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GENETIC DIVERSITY AND BIOLOGICAL CHARACTERIZATION OF *WATERMELON MOSAIC VIRUS* ISOLATES FROM IRAN

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ABSTRACT

During 2017-2018 growing season, some fields of cucurbits in Iran (North and northeast) were surveyed for the presence of *Watermelon mosaic virus* (WMV) and other common cucurbit viruses. RNA extractions from symptomatic leaf samples were tested by RT-PCR using two degenerate primer pairs (CIF/Rev and NIb2F/3R) targeting the two separated partial regions of the Potyvirus genome (CI and NIb respectively); only 5 out of 10 samples were confirmed to be infected with WMV. To verify molecular variability, 5 WMV isolates were cloned and then sequenced. Comparison of the partial CI and NIb genes sequences showed 94.55-99.14% nucleotide identity among isolates, whereas the amino acid identity was 98.71- 100% respectively, suggesting selection for amino acid conservation. Phylogenetic tree based on CI revealed the separation of WMV isolates into two major divergent evolutionary lineages. Phylogenetic analyses grouped Iranian WMV isolates together with isolates from France, Italy, Spain, Turkey, South Korea, and the US in group I. Analysis of the NIb partial sequence showed nucleotide and amino acid identity from 94.83 to 100% and 97.25 to 100% respectively. Phylogenetic analysis based on NIb placed all the studied isolates in one group where most of the world WMV isolates were exist. Biological results showed Iranian WMV isolates infect *C. melo*, *C. lanatus*, *C. moschata* and *C. sativus* causing mild mosaic symptoms on the leaves. Whereas, *C. pepo* plant demonstrated mild to severe mosaic and malformation symptoms on the leaves.
Keywords **:** Genetic diversity. Iran. *Watermelon mosaic virus*. Cucurbits

Introduction

Approximately 35 viral diseases are associated with cucurbits in which at least 10 belong to the genus *Potyvirus* (*Potyviridae*) (Desbiez *et al.,* 2009). One of the most important and destructive potyviruses in cucurbits is *Watermelon mosaic virus* (WMV) (Bananej and Vahdat 2008; Joannon *et al.,* 2010; Massumi *et al.,* 2007). With a broad-spectrum of host range which measured to 170 plant species belong to 27 botanical families (Shukla *et al.,* 1994), WMV has been associated with severe symptoms and important yield losses in several horticultural crops particularly legumes and cucurbits, reached to 49% of *Cucurbita pepo* yield reduction (Delmiglio and Pearson 2006; Moradi 2011; Shoeybi *et al.,* 2009). In nature, WMV is transmitted by aphids in a non-persistent manner to most cucurbit-growing areas, and consisting various symptoms according to the host cultivar and the virus isolate, including mosaic, leaf deformation, vein banding and stunting (Hiebert *et al.,* 1984; Hull 2009). WMV genome is about 10035 nt in length with 760 nm long (Glasa *et al.,* 2011). Comprises flexuous, single positive sense ss RNA molecule, encoding a single large polyprotein precursor, which is proteolytically processed by virus-encoded proteinases to yield as many as 10 functional proteins (Yakoubi *et al.,* 2008). WMV was first reported by Webb and Scott in 1965 (Desbiez and Lecoq 2004). In 1974, WMV was reported for the first time from Iran (Ebrahim-Nesbat 1974). Previous researches showed genetic variation among different isolates of WMV, in which the recombination events are possibly one of the causes. Characterization of the genetic variation, tracing of populations, and geographical distribution of the virus provides a piece of important information on virus evolution and epidemiology, which is crucial to design effective methods for control of viral plant diseases (Rubio *et al.,* 2013). RT-PCR techniques are effective analytical tools to detect and identify plant viruses. The current study was aimed to investigate the partial spreading, genetic diversity, and phylogenetic analysis of 5 different isolates of WMV from Iran, and comparing them with other WMV isolates available in the GenBank. Two degenerate primers pairs which designed within the conserved amino acids of partial nuclear inclusion protein b (NIb) coding region and part of the cytoplasmic inclusion protein gene (CI) were applied (Zheng *et al.,* 2010; Ghasemzade *et al.,* 2012; Ha *et al.,* 2008). Moreover, to have a clear view of the biological properties, the partial host range of the virus was also investigated.

Material and Methods

Sample collection

During 2017-2018 growing seasons, a survey was carried out in cucurbit growing areas of four Iranian counties (Mazandaran, Meyami, Torbat Heydariyeh, and Jimabad) (Table 1). 12 leaf samples with a high incidence of viral symptoms were collected from fields, under the cultivation of cucurbits.

RT-PCR and sequencing

Total RNA was extracted from the samples using RNeasy Mini Kit (Qiagen, Germany) and used as a template for reverse transcription test. RT-PCR analysis was performed by using degenerate primer pairs of CI (CIF/Rev) (Ha et al., 2008) which amplify 700 bp fragments in CI gene and NIb primer pairs (NIb2F/3R) (Zheng et al., 2010) which amplify 350 bp fragments in NIb gene. Synthesis of the complementary DNA (cDNA) was performed at 65°C for 10 min from 3 µl of total RNA, 1µl of dNTPs mix (10mm), 5µl of RNases free distilled water and 1 µl (CIRev) for CI and (NIb3R) NIb respectively. Then 5.5µl of RNases free distilled water, 4µl of 5x M-MLV RT buffer and 0.5µl M-MLV (200 U µl) reverse transcriptase (Takara, Japan) were added to the mixture. The RT reactions were incubated at 30° for 10 min followed by 42°C for 60 min and terminating with 70° for 15 min. The cDNA was amplified by polymerase chain reaction (PCR) using primer pairs (CIF/R) for CI and (NIb2F/4R) NIb respectively. PCR was performed by a thermal profile with an initial denaturing at 94 °C for 4 min, followed by 35 cycles of 94 °C for 30 sec, 50 °C for 30 sec and 72 °C for 90 sec, terminating with 10 min at 72 °C for CI region and 94°C for 2 mins, followed by 35 cycles of 94°C for 45 sec, 45°C for 45 sec, 72°C for 45 sec, and a final polymerization at 72°C for 5 mints for NIb gene. Furthermore, three specific primer pairs were used to possibly detect other common cucurbits viruses (Cucumber mosaic virus, CMV; Green mottle mosaic virus, GMMV and Squash mosaic virus, SqMV) for mix infection. The PCR products were separated by electrophoresis in 1% agarose gel and purified from the gel using the Qiaquick gel extraction kit (Qiagen, Germany). The purified PCR products were ligated into the pTG19-T vector (Vivantis, Malaysia), according to the manufacturer's protocol. The recombinant plasmid vectors were transformed into E. coli strain DH5α, and the positive clones were screened by colony-PCR with M13 and specific primers pairs. The plasmid was purified from recombinant clones by use of plasmid miniprep kit (Qiagen, Germany), two separated clones for each amplicon were sequenced bi-directionally (Macrogen Inc., South Korea). The sequences' results were verified using the BLAST program in the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Sequence and phylogenetic analysis

To perform the phylogenetic relationships among experimental WMV isolates, 10 partial sequences (5 CI and 5 NIb) were deposited into the GenBank and compared separately with the corresponding sequence of (45) WMV GenBank isolates (Table S1) in which *Soybean mosaic virus* (SMV) was used as an outgroup. Clustal Omega (https://www.ebi.ac.uk) was used for percent identity. Multiple sequence alignment was done using BioEdit (version7.2.5) software (http://www.mbio.ncsu.edu/ BioEdit/ bioedit.html). Phylogenetic trees were constructed by the maximum-likelihood (ML) method implemented in MEGA6 (Tamura *et al*., 2013), using 1000 bootstrap replicates in Kimura 2-parameter model. The pairwise nucleotide (nt) sequence identity scores were displayed as color-coded cells using SDT v.1.2 software (Muhire *et al*., 2014).

Biological characteristics

Seeds of zucchini (*Cucurbita pepo*), pumpkin (*Cucurbita moschata*), cucumber (*Cucumis sativus*), melon (*Cucumis melo*), watermelon (*Citrullus lanatus*) and *Chenopodium amaranticolor* plants were sown in mix soil and peatmoss (2:1) in pots (20 cm diameter). The pots were placed in an insect-proof of FUM virology glasshouse at 24-30˚C. The pots were watered daily and fertilized weekly with NPK fertilizer. Based on molecular results, samples infected with only WMV were inoculated to the experimental host plants. Infected leaves tissue were homogenized in 50 mM potassium phosphate buffer (pH 7.0), and filtered through a double layer of muslin, which used as virus inoculum. Leaves of experimental host plants (three-four leaf stage) were dusted with (600 mesh) carborundum and gently rubbed on the upper surface with a cotton swab (Hull 2009), then rinsed off. The inoculated plants were maintained in the greenhouse and monitored for symptoms development. Additionally, two negative control (non-inoculated) seedling, of each experimental plant were kept in the greenhouse, as an indicator of symptom expression.

Results

The results of RT-PCR amplification with two degenerate primers pairs (CI, NIb) were confirmed the infection of 5 (50%) out of 10 samples with WMV which were deposited in the GenBank database under the accession numbers of MN464243- MN464256 (Table 1). The infection rate with other viruses was 30% and 20% for ZYMV and CMV respectively. Neither *squash mosaic virus* nor *green mottle mosaic virus* was detected in surveyed samples.

Sequence comparisons

The sequence identity of partial CI gene among the five Iranian (WMV1, WMV2, WMV3, WMV4, and WMV5) isolates were ranged from 94.55% between [(WMV2 and WMV4) and (WMV3 and WMV4)] to 99.14% (WMV2 and WMV3) at nt level, where it was from 98.71 (WMV2 and WMV4) to 100% (WMV1 and WMV5) at aa level respectively. These results showed that the high variability is existing in the partial sequence of the CI gene at the nt level, nevertheless, most of the mutations are silent, which is indicating of a high selection for aa conservation. By comparing the studied isolates with other sources of WMV from the GenBank, the results showed, the highest (99.71%) nt identity for partial CI gene existed between WMV2 and a French isolate (JF273460). Whereas, the lowest nt (89.48%) identity was found between WMV4 and another French isolate (JF273467). Isolates from France JF273460 and Spain (MH469650 and MH469651) demonstrated the highest level (100%) of identity at aa level with WMV1, WMV3, and WMV5 isolate. While the lowest level of aa identity (96.81%) was observed between WMV5 and KT992077 isolates from South Korea (Table 2).

The pairwise sequence identity of the partial NIb gene, exhibited more identity comparing with corresponding data from CI gene, which showed an identity ranged from 94.83 (WMV3 and WMV4) to 99.70% (WMV2 and WMV3) and from 98.17 [(WMV1 and WMV2) and (WMV1 and WMV3)] to 100% [(WMV2 and WMV3) and (WMV1, WMV4, and WMV5)] at nt and aa levels respectively. Finally, Cg09-640 isolate (JF273467) from France showed 100% identity with (WMV4) at nt level, whereas the lowest nt identity (92.02%) was found between WMV2 and an isolate from Venezuela (KC292915). In comparison with the nt level, most experimental isolates exhibited a high level of aa identity with French isolates ranged from 99 to 100%

while the lowest identity (96.33%) was observed between WMV3 and Hongseong1_2013 isolate (KT992075) from South Korea (Table 2).

Phylogenetic analysis

Phylogenetic analyses, based on partial CI gene with *soybean mosaic virus* (SMV) isolate (AJ507388) as an outgroup showed the clustering of the 50 WMV isolates (5 from this study and 45 from the GenBank) into two varying evolutionary groups; nominated I and II (Fig. 1 and Table S1). Isolates of each group were further divided into two subgroups. All partial CI gene of studied isolates were located in group I. However, two (WMV2, WMV3) isolates were clustered in the subgroup (A), where the majority isolates of this group were from Europe and the others from South Korea, US, and one isolate from Argentina. Whereas, the other three isolates (ZYMV1, ZYMV4, and WMV5) were clustered in the subgroup (B), where it was contained isolates from France, South Korea, Iran, Pakistan, Turkey, and Italy. Isolates belonging to a subgroup (A) shared nt and aa identity from 93.83 to 100% and from 98.28-100% respectively, while subgroup (B) demonstrated identity ranged from 95.12 to 100% (nt), and from 98.28-100% (aa). Whereas, isolates belonging to group I shared nt and aa identity from 90.96 to 100% and from 98.28 -100% respectively, while group II demonstrated identity ranged from 91.34 to 99.86% (nt), and from 96.09 -100% (aa). The genetic distance of the subgroup (A) nt sequence was 0.038, and subgroup (B) it was 0.028, where the genetic distance within the group I was 0.046, and within-group II it was 0.067. However, the genetic distance between the two groups was 0.095, whereas it was 0.039 between 5 surveyed isolates, and the overall mean distance value was 0.061. The result of synonymous (dS) and nonsynonymous (dN) ratio (ω) for the 5 isolates was 0.285 (dN/dS<1), suggesting most nt mutations were silent, encoding similar amino acids. Which shows that this gene is under purifying selection.

Phylogenetic analyses of the 5 partial sequences of NIb gene (Fig 2) plus 45 of their counterparts from the world were clustered all of the studied isolates in one group (I) (Table 2), which was mostly consisted of isolates from Europe, South Korea, Turkey, Iran, and Pakistan respectively, sharing identity ranged from 92.71-100% and from 97.25-100% at nt and aa level respectively. In contrast, the second part of the phylogenetic tree (group II), mainly consisted of GenBank isolates from the USA, Chile, Venezuela, and Iran, and shared nt and aa identity from 98.18- 100% and from 99.08-100% respectively. In comparison with corresponding data of the CI gene, the NIb genetic distance of group I was 0.038, whereas for group II it was 0.010. The between (I, II) group genetic distances was 0.069, whereas the genetic distance between Iranian isolates was 0.032, and the ratio (ω) of dN/dS was 0.316. Which showed that this gene is under purifying selection.

Bioassay and host range test

The reaction between experimental host plants and the 5 studied isolates showed varying degrees of symptom intensity according to virus isolate and plant species. Early systemic symptoms appeared from most of the isolates 14 days post-inoculation (dpi) on zucchini plants, which was consisted of varying degrees of mosaic, blistering, vein clearing, and leaf deformation (Table 3). While no symptoms were observed on melon, watermelon, cucumber, and pumpkin until 21 dpi, and ranged from mild mosaic to asymptomatic. *Chenopodium amaranticolor* plant showed only a chlorotic local lesion with all studied isolates (Fig. 3).

Discussion

Cucurbit crops are commercially grown throughout the world, mainly in the tropical and subtropical regions (Ajuru and Nmom 2017), Iran's ranking is the second country in the world based on cucurbit's yield (Bananej and Vahdat 2008). Watermelon mosaic virus is one of the most common and destructive potyviruses which infect cucurbits worldwide (Desbiez *et al*., 2007; Lecoq and Desbiez 2008), In Iran, it's one of the major viruses of cucurbit plants (Massumi *et al*., 2007). The results of this survey showed that among all (10) collected viral spaceman, the frequency of WMV was (50%), followed by ZYMV (30%) and CMV (20%), whereas no SqMV and CGMMV have been detected in this study. Phylogenetic analysis based on partial CI gene, clustered all studied isolates in group I, with low genetic diversity among them, which is in agreement with previous studies (Sharifi *et al*., 2008). The identity between the two WMV4 and WMV5 isolates from Mazandaran province (North of Iran) was 97.42% whereas it was 99.14% between two isolates from Jimabad (WMV2) and Torbat Heydariyeh (WMV3) (Khorasan Razavi, Northeast of Iran), however the identity between two Iranian provinces (Mazandaran and Khorasan Razavi) (WMV2 and WMV4) isolates was 94.55%, representing an adaptation for geographical location. Despite, all of the studied isolates showed a low level of diversity in the partial NIb gene. However, the topology of the two phylogenetic trees (CI and NIb), showed the separation of the studied isolates as well as the previously reported isolates of WMV from Iran in different subgroups, with relatively low genetic connectivity among each other. Moreover, the highest and lowest identity between studied isolates and GenBank isolates (table 2), showed that the two genes (CI, NIb) of each isolate were more similar to a specific and geographically scattered mosaic of worldwide isolates, which suggesting mixed infections might have occurred between different worldwide isolates in studied areas, which leads to the emergence of severe isolates resulted obviously in the appearance of the variable symptoms in the experimental plants (table 2). Probably the human activities including international trade-offs in plant products and the lack of appropriate quarantine measures were the main reason for observing this high level of diversity. Biological properties such as host range have been used for the differentiation of strains of viruses (Xiao *et al.,* 1993). In the second part of this work, we tried to determine the susceptibility of five different cucurbit host plants to WMV studied isolates in a partial biological assay. According to (Desbiez *et al.,* 2007) there were three groups of WMV isolates based on the NIb-CP motif. Isolates belong to group I usually called (CL isolates) shared KEA motif at N terminal of CP, whereas group two isolates which arose from various part of the world had KET motif, and the third group isolates which were (EM isolates) associated with severe symptoms and had KEKET motif at the CP found in the southeast of France. Inoculated *C. amaranticolor* plants which were inoculated by all studied isolates have just shown local lesion symptoms. The reaction between *C. melo*, *C. lanatus*, *C. moschata*, and *C. sativus* plants with WMV isolates displayed mild to asymptomatic symptoms. Diversity in observed reactions between the host plant and the virus may be somehow due to susceptibility or

resistance of host plants used (Romay *et al.,* 2014), reversibly the different 5 isolates showed mild mosaic to severe leaf deformation with inoculated *C. pepo*, indicating that some studied isolates may belong to group three of the aforementioned category (Desbiez *et al.,* 2007), particularly as some of these isolates showed high identity to French isolates in the phylogenetic tree. Furthermore, zucchini plants showed a high level of susceptibility in comparison with other test plants, which is in agreement with the finding of other researchers (Lecoq *et al.,* 2011; Kamberoglu *et al.,* 2015; Finetti-Sialer *et al.,* 2012). However, more research is needed in order to better understand the reaction between host plant species and the WMV virus.

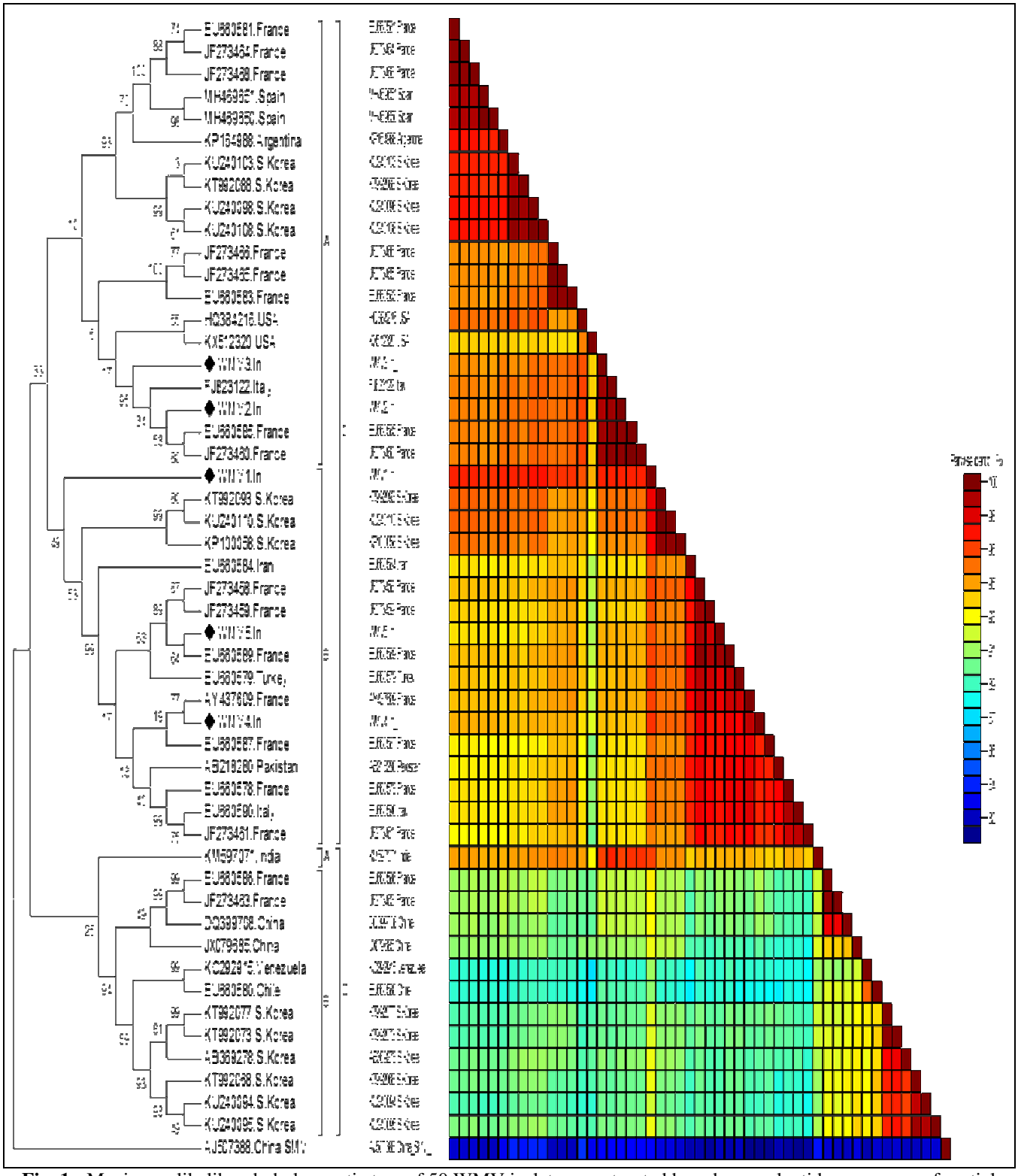

Fig. 1 : Maximum-likelihood phylogenetic tree of 50 WMV isolates constructed based on nucleotide sequences of partial cylindrical inclusion (CI) gene, the studied isolates was marked with ♦. By using MEGA6 software and bootstrapped with 1000 replicates. Bootstrap values ≥ 50% are shown at the branch internodes. Two dimensional nucleotide diversity plots constructed based on SDT MUSCLE alignment.

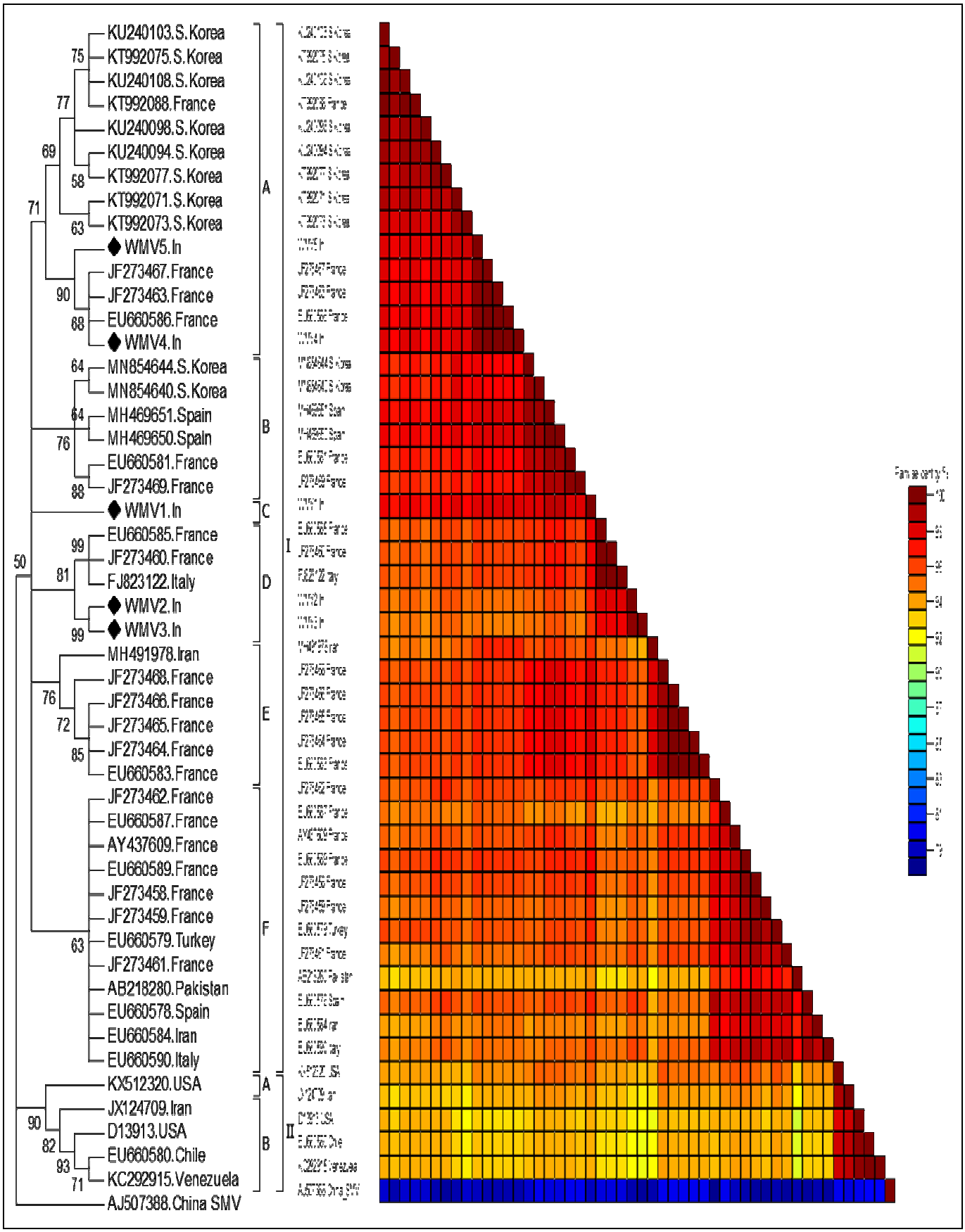

Fig. 2 : Maximum-likelihood phylogenetic tree of 50 WMV isolates constructed based on partial nuclear inclusion b (NIb) gene, the studied isolates was marked with ♦. By using MEGA6 software and bootstrapped with 1000 replicates. Bootstrap values ≥ 50% are shown at the branch internodes. Two dimensional nucleotide diversity plots constructed based on SDT MUSCLE alignment.

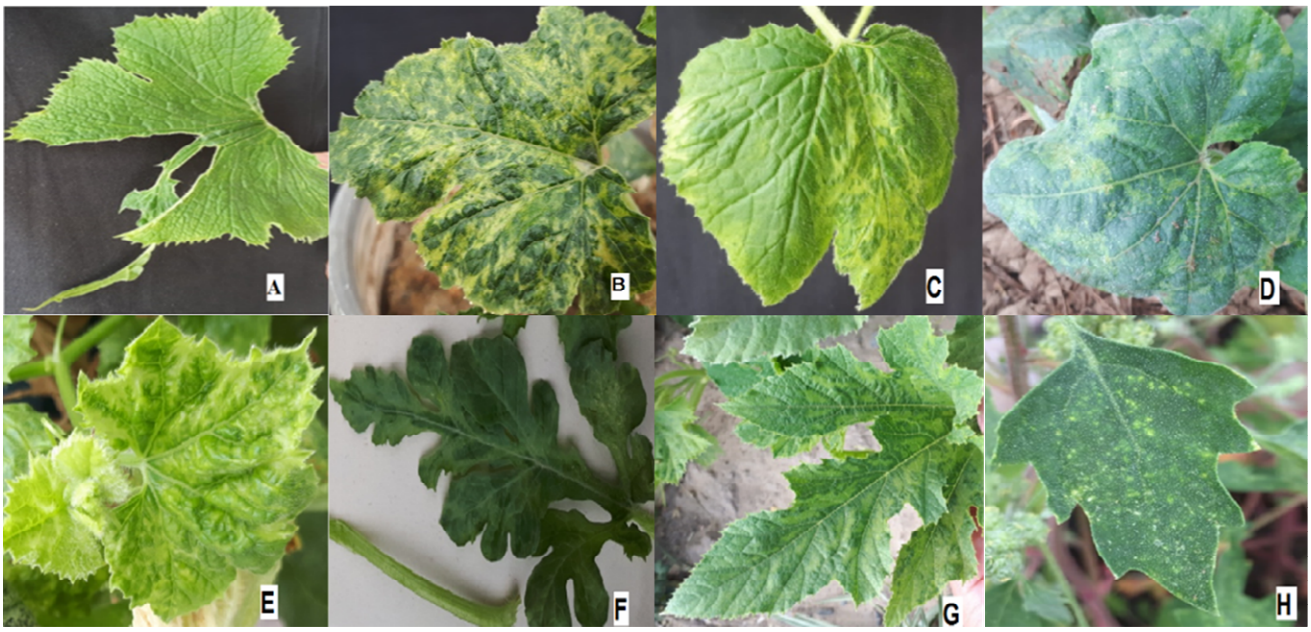

Fig. 3 : Symptoms of different experimental host plants inoculated with Iranian WMV isolates. A (WMV1), B (WMV4) and C: (WMV3) leaf deformation, blistering and mosaic on *Cucurbita pepo*. D: (WMV2) Mild mosaic on *Cucumis sativus*. E: (WMV5) Mild mosaic on *Cucmis melo*. F: (WMV2) Mild mosaic on *Citrullus lanatus*. G: (WMV1) Mild mosaic on *Cucurbita moschata*. H: Local lesion on *Chenopodium amaranticolor*.

Table 1 : Sources and the accession number of the five Iranian WMV isolates reported in this study.

Isolate	CI Accession number	NIb Accession number	city	source
WMV1	MN464243	MN464252	Meyami	melon
WMV2	MN464244	MN464253	Jimabad	melon
WMV3	MN464245	MN464254	Torbat Heydariyeh	cucumber
WMV4	MN464246	MN464255	Mazandaran-Sari	pumpkin
WMV5	MN464247	MN464256	Mazandaran-Kia Pey	watermelon

Table 2: Percent nucleotide/amino acid sequence identity of the CI and NIb partial genes of studied isolates and coresponding sequences from the GenBank.

Studied Isolate	**GenBank isolate nt/aa % identity (CI)**		**GenBank isolate nt/aa % identity (NIb)**	
WMV1	98.1% KU240110 100% MH469651	91.0% KC292915 97.8% JX079685	98.7% MH469650 100% JF273465	93.9% KC292915 98.1% JX124709
WMV2	99.7% JF273460 99.5% JF273460	90.0% KC292915 97.0% EU660580	97.8% FJ823122 98.1% JF273465	92.0% KC292915 96.4% JX124709
WMV3	99.4% JF273460 100% JF273460	90.0% KC292915 97.4% JX079685	97.5% FJ823122 98.1% MH469650	92.1% KC292915 96.3% KT992093
WMV4	99.0% AY437609 99.5% MH469650	89.4% JF273467 99.5% DQ399708	100% JF273467 100% KU240103	93.3% JX124709 98.1% KT992093
WMV5	99.6% EU660589 100% MH469650	89.8% EU660580 96.8% KT992077	99.7% JF273467 100% JF273466	93.3% EU660580 98.1% JX124709

Table 3: Type of the reaction between experimental host plants and 5 WMV isolates from this study.

Isolate	***Cucurbita pepo***	***Cucumis sativus***	***Cucmis melo***	***Citrullus lanatus***	***Cucurbita moschata***	***Chenopodium amaranticolor***
WMV1	leaf deformation	mild mosaic	mild mosaic	mild mosaic	mild mosaic	local lesion
WMV2	mild mosaic	mild mosaic	mild mosaic	mild mosaic	asymptomatic	local lesion
WMV3	mild mosaic	asymptomatic	mild mosaic	mild mosaic	asymptomatic	local lesion
WMV4	blistering and mosaic	asymptomatic	asymptomatic	mild mosaic	asymptomatic	local lesion
WMV5	blistering and mosaic	asymptomatic	mild mosaic	mild mosaic	asymptomatic	local lesion

Table 4 : List of the Watermelon mosaic virus isolates restored from GenBank for CI and NIb phylogenetic analyses, with their Sources and accession numbers.

Isolate	Source	Accession number	Genomic region
FMFOO-LL1	France	EU660581	CI, NIb
C07-014	France	JF273464	CI, NIb
CO7-284	France	JF273468	CI, NIb
FMF00-LL2	France	EU660578	CI, NIb
A08-170	France	JF273466	CI, NIb
A08-160	France	JF273465	CI, NIb
FMF03-141	France	EU660583	CI, NIb
C05-465	France	JF273460	CI, NIb
C05-270	France	EU660585	CI, NIb
C05-463	France	JF273458	CI, NIb
C05-464	France	JF273459	CI, NIb
C05-337	France	EU660589	CI, NIb
WMV-Fr	France	AY437609	CI, NIb
C06-188	France	EU660587	CI, NIb
C07-349	France	JF273461	CI, NIb
FBR04-37	France	EU660586	CI, NIb
C06-257	France	JF273463	CI, NIb
Vera	Spain	MH469650	CI, NIb
Vera-crtB	Spain	MH469651	CI, NIb
Yeongju-7-2,2013	South Korea	KT992088	CI, NIb
Sangju2_2012	South Korea	KU240103	CI, NIb
Jeongeup1-1_2012	South Korea	KU240098	CI, NIb
Yeongju5_2012	South Korea	KU240108	CI, NIb
Yeongyang8-1_2013	South Korea	KT992093	CI
Yeongyang3_2012	South Korea	KU240110	CI
Naju2-2_2013	South Korea	KT992077	CI, NIb
19-FEB-2009	South Korea	AB369278	CI, NIb
Bonghwa7-2_2014	South Korea	KT992068	CI
Buan2-1_2012	South Korea	KU240094	CI, NIb
Gimcheon1_2012	South Korea	KU240095	CI
Eumseong15_2014	South Korea	KT992073	CI, NIb
Dendrobium	USA	HQ384216	CI
passiflora	USA	KX512320	CI, NIb
Lecce	Italy	FJ823122	CI, NIb
ITA00-G	Italy	EU660590	CI, NIb
IR02-54	Iran	EU660584	CI, NIb
TURK91	Turkey	EU660579	CI, NIb
WMV-CHN	China	DQ399708	CI
WMV-ShanXi	China	JX079685	CI
CHI87-620	Chile	EU660580	CI, NIb
VE10-099	Venezuela	KC292915	CI, NIb
Cheongsong5_2013	South Korea	KT992071	NIb
Hongseong1_2013	South Korea	KT992075	NIb
SDE FF	Argentina	KP164988	CI
Cg09-640	France	JF273467	NIb
C04-106	France	JF273469	NIb
Ir-Na	Iran	MH491978	NIb
DJ-sq3	South Korea	MN854640	NIb
HS-me4	South Korea	MN854644	NIb
C06-526	France	JF273462	NIb
Znl-sq167	Iran	JX124709	NIb
Khl-ct180	Iran	JX124711	NIb
isolate USA	USA	D13913	NIb
SangJu6-1	South Korea	KP100058	CI
RKG2	India	KM597071	CI
WMV-Pk	Pakistan	AB218280	CI

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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