Asian Journal of Plant Pathology

ISSN 1819-1541

Academic Journals Inc.

www.academicjournals.com

Asian Journal of Plant Pathology 5 (3): 115-125, 2011
ISSN 1819-1541 / DOI: 10.3923/ajppaj.2011.115.125

Diagnosis and Molecular Variability of *Watermelon mosaic virus* Isolates from North, East, North-east and North-west Regions of Iran

Zohreh Moradi
Department of Plant Pathology, College of Agriculture, Ferdowsi University of Mashhad, Khorasan, Iran

ABSTRACT

Watermelon Mosaic Virus (WMV) is a *Potyvirus* with a worldwide distribution. This virus causes serious economic losses in many cucurbits in Iran. To study relative incidence and molecular variability of WMV, 620 samples of cucurbit plants were collected from the different fields and cucumber greenhouses in some regions of Iran. Three hundred and three samples were infected by WMV in DAS-ELISA using specific polyclonal antibody (The rate of infection was 48.8%). In RT-PCR assay with specific primers of WMV, a single band of about 825 bp in length was produced from the samples. The Coat Protein (*CP*) region of the genome from 15 representative isolates were sequenced and compared with the sequences available in GeneBank. The identity of WMV CP nucleotide sequences of the 15 Iranian isolates ranged from 95.8 to 99.2%. According to this research Iranian isolates have high variation in *CP* gene. In most cases, geographical isolation is consistent with the phylogenetic grouping. Molecular weights of coat protein using SDS-PAGE were estimated at 34100 Da. Data shown in this study represent the occurrence of WMV based on ELIZA, RT-PCR and *CP* gene analyses in some region of Iran. These results make it possible to have a better understanding of the development of WMV disease in Iran.

Key words: *Watermelon mosaic virus*, RT-PCR, coat protein sequence, molecular variability, phylogenetic analyses

INTRODUCTION

Watermelon Mosaic Virus (WMV) is a member of the genus *Potyvirus* (family Potyviridae) and consists of flexuous, filamentous particles, approximately 760 nm long. The genome is a positive-sense, single-stranded RNA and the size of RNA is about 10035-nt long. The genomes of *Potyviridae* have a single Open Reading Frame (ORF) that is translated into a single large polyprotein which is hydrolyzed, after translation, into several proteins by virus-encoded proteinases (Riechmann *et al*., 1992; Al-Saleh *et al*., 2009).

At the molecular level, WMV is closely related to SMV (*Soybean mosaic virus*) in most of its genome (in spite of its different and much broader host range) and could consider as a divergent strain of this virus. Moreover, it also appears to share a recombination with *Bean common mosaic* Virus (BCMV) in the P1 coding region. The first report of full sequenced WMV was from France (WMV-Fr) (Desbiez and Lecoq, 2004) followed by China (WMV-CHN) (Wu *et al*., 2006) and Pakistan (WMV-PK) (Ali *et al*., 2006). WMV is probably distributed worldwide mostly in temperate regions and was first reported in *Citrullus lanatus* by Webb and Scott (1965) and Purcifull *et al*. (1984). The geographical occurrence of the virus is variable and depends on climatic conditions (Desbiez *et al*., 2007).

The Cucurbitaceae family includes several species of horticultural crops. Iran cultivates the second-largest total cucurbit acreage (770,000 ha) in the world, exceeded only by China.

(Massumi *et al*., 2007). WMV can cause economically important diseases in several horticultural crops, mostly cucurbits and legumes, resulting in quality and yield losses. It can experimentally infect more than 170 plant species belonging to 27 families, including many weeds that can host the virus between crops (Shukla *et al*., 1994). In Iran, host range of this virus is limited to cucurbitaceous plants but in the East also other plants such as *Habenaria* and *Vanilla* have been reported as host (Gara *et al*., 1997; Wang *et al*., 1993).

The studies of WMV variation at the nucleotide and amino acid levels focused on analysis of the sequence of the P1, *CP* and Cylindrical Inclusion (*CI*) genes (Sharifi *et al*., 2008). The first report of WMV genetic diversity in Iran was related to Desbiez *et al*. (2007) that reported a 218-nucleotide sequence of the *CP* N-terminal region of eight isolates from Iran. Sharifi *et al*. (2008) analyzed 18 isolates from central and southern regions of Iran based on *CP* gene. Following Shoeibi *et al*. (2009) analyzed 4 isolates from Golestan province and one isolate from Mashhad and Shiraz (from north east and southern regions of Iran, respectively).

Although, WMV has long been known to occur in several regions of Iran (Sharifi *et al*., 2008), there are few reports on its occurrence in North, East, Northeast and Northwest of Iran. The objective of the present study was amplification of *CP* gene and evaluation of the genetic variation among the *CP* sequences of the chosen isolates collected from several regions of Iran. Information about geographically diverse range of WMV isolates encouraged to originate facts on the molecular variability of this virus.

MATERIALS AND METHODS

Viral sample sources: WMV isolates were collected from different geographical locations in the northwest (provinces of East Azarbayjane, West Azarbayjane and Ardabil), north (provinces of Mazandaran and Golestan), Northeast and East (provinces of Razavi Khorasan, Northern Khorasan and South Khorasan) of Iran (Table 1), from June 2009 to October 2011. Samples (n = 620) consisted of young fully expanded leaves showing Symptoms such as systemic mosaic, severe yellow mosaic, vein clearing and blistering and including cucumber, *Cucurbita pepo* varieties, watermelon (*Citrullus lanatus*) and melon (*Cucumis melo* varieties including Cantaloupe, snakemelon, longmelon and local cvs.). The samples were stored at 80°C until they were used.

Table 1: Accession Number, Origin, name of isolates and host of 15 sequenced isolates of *Watermelon mosaic virus*

Provice-region	Host	Accession No.	Isolate
Razavi Khorasan-Torghabe	*Cucumis melo*	JN166702	Kh.R.To
Razavi Khorasan-Sabzevar	*Cucurbita pepo*	JN166697	Kh.R.Sab
Northen Khorasan-Shirvan	*Cucurbita pepo*	JN166700	Kh.N.Sh
Northen Khorasan-Bojnord	*Cucurbita pepo*	JN166708	Kh.N.Boj
Northen Khorasan-Jajarm	*Cucumis sativus*	JN166710	Kh.N.Ja
South Khorasan-Birjand	*Cucurbita pepo*	JN166707	Kh.S.Bir
South Khorasan-Ghaenat	*Cucurbita pepo*	JN166709	Kh.S.Gh
South Khorasan-Nahabandan	*Cucumis melo* L.	JN166711	Kh.S.Nah
East Azarbayjane-Bonab	*C. moschata*	JN166701	AE.Bon
West Azarbayjane-Uromia	Pumpkin	JN166703	AW.Uro
Mazandaran-sari	*Cucurbita moschata*	JN166698	Maz.Sar
Mazandaran-behshahr	Watermelon	JN166706	Maz. Beh
Mazandaran-Savadkooh	*Cucurbita pepo*	JN166699	Maz.Sav
Golestan-Aliabad	Watermelon	JN166704	Gol.Ali
Ardabil -Meshkinshahr	*C. moschata*	JN166705	Ard.Me

Double-antibody Sandwich (DAS)-ELISA: DAS-ELISA was performed using the specific polyclonal antibody (SEDIAG S.A, Strasbourg, France) following the general protocol of Clark and Adams (1977). Extracts from healthy *C. pepo* were used as negative control in all tests.

Virus isolation and propagation: Virus isolation biologically was carried out using single local lesion assay following two passages onto *Chenopodium amaranticolor* CosteandReyn. Then virus was propagated in *C. melo* and *Cucurbita pepo* (squash cvs. Black knight and Local) for further investigation. ELISA positive samples were homogenized in 50 mM potassium phosphate buffer (pH 7.0) and the extract was rubbed onto leaves dusted with carborundum powder. Plants were maintained in an insect-proof screen house at 24-27°C and controlled at least for 4 weeks after inoculation. ELISA test was used addition to symptoms observation for checking the presence of the virus.

Protein analysis: The molecular weight of the viral coat protein was estimated by discontinuous SDS-PAGE (Sodium dodecyl sulfate poly acryl amid gel electrophoresis) as described by Laemmli (1970). A purified virion suspension and plant samples infected with the virus were mixed with the sample buffer, boiled at 100°C for 5 min and subjected to electrophoresis on 12% polyacrylamide gel along with poly peptide size standard. The gel was stained with coomassie blue. The molecular weight of polypeptides from WMV was determined by comparison with the size standards.

Total RNA extraction and RT-PCR: Total RNA was extracted from pulverized tissue using AccuZol™ Reagent (Bioneer, Alameda, CA) from fresh, systemically infected cucurbit leaves according to the manufacturer's instructions. After precipitating with ethanol, total RNA was re-solubilized in 25 μL of RNase-free water. RNA samples were tested for the presence of WMV using specific primers designed to amplify a fragment of the coat protein gene. Forward primer (5'- GAA TCA GTG TCT CTG CAA TCA GG -3') and reverse primer (5'- ATT CAC GTC CCT TGC AGT GTG -3') (Sharifi *et al*., 2008) corresponding to nucleotides (nt) 8926 to 8948 and nt 9727 to 9747 of WMV-Fr (GenBank accession number EU660584), respectively, were used to amplify a 825-bp fragment covering the *CP* region. Reverse Transcription (RT) reaction was performed as follows: 1 μL of reverse primer (20 picomoles) and 1 μL of RNA sample were added to 8.5 μL of Diethyl Pyrocarbonate (DEPC)-treated water. The mixture was incubated at 65°C for 10 min and chilled on ice for 3 min to denature the RNA. Then 3.5 μL of DEPC-treated water, 4 μL of 5x M-MLV RT buffer, 2 μL of dNTPs mix (10 mm) and 1 μL M-MLV (200 U μL) reverse transcriptase (Promege, USA) were added to mixture. The RT reactions were incubated at 42°C for 60 min followed by 95°C for 3 min to terminate the RT reaction. Viral cDNA was then amplified by PCR. The PCR reaction was performed using 2.5 μL of cDNA, 13.5 μL DEPC-treated water, 5 μL of 5x GoTag polymerase buffer, 2.5 μL 10x MgCl2, 0.5 μL of each forward and reverse primers (20 pmol), 0.75 μL of dNTP mix (10 mm) and 0.125 μL of GoTag polymerase (2.5 U μL) (Promege, USA). Following program was used for PCR: A first denaturation for 3 min at 94°C was performed and followed by 35 cycle of denaturation for 60 s at 94°C, 60 sec of annealing at 63°C, extension for 1 min at 72°C and a final extension step at 72°C for 10 min. PCR products were analyzed by electrophoresis in 1.7% agarose gel and visualized by ethidium bromide staining.

Table 2: GenBank accession numbers and origin of previously reported WMV Isolates/strains[a] used for phylogenetic comparison of the 789 nucleotides long fragment of genome including *CP* gene

Genome region	No. of nucleotides	Origin	Host	Accession No.	Isolate
	3308	USA	Melon	D13913	WMV
Comp.	10037	South Korea	Watermelon	AB369278	WMV
CP-UTR	1157	Australia	Melon	D00535	WMV
Comp.	10035	France	Watermelon	NC_006262	WMV-Fr
Nib-CP-UTR	1154	China	Melon	EF127832	WMV-ch99/69
Comp.	10037	China	Watermelon	DQ399708	WMV-CHN
Comp.	10037	NewZealand	Watermelon	AY995215	WMV
Comp.	10039	Pakistan	*C. melo* var flexuosus	AB218280	WMV-Pak
NIb-CP	1167	Japan	Pumpkin	AB353119	WMV
CP	852	China	Snake gourd	AY464948	WMV-HLJ
Nib-CP-UTR	2045	Pakistan	Snake gourd	AB127934	WMV Pak
CP	873	Spain	*C. melo*	AJ579524	WMV- MAL99.5
CP	873	Spain	*C. melo*	AJ579509	WMV-MAD95.6
CP	873	Spain	*C. melo*	AJ579493	WMV -BAR99.2
CP	873	Spain	*C. melo*	AJ579491	WMV -BAR95.1
CP	873	Spain	*C. melo*	AJ579486	WMV -ZAR99.1
Nib-CP	1602	Spain	*C. melo*	AF551334	WMV -M116
Nib-CP	1180	Japan	*C. melo*	AB001994	WMV-Habenaria
CP	843	Israel	*C. melo*	AF322376	WMV
Nib-CP	1656	Tonga	*C. melo*	L22907	WMV Tonga
CP	822	kerman-Jiroft	*Citrullus colocynthis*	EU667627	KER.JI.1
CP	822	Kerman-Kerman	*Cucumis melo* L.	EU667644	KER.KE.1
CP	822	Yazd-Sadogh	*C. melo* L.	EU667635	YAZ.SH.1
CP	822	Yazd-Mohsenabad	*Cucumis sativus*	EU667638	Yaz.MO.1
CP	822	Yazd-Mohsenabad	*Cucurbita pepo*	EU667630	Yaz.MO.2
CP	822	Yazd-Taft	*Cucurbita pepo*	EU667632	Yaz.TA.1
CP	822	Esfahan-esfahanak	*C. moschata*	EU667637	ESF.ES.1
CP	822	Esfahan-esfahanak	*C. melo* L.	EU667640	ESF.ES.2
CP	822	Esfahan-esfahanak	*Cucumis sativus*	EU667633	ESF.ES.3
CP	822	Esfahan-Gaz	*Cucumis sativus*	EU667641	ESF.GA.1
CP	822	Esfahan-Gaz	*C. maxima*	EU667634	ESF.GA.2
CP	822	Esfahan-Gaz	*Cucumis sativus*	EU667643	ESF.GA.3
CP	822	Uromiae-Oshnavia	*C. melo* L.	EU667631	URO.OS.1
CP	962	Shiraz	Summer squash	GQ421161	Shiraz (cp)
CP	963	Mashhad	Summer squash	GQ421158	Mashhad (cp)

[a]: The WMV isolates were identified by location, plant host and series number

Sequencing and phylogenetic analysis: RT-PCR products of WMV isolates were then purified from 1% agarose gels using the DNA gel extraction kit (bioneer, korea) and nucleotide sequencing reactions were performed by MWG Company (Biotech, Germany) using dideoxy nucleotide chain termination method with specific forward and reverse primers. Then, these and 34 isolates from the GenBank were analyzed to determine their phylogenetic relationships.

The phylogenetic analysis of the Iranian isolates was conducted by comparing the 789 bp of the *CP* gene with those of the other isolates of WMV obtained from GenBank (Table 2). Nucleotide sequence analysis and translation to the corresponding amino acid sequence were performed using

DNAMAN software (version 4.02) package (BBA, Germany). The nucleotide and deduced amino acid sequences were compared with the equivalent sequences of WMV. Multiple alignments of the nucleotide and amino acid sequences were carried out using the ClustalW program in BioEdit (version 7.0.9.) software and DNAMAN software (version 4.02). Phylogenetic tree for grouping based on nucleotide sequences were constructed by MEGA 4.1 software program using the neighbor-joining method. Tree branches were bootstrapped with 1000 replications and bootstrap values less than 55% were condensed on nodes (Fig. 4).

RESULTS

Virus isolate: Among 620 samples collected from different areas of Iran, 303 samples were positive in ELISA test (The rate of infection was 48.8%). The symptoms consisted of varying degrees of mosaic with leaf blistering and leaf deformation or stunting. Symptoms were diverse in different Cucurbit plants and related to host plant age and time of infection (Fig. 1a-d).

SDS-PAGE: The size of the viral coat protein of some of the isolates was determined about 34,100 Da while no band was found in the healthy plant extracts (Fig. 2) and this was similar to standard protein molecular weight respect to other WMV isolates described by researchers in other parts of the world.

RT-PCR, sequence data and identity matrix: RT-PCR was carried out using the primers WMV/F and WMV/R (described previously) which resulted in a fragment of approximately 825 nts (Fig. 3). Sequence information for these 15 isolates has been submitted to NCBI-GenBank with the accession numbers from JN166697 to JN166711 (Table 1).

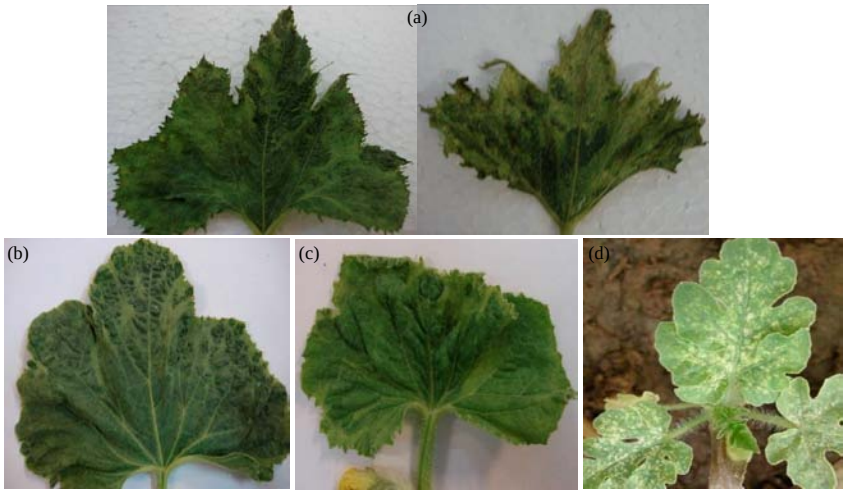

Fig. 1(a-d): WMV Symptoms on different cucurbit plants. (a) Mosaic, blistering, vein banding and malformation of leaves on zucchini, (b) Mosaic and blistering on *C. melo*., (c) Yellowing, blistering and malformation on *C. sativus* and (d) Sever systemic mosaic on *Citrullus lanatus* leaf blade

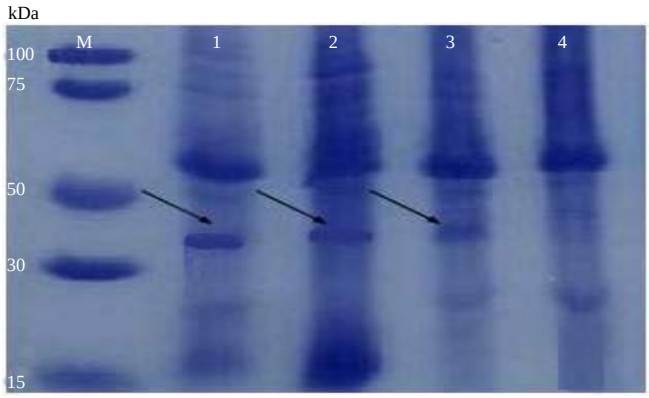

Fig. 2: Denaturing polyacrylamide gel electrophoresis of infected plants (line 1, 2, 3) related to Khorasan provinces isolates, healthy plant (zucchini leaf) (4) and molecular weight marker proteins (M). The protein band in gel was stained with Coomassie Blue

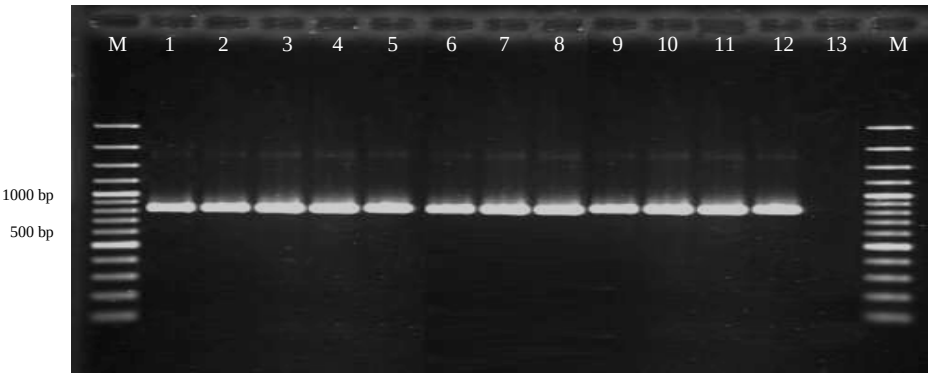

Fig. 3: Electrophoresis pattern of DNA fragments amplified by RT-PCR in 1.7% agarose gel related to twelve selected WMV isolates collected from different location of Iran (lanes 1-12) 13: healthy Zucchini plant extract as negative control. M: 100 bp DNA marker (Fermentas)

Phylogenetic analysis: Phylogenetic tree based on multiple sequence alignment of *CP* divided all WMV isolates into two large groups: group 1 included 4 subgroups: Sg1, Sg2, Sg3, Sg4 (Fig. 4). Group 1 included Middle East and European isolates, only one isolate from Australia placed in this group. Most of Iranian isolates were classified in the subgroup 1 which can be divided into many clades. 13 out of 15 isolates including Kh.R.To, Kh.R.Sab (collected from Razavi Khorasan province), Kh.N.Sh, Kh.N.Boj, Kh.N.Ja (collected from Northern Khorasan province), Kh.S.Bir, Kh.S.Gh, Kh.S.Nah (collected from South Khorasan province), AE.Bon (collected from East Azarbayjane province), Maz.Sar Maz. Beh, Maz.Sav (collected from Mazandaran province), Ard.Me (collected from Ardabil province) have been reported for the first time herein. Pakistan isolates were classified in subgroup 2. Geographically, only Subgroup 3 is a heterogeneous group and included isolates of center parts of Iran (Yazd, KERJI1), Tonga, USA and New Zealand. Exception Shiraz isolate in subgroup 4 which has been located with Japan isolates, geographical isolation is

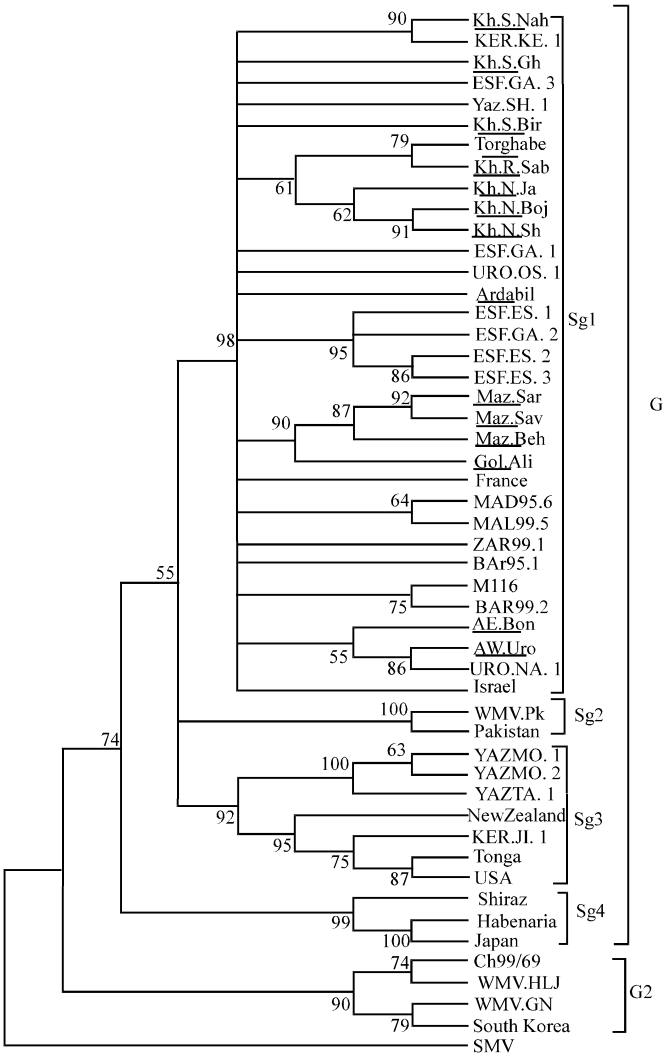

Fig. 4: Phylogenetic tree constructed from the alignment of nucleotide sequences of coat protein gene of 45 WMV isolates (Table 1 and 2) using neighbor-joining method based on 1000 replicates. The numbers indicate bootstrap percentage. Bootstrap values higher than 55 are indicated on nodes and the nodes less than 55 were condensed. The isolates which have been sequenced here, were underlined. SMV (*Soybean mosaic virus*) included as out-group

consistent with the phylogenetic grouping (Shoeibi *et al*., 2009). Isolates in group 2 included South Korea, Poland and China isolates. Eighteen isolates in subgroup 1 were collected from different regions of Khorasan provinces formed a distinct small group according to the geographical region.

Phylogenetic analyses based on ClustalW Multiple alignments, demonstrated that 15 isolates share 95.8 to 99.2% nucleotide sequence identity with each other. Identity of 15 Iranian isolates in the amino acid levels ranged between 95.2 to 100% (Table 3). The percentage similarities for nucleotide between Iranian isolates and the other isolates of WMV recorded in NCBI ranged from 91 to 99%. The DAG box (Asp-Ala-Gly) which is critical for *Potyvirus* transmission by aphids, was detected in the all Iranian isolates coat protein gene.

Table 3: Comparison of the Percent nucleotide (bottom and left, in bold) and amino acid (top and right) identities of 825 nt *CP* sequences between selected WMV isolates used in this study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Torghabe	*	98.5	99.6	99.2	99.6	97.8	99.2	97.4	97.4	97.0	96.7	97.8	98.5	98.1	98.1
2. Kh.R.Sab	98.9	*	98.1	98.5	98.1	96.3	97.8	96.0	97.0	96.7	96.3	97.8	97.4	96.7	96.7
3. Kh.N.Sh	99.2	98.1	*	99.6	100.0	98.1	98.9	97.8	97.8	97.4	97.0	98.1	98.9	98.5	98.5
4. Kh.N.Ja	98.1	97.2	98.4	*	99.6	97.8	98.5	97.4	98.1	97.8	97.4	98.5	98.5	98.1	98.1
5. Kh.N.Boj	98.8	97.9	99.6	98.3	*	98.1	98.9	97.8	97.8	97.4	97.0	98.1	98.9	98.5	98.5
6. Kh.S.Gh	97.6	96.7	97.2	96.4	97.1	*	97.0	96.3	96.0	96.3	96.0	96.3	97.0	96.7	96.7
7. Kh.S.Bir	98.3	97.2	97.7	96.6	97.3	97.8	*	96.7	97.4	97.0	96.7	97.8	98.5	98.1	98.9
8. Kh.S.Nah	97.0	96.0	96.6	95.8	96.2	97.1	96.5	*	95.6	95.2	94.9	96.0	96.7	97.0	96.3
9. Maz.Beh	98.1	97.5	97.9	97.8	97.8	97.3	97.3	96.2	*	98.1	98.5	98.5	98.9	97.8	97.8
10. Maz.Sar	97.8	97.2	97.7	97.6	97.6	97.2	97.1	96.0	99.3	*	99.6	98.1	98.5	97.4	97.4
11. Maz.Sav	97.7	97.1	97.6	97.5	97.5	97.1	97.0	95.9	99.4	99.9	*	97.8	98.1	97.0	97.0
12. Gol.Ali	97.9	97.6	97.8	97.7	97.9	97.2	97.2	96.4	99.2	98.9	98.8	*	98.9	98.1	98.1
13. AE.Bon	97.9	96.8	97.8	97.5	97.5	97.2	97.5	96.1	98.4	98.2	98.1	98.1	*	98.9	98.9
14. Ardabil	97.6	96.5	97.5	96.6	97.1	96.6	97.1	96.0	97.6	97.3	97.2	97.5	97.7	*	98.5
15. AW.Uro	97.6	96.8	97.5	96.8	97.5	96.7	97.3	95.8	97.9	97.7	97.6	98.1	98.2	97.3	*

DISCUSSION

Virus infections are a major limiting factor in cucurbit production in Iran (Moradi and Jafarpour, 2011). *Watermelon Mosaic Virus* (WMV) causes serious economic losses in Iran in many cucurbits. Cucurbitaceous crops are grown commercially throughout the world, have been destroyed completely locally or epidemically when infected by plant viruses (Yoon *et al*., 2008).

The RT-PCR technique is highly sensitive and is found to be a reliable method in comparison to biological indexing and DAS-ELISA. Using RT-PCR the virus could be detected in some plants which were found to be negative on DAS-ELISA (Sidaros *et al*., 2009). Also, PCR became widely used as a diagnostic method for infection by plant viruses belonging to several different groups such as the *geminiviruses*, *luteoviruses* and *potyvirus* groups (Al-Saleh *et al*., 2010; Sanchez *et al*., 2007).

The *CP* gene is the gene most frequently used for studies of genetic diversity in *Potyviruses* (El-Araby *et al*., 2009). Most of the genes that used to identify WMV were using the Polyprotein (P1) gene or the Coat Protein *CP*) gene (Salem *et al*., 2007) and hence, in the study, *CP* gene sequences were used for comparison and analysis.

Among different cucurbit plants, zucchini squash and pumpkin has shown the highest percentage of WMV infection and the lowest of WMV infection is related to cucumbers (data not shown). The most typical symptoms in zucchini squash were observed with systemic mosaic and varying degrees of blistering on leaves and this was in agreement with Shoeibi *et al*. (2009). In many cases the symptoms mix with other cucurbit infecting viruses (Moradi and Jafarpour, 2011).

According to the results of this study and those of Sharifi *et al*. (2008) the isolates of group 1 are the most widespread isolates of WMV and maximum molecular variation occurs in Eastern Asia (Shoeibi *et al*., 2009). The deduced coat protein consisted of 261 amino acids and also, all Iranian isolates had a DAG amino acid triplet which is required in aphid transmissibility this in agreement with Sharifi *et al*. (2008).

Data shown in this study represent the occurrence of WMV based on ELIZA, RT-PCR and *CP* gene analyses in some region of Iran. Previous studies were in the central and southern regions (Sharifi *et al*., 2008) but did not provide information about it in northwest, north, east and northeast of Iran at the molecular level.

Phylogenetic analyses were done by the Neighbor-joining (NJ) method implemented with ClustalW alignment which was used to compare the similarities between the coding nucleotides and their encoded protein. Sequencing results of a coat portion confirmed the RT-PCR analysis and Slight variations were observed between the results obtained in our study with those obtained in previous investigations. The minor differences observed in the nucleotide sequence homology between the Iranian isolates may suggest that all these isolates are closely related. In most cases, geographical isolation is consistent with the phylogenetic grouping.

Plant pathogenic viruses are responsible for increasing economic losses worldwide. There is no chemical treatment to eliminate a plant of virus infection (Youssef and Shalaby, 2009); however, chemotherapy directed to plant viral diseases has evolved significantly. So, identification and analysis of its genetic diversity of isolates and strains of WMV, presenting the appropriate solution to its control.

Determining variability within a virus group and understanding mechanism and factors affecting this variability are of considerable agronomic significance, particularly for determining resistance gene deployment strategies, since natural resistance genes can be rapidly overcome by adapted virus strains. In addition, variability of virus strain, particularly within the capsid protein, raises a problem for the development of reliable diagnosis techniques based on the antigenic properties of the coat protein (Safaeizadeh, 2008). These results make it possible to have a better understanding of the development of WMV disease in Iran.

CONCLUSION

The present study is one of several investigations regarding the significance of pathogenicity of this virus and aims at isolation, biological and molecular characterization of Iranian isolates of WMV which were isolated from cucurbit plants. In summary, the nucleotide sequences of *CP* gene of 15 WMV isolates was determined and demonstrated their identity and phylogenetic relationships to the other isolates of WMV recorded in NCBI. The identity of WMV *CP* nucleotide sequences of the 15 Iranian isolates ranged from 95.8 to 99.2%. The results obtained from this study will help managing this disease in cucurbit crops and probably in other crops that were reported to be infected with it, as well.

ACKNOWLEDGMENTS

The author wish to thank plant pathology laboratory manager at the Ferdowsi University of Mashhad. Also, the author is grateful to Dr. L. Prevotat for the WMV antiserum and the help of plant virologist, Dr. M. Mehrvar and those who help in technical works.

REFERENCES

Al-Saleh, M.A., I.M. Al-Shahwan, M.A. Amer and O.A. Abdalla, 2009. Etiology of a mosaic disease of radish and lettuce and sequencing of the coat protein gene of the causal agent in Saudi Arabia. Int. J. Virol., 5: 131-142.

Al-Saleh, M.A., I.M. Al-Shahwan, O.A. Abdalla and M.A. Amer, 2010. Identification and coat protein nucleotide sequence of turnip mosaic *Potyvirus* from *Eruca sativa* in Saudi Arabia. Asian J. Plant Pathol., 4: 116-127.

Ali, A., T. Natsuaki and S. Okuda, 2006. The complete nucleotide sequence of a Pakistani isolate of *Watermelon mosaic virus* provides further insights into the taxonomic status in the *Bean common mosaic virus* subgroup. Virus Genes, 32: 307-311.

Clark, M.F. and A.N. Adams, 1977. Characteristics of the microplate method of enzyme-Linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol., 34: 475-483.

Desbiez, C. and H. Lecoq, 2004. The nucleotide sequence of *Watermelon mosaic virus* (WMV, *Potyvirus*) reveals interspecific recombination between two related *Potyvirus*es in the 5' part of the genome. Arch. Virol., 149: 1619-1632.

Desbiez, C., C. Costa, C. Wipf-Scheibel, M. Girard and H. Lecoq, 2007. Serological and molecular variability of *Watermelon mosaic virus* (genus *Potyvirus*). Arch. Virol., 152: 775-781.

El-Araby, W.S., I.A. Ibrahim, A.A. Hemeida, A. Mahmoud, A.M. Soliman, A.K. El-Attar and H.M. Mazyad, 2009. Biological, serological and molecular diagnosis of three major potato viruses in Egypt. Int. J. Virol., 5: 77-88.

Gara, I.W., H. Kondo, T. Maeda, N. Inouye and T. Tamada, 1997. Stunt disease of *Habenaria radiata* caused by a strain of *Watermelon mosaic virus*. Ann. Phytopathol. Soc. Japan, 63: 113-117.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Massumi, H., A. Samei, A.H. Pour, M. Shaabanian and H. Rahimian, 2007. Occurrence, distribution and relative incidence of seven viruses infecting greenhouse-grown cucurbits in Iran. Plant Dis., 91: 159-163.

Moradi, Z. and B. Jafarpour, 2011. First report of coat protein sequence of Cucumber green mottle virus in cucumber isolated from Khorasan in Iran. Int. J. Virol., 7: 1-12.

Purcifull, D., E. Hiebert and J.R. Edwardson, 1984. *Watermelon mosaic virus* 2. CMI/AAB Descr. Pl. Viruses No. 293, pp: 7.

Riechmann, J.L., S. Lain and J.A. Garcia, 1992. Highlights and prospects of *Potyvirus* molecular biology. J. Gen. Virol., 73: 1-16.

Safaeizadeh, M., 2008. Comparative biological and molecular variability of Zucchini yellow mosaic virus in Iran. Asian J. Plant Pathol., 2: 30-39.

Salem, T.Z., S.M. El-Gamal and A.S. Sadik, 2007. Use of helper component proteinase gene to identify a new Egyptian isolate of *Watermelon mosaic Potyvirus*. Int. J. Virol., 3: 107-116.

Sanchez, F., M. Rodriguez-Mateos, A. Tourino, J. Fresno and C. Gomez-Campo *et al*., 2007. Identification of new isolates of Turnip mosaic virus that cluster with less common viral strains. Arch. Virol., 152: 1061-1068.

Sharifi, M., H. Massumi, J. Heydarnejad, A.H. Pour, M. Shaabanian and H. Rahimian, 2008. Analysis of the biological and molecular variability of *Watermelon mosaic virus* isolates from Iran. Virus Genes, 37: 304-313.

Shoeibi, S., M. Masumi, S. Nasrollahnezhad, S. Heydari, K. Izadpanah and A. Ahmadikhah, 2009. Sequencing of six Iranian isolates of *Watermelon mosaic virus* and phylogenetic comparison of Iranian isolates with other isolates of the world. J. Plant Pathol., 45: 34-37.

Shukla, D.D., C.W. Ward and A.A. Brunt, 1994. The Potyviridae. 1st Edn., CAB International, Wallingford.

Sidaros, S.A., S.A. El-Kewey, H.A. Amin, E.A.H. Khatab, A.A. Emeran, S.A. El-Khalik and M.A.S. El-Kady, 2009. Cloning and sequencing of a cDNA encoding the coat protein of an Egyptian isolate of pepper mild mottle virus. Int. J. Virol., 5: 109-118.

Wang, Y.Y., D.L. Beck, R.C. Gardner and M.N. Pearson, 1993. Nucleotide sequence, serology and symptomatology suggest that vanilla necrosis *Potyvirus* is a strain of *Watermelon mosaic virus* II. Arch. Virol., 129: 93-103.

Webb, R.E. and H.A. Scott, 1965. Isolation and identification of *Watermelon mosaic virus* 1 and 2. Phytopathology, 55: 895-900.

Wu, Y.F., J.X. Zhang, R. Wang, Z.P. Lue, X.M. Wang and D. Yang, 2006. Complete genome of *Watermelon mosaic virus* strain WMV-CHN. Accession No. DQ399708. http://www.ncbi.nlm.nih.gov/nuccore/88954063.

Yoon, J.Y., G.S. Choi, S.K. Choi, J.S. Hong and J.K. Choi *et al.*, 2008. Molecular and biological diversities of Cucumber green mottle mosaic virus from cucurbitaceous crops in Korea. J. Phytopathol., 156: 408-412.

Youssef, S.A. and A.A. Shalaby, 2009. Single-step multiplex reverse transcription-polymerase chain reaction (m-RT-PCR) for simultaneous detection of five RNA viruses affecting stone fruit trees. Int. J. Virol., 5: 100-108.