.

Results

Sampling and biological assays

All 215 collected samples were suspected to be infected with potyvirus species based on the symptoms. These symptoms included deforming and reduction in leaf size, blistering, mild and severe mosaic, fruit deformation, and stunting (Fig. 1). The common symptoms on squash plants included deformation and leaf-rolling of leaves, blistering, filiform leaves, mosaic, and reduction of fruits. Also, severe mosaic and vein-clearing were observed on cucumber samples. Mosaic, mottling, and deformation of leaves and fruit were common symptoms on watermelon samples. Infected melon plants mainly showed mottling, blistering, and deformation of leaves. Following mechanical inoculation of the indicator plants with sap from the symptomatic cucurbit leaves, a variety of symptoms developed on the plants (Fig. 1).

RT-PCR and phylogenetic analysis

Seventy out of 215 symptomatic leaf samples were checked randomly by RT-PCR. Twenty-seven DNA fragments with 350 bp in length covering the internal region of the NIb region were amplified from potyvirus-infected plants using Nib2F/ Nib3R primer pair (Fig. 2). Finally, five different partial NIb sequences isolated from different cucurbit crops such as two squash, watermelon, melon, and cucumber were deposited in the NCBI GenBank database with the accession numbers MH491978, MH491979, MH491980, MH491981, and MH491982, respectively. These included isolates Ir-Na (watermelon), Ir-Ma (Melon), Ir-Ng (Squash), Ir-Pi (Squash), and Ir-Ur (Cucumber) from Nalus, Mahabad, Naghadeh, Piranshahr, and Urmia, respectively (Tables 3 and 4). Comparison of five NIb sequences with the available corresponding sequences at the NCBI GenBank database showed that isolates Ir-Pi, Ir-Ng, Ir-Ma, and Ir-Ur belonged to *Zucchini yellow mosaic virus* (ZYMV) whereas isolate Ir-Na was recognized as *Watermelon mosaic virus* (WMV). Two phylogenetic trees were constructed by Maximum-Likelihood in which Ir- Na isolate was placed in CL together with five isolates from France, one isolate from India, two isolates from the USA, and three isolates from South Korea (Fig. 3). Also, ZYMV isolates were placed in both subgroups I and II belonging to group A (Fig. 4).

Discussion

Despite the occurrence of viral infections in cucurbit crops, there has been little research conducted on infections with ZYMV and WMV in West Azerbaijan. Therefore, one aim of this study was the determination of geographical origins of the isolates especially from the locations where no detailed studies have already been done. Based on molecular assays, we found that these regions were highly contaminated by two or more potyvirus species.

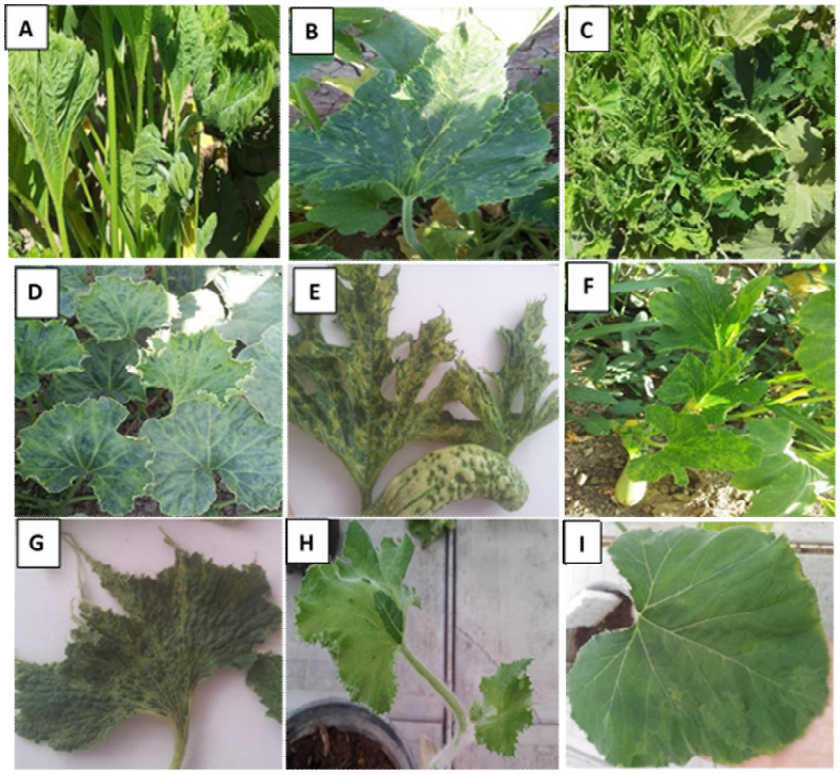

Fig. 1. Disease symptoms are caused by ZYMV and WMV on cucurbit crops. (A) upward leaf-rolling on *Cucurbit*a sp. caused by ZYMV (B) severe mosaic accompanied by chlorotic spots on *Cucurbit*a sp. caused by ZYMV (C) leaf narrowing on *Cucurbit*a sp. caused by ZYMV (D) mottling and vein clearing on *Cucurbit*a sp. caused by WMV (E) sever mosaic, chlorotic spots and blistering on cucumber leaves and fruit caused by WMV (F) chlorotic spots and twisted leaves on *Cucurbit*a sp. caused by WMV (G) leaf blister on *Cucurbit*a sp. 20 days post-inoculation (dpi) caused by ZYMV (H) dark green spot and leaf distortion on *Cucurbit*a sp. 15 dpi caused by WMV (I) mild mosaic on *Cucurbit*a sp. 20 dpi caused by ZYMV.

Twenty-seven out of 70 field samples were positive in the RT-PCR suggesting that the rate of infection was 38.57%. Based on the specific primer pairs used for potyviruses detection, we can strongly claim that all these 27 isolates belonged to the potyvirus genus. In other words, the accurate detection of distinct species is easily possible without more investigation such as using specific antibodies or sequencing all isolates, unless there are very closely related species at the sequenced region. This study proved the efficiency of NIb2F2/NIb3R in the identification of potyviruses and revealed the extent of infections with two destructive virus species in northern Iran, which is following previous studies (Bananej *et al.*, 2008, Sharifi *et al.*, 20008: Nematollahi *et al.*, 2021). The most important step to manage viral infections is prevention, therefore, prior detection of viruses is essential (FAOSTAT, 2019). In this study, we proved the presence of two destructive potyvirus species as the first step to further investigations.

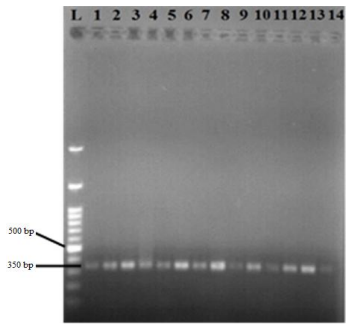

Fig. 2. Electrophoresis pattern of RT-PCR products in 1% agarose gel. Lane L: Gene Ruler 100 bp DNA Ladder plus (MBI Fermentas, Germany), Lane 1-14: DNA bands of the expected size (350bp) with Nib2F / Nib3R primer pair from symptomatic squash, cucumber, melon, and watermelon samples.

Biological and molecular methods have been used for the identification of viruses. Collecting different cucurbit crops such as cucumber, melon, watermelon, and squash revealed differences in symptoms and a wide host range of the two potyviruses, ZYMV and WMV.

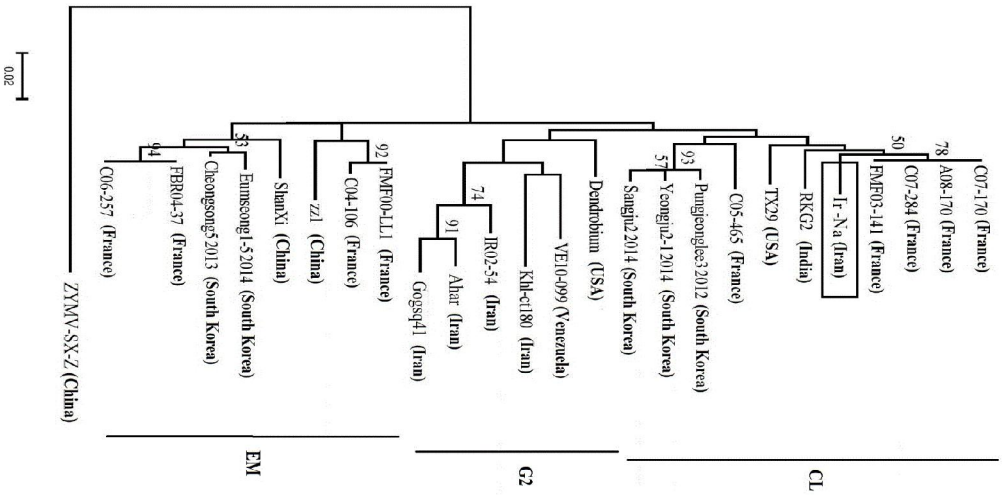

Fig. 3. Maximum-likelihood tree based on nucleotide sequences of the amplified 350 bp fragment from 26 *Watermelon mosaic virus* isolates including isolated from Iran (bolded). Multiple sequence alignments were generated with the MEGA program Version 6. Bootstrap values (>50%) are shown on the nodes. *Zucchini yellow mosaic virus* (KP710870) was included as an outgroup.

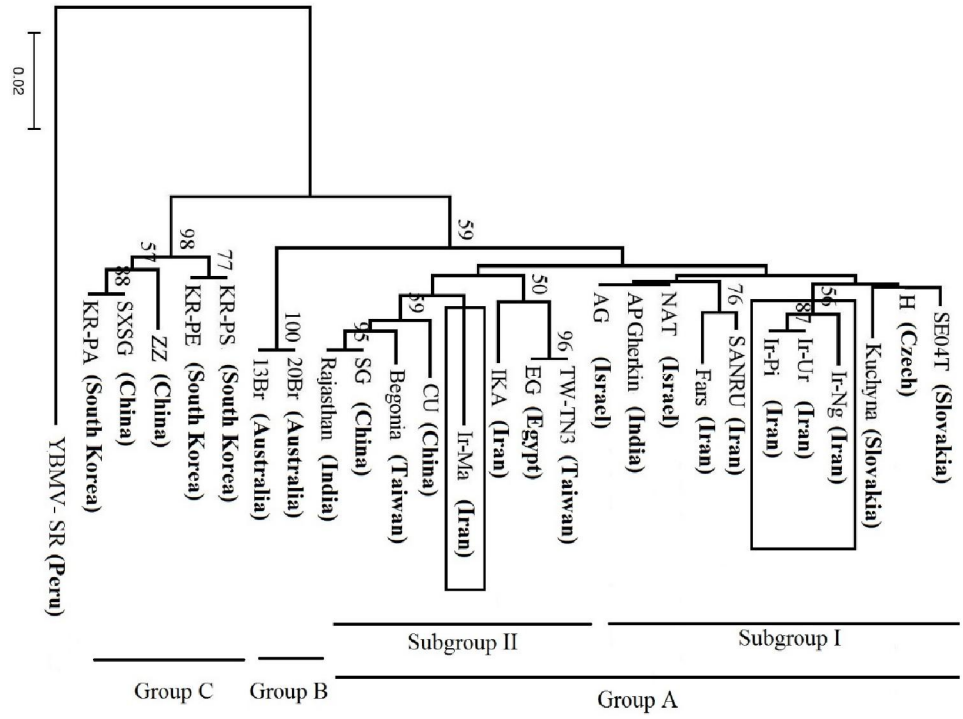

Fig. 4. Maximum-likelihood tree constructed based on nucleotide sequences of the amplified 350 bp fragment from 27 *Zucchini yellow mosaic virus* isolates including isolates from Iran (bolded). Multiple sequence alignments were generated with the MEGA program Version 6. Bootstrap values (>50%) are shown on the nodes. *Yam bean mosaic virus* (JN190431) was included as an outgroup.

Among the samples, the mosaic was the dominant symptom, however, no viral infection was detected in many symptomatic samples which might suggest the presence of other virus species. In some regions such as Naghadeh and Bukan, the viral symptoms were more frequent compared with the other regions. This might be because of more abundance of aphids transmitting the viruses. Indeed, the climate was warmer and more humid in these locations which are more desirable for the aphids. Also, according to our knowledge, the seeds used in these locations were not certified virus-free seeds.

Table 3. Name and origin of ZYMV isolates included in the phylogenetic analysis and accession numbers of the newly-generated sequences. The new Iranian isolates of this research are bolded.

Isolate name	Geographical location	Original host	Accession no.
Kuchyna	Slovakia	Squash	DQ124239
H	Czech Republic	Squash	KF976712
AP Gherkin	India	*Cucumis anguria*	KT778297
AG	Israel	-	EF062583
NAT	Israel	-	EF062582
Begonia	Taiwan	Begonia	AM422386
Rajasthan	India	Muskmelon	KJ425470
SANRU	Iran	*Cucurbita pepo*	KU198853
SE04T	Slovakia	*Cucurbita pepo*	KF976713
KR-PS	South Korea	*Cucurbita moschata*	AY279000
KR-PE	South Korea	*Cucurbita moschata*	AY278999
EG	Egypt	Squash	LC153709
SXSG	China	*Luffa aegyptiaca*	KX249747
KR-PA	South Korea	*Cucurbita moschata*	AY278998
ZZ	China	*Sesamum indicum (sesame)*	KX421104
Fars	Iran	Squash	JN183062
TW-TN3	Taiwan	-	AF127929
CU	China	Cucumber	AJ307036
SG	China	-	AJ316228
IKA	Iran	Squash	KU528623
13Br	Australia	Zucchini	KY225555
20Br	Australia	Zucchini	KY225550
Ir-Ma	**Iran-Mahabad**	**Melon**	**MH491979**
Ir-Ng	**Iran-Naghadeh**	**Squash**	**MH491980**
Ir-Pi	**Iran-Piranshahr**	**Squash**	**MH491981**
Ir-Ur	**Iran-Urmia**	**Cucumber**	**MH491982**
Yam bean mosaic virus isolate SR	Peru	Yam bean	JN190431

Table 4. Name and origin of WMV isolates included in the phylogenetic analysis and accession numbers of the newly-generated sequence. The isolate obtained in this survey is in bold.

Isolate name	Geographical location	Original host	Accession no.
C07-170	France	Melon	JF273464
A08-170	France	Zucchini	JF273466
C07-284	France	Zucchini	JF273468
FMF03-141	France	-	EU660583
RKG2	India	Watermelon	KM597071
TX29	USA	Watermelon	KU246036
C05-465	France	Zucchini	JF273460
Pungjeonglee3_2012	South Korea	-	KU240102
Yeongju2-1_2014	South Korea	-	KT992084
Sangju2_2014	South Korea	-	KT992078
Dendrobium	USA	*Dendrobium anosmum* (orchid)	HQ384216
VE10-099	Venezuela	*Cucumis anguria L.*	KC292915
Khl-ct180	Iran	*Cantaloupe*	JX124711
IR02-54	Iran	-	EU660584
Ahar	Iran	Squash	JF707768
Gog-sq41	Iran	Squash	JX124710
FMF00-LL1	France	_	EU660581
C04-106	France	Melon	JF273469
ZZ1	China	*Sesamum indicum*	KM978929
ShanXi	China	Watermelon	JX079685
Eumseong1-5_2014	South Korea	*Panax ginseng C.A. Meyer*	KT992073
Cheongsong5_2013	South Korea	*Panax ginseng C.A. Meyer*	KT992071
FBR04-37	France	-	EU660586
C06-257	France	Melon	JF273463.
Ir-Na	**Iran-Nalus**	**Watermelon**	**MH491978**
ZYMV- SX-Z	China	*Glycine max*	KP710870

According to the previous literature, we used *cucurbita pepo* as the indicator plant (Antignus, 2012). Based on biological properties, the Iranian ZYMV isolates caused different types of symptoms such as mosaic and blistering whereas inoculation with WMV isolates caused dark

green spot and leaf distortion on the indicator plant which are similar to the previously published reports (Dunham *et al*., 2014). In this study, we used a pair of universal degenerate primers designed to amplify 350 bp of internal NIb in potyvirus. We found that the results achieved by the PCR accompanied by the biological tests were valuable to the rapid detection of the potyvirus species. Seventy samples were tested in the RT-PCR for amplification of the expected ~350 bp fragment. As a result, infections in 27 samples by two virus species were verified. Compared to serological detection, RT-PCR with broad-spectrum primers offers cost-effectiveness, specifically in developing countries where using antibodies for serological tests is not affordable (Massumi *et al*., 2011).

According to the previous surveys, all WMV isolates clustered into three phylogroups (Fig. 3). In agreement with these studies, all WMV isolates were categorized into three phylogroups including CL, G2, and EM. CL is predominant and contains the classical isolates, the G2 isolates originated from different parts of the world with no particular biological feature, and the EM isolates were recently found in the southeastern part of France with severe symptoms on zucchini (Sokhandan-Bashir *et al*., 2013). The CL group was the largest group including five isolates from France, one isolate from India, two isolates from the USA, and three isolates from South Korea. Our single isolate Ir-Na was placed in CL close to the Indian and French isolates whereas none of the Chinese isolates was found in this phylogroup. Phylogroup G2 included six isolates including four isolates from Iran, one isolates from Venezuela and one isolates from the USA. Phylogroup EM also consisted of several isolates belonging to different geographical regions such as France, South Korea, and China.

Previous studies revealed that ZYMV isolates were divided into several major groups based on coat protein and NIb ORF (Desbiez *et al*., 2002; Zhao *et al*., 2003; Glasa *et al*., 2011). In this study, ZYMV isolates are classified into three main groups previously designated as groups A, B, and C based on the nucleotide sequences of NIb-CP gene fragment (Fig. 4) (Glasa *et al*., 2011). All our ZYMV isolates were categorized in this group. Group A was further divided into two subgroups I and II. Eleven isolates were placed in Subgroup I including one isolate from Czeck, two isolates from Slovakia, five isolates from Iran, two isolates from Israel, and one isolate from India. Three isolates Ir-Ng, Ir-Pi, and Ir-Ur formed a distinct clade in subgroup I close to two Iranian isolates SANRU and Fars. Similar results were seen in previous studies. Massumi *et al*. (2011) showed that twenty-one ZYMV collected from 13 Iranian provinces belonged to group A which is following the results obtained in our study. On the other hand, seven isolates were placed in subgroup II including one isolate from Egypt, two isolates from Iran, two isolates from Taiwan, and two isolates from China. Isolate Ir-Ma were placed in this subgroup (II) close to the Chinese isolate (CU). According to Bananej *et al*. (2008), most of the Iranian isolates belonged to Group A, but a recent study by Maghamnia *et al*. (2018) revealed that some Iranian isolates fall into Group C indicating genetic variation among Iranian ZYMV isolates (Fig. 4). Group B consisted of three Indian and one Australian isolate. Finally, group C consisted of five isolates including three isolates from South Korea and two isolates from China.

Iran now is one of the important countries for the production of cucurbits in Asia, which explains the fact that further studies are needed to be performed on the natural distribution of threatening virus species. Due to the reason that these two viruses can be transmitted by aphids, the development of strategies for the detection and management of these two destructive virus species is required.

Conclusion

Our analyses provide further evidence of potyvirus infection in west-northern Iran. Based on the result it can be concluded that the cucurbit fields are infected by two important potyviruses including WMV and ZYMV. Phylogenetic analysis revealed that the newest ZYMV isolates were placed in Group A with Old Iranian isolates. It could be concluded that the aphids may transmit ZYMV between the near regions. Also, WMV isolates grouped with other Iranian isolates in the CL subgroup.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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J Genet Resour2021;7(1): 106-115 Homepage: http://sc.journals.umz.ac.ir/
RESEARCH ARTICLE DOI: 10.22080/jgr.2021.20641.1232
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ردیابی و خصوصیات مولکولی دو گونه پوتی‌ویروس از مزارع کدوئیان شمال غرب کشور

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چکیده

کدوییان متعلق به تیره Cucurbitaceae و یکی از مهمترین محصولات زراعی کشت شده در سطح وسیع در جهان و ایران هستند. به منظور ردیابی و تجزیه و تحلیل تبارزایی *Potyvirus* ها، تعداد ۲۱۵ نمونه برگی از خیار، کدو، طالبی و هندوانه که دارای علایمی نظیر تغییر شکل، کاهش اندازه برگ، تاول برگی، موزائیک خفیف تا شدید ، تغییر شکل میوه و کوتولگی از ده منطقه زیر کشت این محصولات در استان آدربایجان غربی و در طی سال‌های ۲۰۱۵-۲۰۱۶ جمع آوری گردید. با تلقیح عصاره گیاهان علایم دار روی *Cucurbita pepo* به عنوان گیاه محک طیف وسیعی از علایم ویروسی مشاهده گردید. سپس RNA از هفتاد نمونه علایم دار استخراج گردید و بخشی از ژن RNA Polymerase وابسته به RNA (Nib) به طول ۳۵۰ جفت باز با استفاده از آغازگرهای عمومی (NIb2F/NIb3R) در واکنش RT-PCR مورد تکثیر قرار گرفت. نتایج نشان داد که ٪۳۸/۷۵ از نمونه ها به پوتی ویروس ها آلوده بودند. پنج فرآورده PCR متعلق به میزبان‌های مختلف با طول مورد انتظار ۳۵۰ نوکلوتید تعیین توالی شد. یک نمونه (Ir-Na: MH491979) به عنوان ویروس موزائیک هندوانه شناسایی شد در حالیکه چهار نمونه دیگر(Ir-Ma:MH491979 Ir-Ng:MH491980 Ir-Pi: MH491981, Ir-Ur: MH491982) به عنوان ویروس موزائیک زرد کدو شناسایی شدند. در تجزیه و تحلیل تبارزایی براساس Maximum-Likelihood سه جدایه (Ir-Ng, Ir-Pi, and Ir-Ur) ویروس موزاییک زرد کدو در زیر گروه I قرار گرفتند در حالیکه یک جدایه Ir-Ma این ویروس در زیرگروه II از گروه A قرار گرفتند . همچنین جدایه Ir- Na ویروس موزائیک هندوانه نیزدر زیرگروه CL قرار گرفت.

واژگان کلیدی: ردیابی، آغازگر عمومی، ویروس موزاییک زرد کدو، ویروس موزاییک هندوانه

Please cite this paper as: Morteza F, Nematollahi S, Koolivand D. 2021. Detection and molecular characterization of two Potyvirus species on cucurbits from Northwestern of Iran. *J Genet Resour* 7(1): 106-115. doi: *10.22080/jgr.2021.20641.1232.*