تنوع ژنتیکی و تجزیه و تحلیل فیلوژنتیکی جدایه‌های ویروس موزاییک ایرانی ذرت براساس پراکنش جغرافیایی، میزبان و نوع علائم

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چکیده

ویروس موزاییک ایرانی ذرت یک گونه منحصر به فرد در جنس نوکلئورابدوویروس می باشد. تنوع ژنتیکی جدایه های مختلف ویروس موزاییک ایرانی ذرت جمع آوری شده از مناطق جغرافیایی مختلف ایران و از میزبان های مختلف ویروس با علایم متفاوت در سال های ۱۳۹٤ و ۱۳۹۵ مورد بررسی و مطالعه قرار گرفت. توالی مربوط به ژن های نوکلئوکپسید، گلیکوپروتیین و یک قطعه با اندازه ۵۰۰ جفت باز از ژن پلی مراز و نواحی غیر کد شونده بین ژن ها تعیین شد. تنوع ژنتیکی در نواحی ژن های نوکلئوکپسید و گلیکوپروتیین به میزان ۱/۳ در صد و درژن پلی مراز ۰/۸ درصد بود. تنوع ژنتیکی در نواحی غیرکد کننده بیشتر از نواحی ژنی بودکه در ناحیه بین ژن فسفوپروتیین و ژن ۳ به بیش از ۱٤٪ می رسید. در تجزیه و تحلیل های فیلوژنتیکی جدایه ها، ارتباطی بین گروه بندی فیلوژنتیکی و منطقه جغرافیایی، دامنه میزبانی و نوع علایم ویروسی دیده نشد. نسبت جانشینی های نامترادف به مترادف کمتر از یک بود که بیانگر وجود فشار انتخاب منفی بر روی ژن های مورد مطالعه بود. با توجه به این که ذرت یک گیاه وارداتی به ایران است و کشت آن در سال های اخیر توسعه یافته است به نظر می رسد ویروس موزاییک ایرانی ذرت از علف های هرز و یا میزبان های زراعی به ذرت منتقل شده است. احتمالا وابستگی شدید ویروس موزاییک ایرانی ذرت به زنجرک ناقل به عنوان تنها راه انتقال شناخته شده برای ویروس، می تواند دلیل پایین بودن تنوع ژنتیکی ویروس باشد. تنوع ژنتیکی پایین در ویروس موزاییک ایرانی ذرت، می تواند به عنوان ابزاری مفید، جهت تولید واریته هایی با مقاومت پایدار استفاده شود.

کلیدواژه: تنوع ژنتیکی، گلیکوپروتئین، ویروس موزاییک ایرانی ذرت، نواحی غیرکدشونده، نوکلئوپروتئین، نوکلئورابدوویروس، فشار انتخاب

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Genetic diversity and phylogenetic analysis of Maize Iranian mosaic virus isolates based on geographical distribution, host and type of symptoms

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Abstract

Maize Iranian mosaic virus (MIMV) is a distinct member of the genus *Nucleorhabdovirus*. The genetic diversity of maize Iranian mosaic virus (MIMV) isolates collected from different geographical regions of Iran, different hosts and different symptoms was determined during 2015-2016. Selection pressure analyses were also performed. Sequences of the nucleocapsid protein, glycoprotein, and a 500 bp fragment of polymerase genes and non-coding regions were determined. Genetic diversity in coding regions was less than 1.3 % in nucleocapsid protein and glycoprotein genes and 1 % in polymerase gene. The diversity in non-coding sequences was higher compared to ORFs especially between P and ORF 3 (up to 14.7 %). In phylogenetic analysis no correlation was found between MIMV isolates and geographical regions, type of symptoms and host differences. The ratio of non-synonymous to synonymous polymorphic sites indicated purifying selection has acted upon the analyzed genes. Likely, the dependence of MIMV on planthoppers for spreading can explain its low diversity. The information on the low genetic diversity of MIMV will be helpful in selection of maize cultivars with durable resistance.

Keywords: Genetic diversity, glycoprotein, maize Iranian mosaic virus, non-coding regions, nucleocapsid protein, *Nucleorhabdovirus*, selection pressure

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Introduction

Maize Iranian mosaic virus (MIMV), infects maize, barley, wheat, rice and some gramineous weeds in Iran (Izadpanah et al. 1983b; Izadpanah and Parvin 1979). Based on serological, physicochemical, biological and molecular properties, this virus assigned as the species <*Maize Iranian mosaic virus*> which belongs to the genus *Nucleorhabdovirus* (Izadpanah 1989; Izadpanah et al. 1983b; Massah et al. 2008; Dietzgen et al. 2011). The most common viruses infecting maize in Iran are MIMV, maize rough dwarf virus, Iranian Johnson grass mosaic virus and maize dwarf mosaic virus (Afsharifar and Izadpanah 1994; Moini and Izadpanah 2001; Izadpanah et al. 1983a). The obvious symptoms of MRDV are fine enations on leaves and severe stunting, While MIMV produces chlorotic and necrotic streaks and stripes along the veins in leaves and sheaths. Other aforementioned viruses induce common mosaic on leaves (Afsharifar and Izadpanah 1994; Izadpanah and Parvin 1979; Izadpanah et al. 1983a). The symptoms of MIMV were observed in different severities on the same maize cultivar (personal observation).

MIMV is transmitted by planthoppers *Laodelphax striatellus* and *Unkanodes tanasijevici* in a persistent propagative manner (Izadpanah et al. 1983b). The MIMV genome has been completely sequenced (Massah et al. 2008; Ghorbani et al. 2017). It encodes six genes similar to other rhabdoviruses in the order 3′-N-P-gene 3-M-G-L-5′. The genes are flanked by noncoding regions that are bordered by transcription initiation and polyadenylation signals. The six coding regions of MIMV encode nucleocapsid (N) protein, phosphoprotein (P), putative movement protein (P3), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase (L) (Massah et al. 2008).

Recent studies on plant rhabdoviruses have provided voluminous data on their genetic structure. This has facilitated studies on genetic diversity of these viruses. For instance, different isolates of strawberry crinkle virus (SCV) from Europe shared more than 89% identity in nucleotide sequence of L gene (Klerks et al. 2004). In taro vein chlorosis virus (TaVCV), nucleotide identity of N and L genes in different isolates were more than 80.7% and 72.6%, respectively (Revill et al. 2005). Comparison of eggplant mottled dwarf virus (EMDV) isolates from Greece and Cyprus showed homology ranges of 78-86% in coding and 59-91% in noncoding regions (Pappi et al. 2015). Different isolates of Lettuce necrotic yellows virus (LNYV) from Australia and New Zealand shared more than 80% identity in nucleotide sequence of N gene (Callagan and Dietzgen 2005).

Phylogenetic analysis of partial L gene sequences determined two subgroups among eight European isolates of SCV (Klerks et al. 2004). Some nucleotide sequence diversity was also found when 1kb sequences of the N and L genes of TaVCV isolates from six Pacific Islands countries were compared (Revill et al. 2005). Phylogenetic analysis of the complete N gene of 12 LNYV isolates from different host plants across Australia revealed two distinct subgroups (Callagan and Dietzgen 2005). Study on the genetic diversity of EMDV showed three distinct subgroups among isolates collected from different plant species and geographical areas (Pappi et al. 2015). The objective of the present study was to determine the genetic diversity of MIMV isolates based on the type of symptoms, geographical location and hosts in Iran. For this purpose the N, G and a 500 bp fragment from L genes as well as the non-coding regions were sequenced and compared. The genetic diversity between isolates were determined by maximum-likelihood (ML) phylogenetic reconstructions. Genetic diversity, recombination, and selection pressure analyses were also performed.

Materials and methods

Sampling

MIMV isolates used in this study were collected from major maize growing regions of Iran including Isfahan, Fars, Chaharmahal-Bakhtiari and Kohguiluyeh-Boyerahmad provinces and from various plants during 2015-2016. Maize plants showing specific MIMV symptoms including chlorotic and necrotic streaks and stripes along the veins in leaves and sheaths were studied for possible genetic variation (Izadpanah et al. 1983b; Izadpanah and Parvin 1979). In addition to maize, MIMV isolates from some other hosts were also studied (Table 1). The leaves of infected plants were collected and used for total RNA isolation.

Table 1. MIMV isolates from different geographical regions used in this study.

Isolate	Accession number			Host	Symptom type	Location
	N gene	G gene	L gene			
E1	MG367440	MG242351	MG242381	*Zea mays*	2*	Isfahan/Iran
E2	MG367441	MG242352	MG242382	*Z. mays*	3	Isfahan/Iran
E16	MG367438	- [a]	-	*Z. mays*	2	Isfahan/Iran
E18	MG367439	-	MG242380	*Z. mays*	3	Isfahan/Iran
E36	MG367442	MG242353	MG242383	*Z. mays*	1	Isfahan/Iran
E100	MG367436	MG242349	MG242379	*Z. mays*	2	Isfahan/Iran
E106	MG367437	MG242350	-	*Z. mays*	3	Isfahan/Iran
EL1	MG367443	MG242354	MG242384	*Z. mays*	2	Isfahan/Iran
Fa1	MG367445	MG242358	MG242387	*Z. mays*	1	Fars/Iran
Fa10	-	MG242356	-	*Z. mays*	2	Fars/Iran
Fa17	MG367444	MG242357	MG242386	*Z. mays*	2	Fars/Iran
Fa20	-	MG242355	MG242385	*Z. mays*	3	Fars/Iran
Fa35	MG367446	-	MG242388	*Z. mays*	2	Fars/Iran
Gr1	-	MG242372	MG242374	*Cynodon dactylon*	n/a	Isfahan/Iran
Gr9	-	MG242378	MG242376	*Panicum miliaceum*	n/a	Isfahan/Iran
Gr79	MG367447	MG242377	MG242375	*Echinochloa crus-galli*	n/a	Fars/Iran
Mar30	MG367448	MG242359	MG242389	*Z. mays*	2	Fars/Iran
Mar45	MG367449	MG242360	MG242390	*Z. mays*	2	Fars/Iran
Mar50	MG367450	MG242361	MG242391	*Z. mays*	1	Fars/Iran
Sh4	-	MG242362	-	*Z. mays*	3	Chaharmahal-Bakhtiari/Iran
Sh6	MG367455	MG242366	MG242396	*Z. mays*	2	Chaharmahal-Bakhtiari/Iran
Sh15	MG367453	-	MG242392	*Z. mays*	2	Chaharmahal-Bakhtiari/Iran
Sh20	MG367454	MG242365	MG242395	*Z. mays*	2	Chaharmahal-Bakhtiari/Iran
Sh104	MG367451	MG242363	MG242393	*Z. mays*	3	Chaharmahal-Bakhtiari/Iran
Sh111	MG367452	MG242364	MG242394	*Z. mays*	3	Chaharmahal-Bakhtiari/Iran
Ya5	MG367458	MG242369	MG242397	*Z. mays*	2	Kohguiluyeh-Boyerahmad /Iran
Ya53	MG367456	MG242367	MG242398	*Z. mays*	2	Kohguiluyeh-Boyerahmad /Iran
Ya55	MG367457	MG242368	MG242399	*Z. mays*	2	Kohguiluyeh-Boyerahmad /Iran
Ya62	MG367459	MG242370	MG242400	*Z. mays*	2	Kohguiluyeh-Boyerahmad /Iran
Ya65	MG367460	MG242371	MG242401	*Z. mays*	2	Kohguiluyeh-Boyerahmad /Iran
Ri13	MG367461	MG242348	MG242373	*Oryza sativa*	n/a	Isfahan/Iran

*Symptom types are: 1, fine chlorotic stripes withmild intensity; 2, fine chlorotic stripes with severe intensity; 3, wide chlorotic stripes with severe intensity.
a: Nucleotide sequences not determined.
n/a: not included in symptom type clustering.

Total RNA extraction

Total RNA was isolated according to Channuntapipat *et al.* method with minor modification as follows: 100 mg of leaf tissue were ground to a fine powder in liquid nitrogen and mixed with 800 µl of extraction buffer (0.1 M LiCl, 0.1 M Tris HCl, pH 8.0, 0.01 M EDTA, and 1%SDS); 400 µl of phenol (saturated with Tris HCl, pH 8.0) wereadded and vortexed for 1 min. Then, 380 µl of chloroform and 16 µl of isoamylalcohol were added, and the mixture was centrifuged at 13000 rpm at 4 ºC for 17 min. The aqueous phase was collected, an equal volume of 4 M LiCl added, and incubated at –20°C for 3–4 h. The nucleic acid was pelleted by centrifugation at 13000 rpm at 4°C for 15 min, dissolved in 400 µl of sterile water and reprecipitated with 2.5 vol. of ethanol in the

Table 2. Primers used in this study

Primer Name	Target Gene/ noncoding sequence	Product Size	Primer sequence
MIMV-N	N	1480bp	CTTGGTGAGTTCTCCGCAGC CAATGGACACCGACACAGGAGC
MIMV-NP	N-P	597bp	CTTGGTGAGTTCTCCGCAGC CAATGGACACCGACACAGGAGC
MIMV-P3	P-3	664bp	CACGACATGCTCACTGGGCTCC TTGAATACGGCGAGTCGGA
MIMV-3M	3-M	541bp	TCCTGGAGTGTGTGCCCGTAGCTC TGATCCCGCTAAAGACTGCCGCT
MIMV-MG	M-G	698bp	CCACCTCTGTAGAGTTGGACGCCA ATTCAGTGGCAGAGTCGAGCTGCTG
MIMV-G	G	1782bp	AGTCCTCAGCAGCAGGAACAGTCATCTTGGCTGATGGGCT C
MIMV-GL	G-L	813bp	CAATGTCTCGGACTGCAGAGCACCGAAGGGATTTGGACGG CACT
MIMV-L	L	500 bp	CTCAGACCACACGCGGAGATG TGACCCATGTCTCAGAGGCT

presence of 0.3 M NaAc (pH 5.2). The total RNA was recovered by centrifugation at 13000 rpm at 4°C for 15 min, washed twice with cold 70% ethanol and dissolved in 30 µl of DEPC treated water (Channuntapipat et al. 2001).

Designing oligonucleotide primers

The sequencing covered MIMV genes, N, G, and a 500 bp fragment of L as well as non-coding sequences of various isolates. Primers were designed based on the sequence of MIMV available in GenBank (accession no.NC 011542) (Table 2).

RT-PCR

To synthesize the cDN, 1µg of total RNA was mixed with each specific reverse primer (1µM) in a total of 10 µl reaction. The mixture was incubated at 70°C for 10 min and chilled on ice. Four µl of 5X reaction buffer, 1 µl dNTP (10 mM) and 100 unit of reverse transcriptase (M-MLV RT, Thermoscientific) were added to each sample. Reactions were incubated at 42°C for 45 min. cDNA synthesis was terminated by heating at 85˚C for 5 min.

The cDNAs were used as template in PCR reactions. PCR was carried out in a final volume of 30 µl, containing 200 units of *Taq* DNA polymerase (CinnaGen, Tehran, Iran) 3 µl of 10x PCR buffer, 20 mM $MgCl_2$, 0.2 mM dNTPs and 1 mM of each reverse and forward primers. Cycling conditions consisted of a cycle of 95°C for 3min, followed by 35 cycles of 95°C for 30 s and 53-58°C (based on Tm of primer pairs) for 45 s, 72°C for 1 min and a final single cycle of 72°C for 10 min. The amplicons were analyzed on a 1.5 % agarose gel. PCR products were sequenced directly in both directions (Seqlab, Germany). The achieved sequences were deposited in the GenBank database under the accession numbers as listed in Table 1.

Data analysis

Genetic diversity among isolates were calculated using multiple alignment of nucleotide and deduced amino acid sequences of N and G genes, a 500 bp fragment of polymerase gene and noncoding sequences. Multiple alignments were generated using ClustalW and amino acid and nucleotide sequence identities were determined.

Maximum-likelihood phylogenetic trees were generated with the general time-reversible (GTR) model and four rate categories, using the SPR tree search algorithm and 1000 bootstrap replicates for accuracy of the confidence of tree branches with the SEAVIEW software. Presence of recombinations was assessed with RDP and Boot scan methods by RDP4 software.

Positive selection analysis was performed for N and G genes and the mean ratio of non-synonymous substitutions per non- synonymous site (dN) to synonymous substitutions per synonymous site (dS) was estimated to assess the

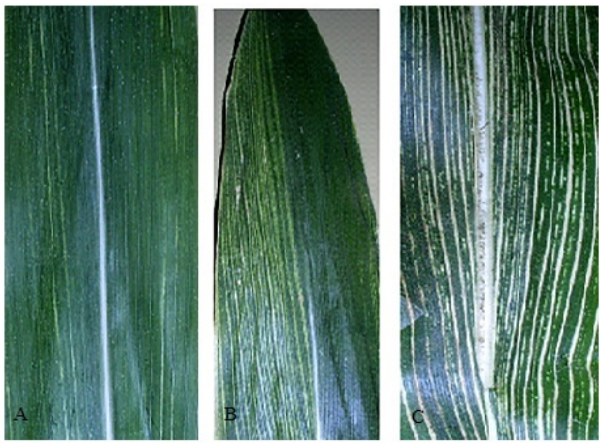

Fig. 1. MIMV symptom types: A, fine chlorotic stripes with mild intensity; B, fine chlorotic stripes with severe intensity; C, thick chlorotic stripes with severe intensity.

selection pressures acting on the gene. Analyses were performed in Datamonkey Adaptive Evolution Server (http://www.datamonkey.org). To detect the positive selection, single-likelihood ancestor counting (SLAC), fixed- effects likelihood (FEL) and random-effects likelihood (REL) methods were used with GTR model and a statistical significance threshold of 0.05 or Bayes factor 50.

Results

Detection of MIMV in collected samples

MIMV collected samples consisted of 140 samples from maize and 95 samples from non-maize plants including *Hordeum vulgare, Hordeum murinum, Oryza sativa, Secale montanum, Triticum aestivum, Echinochloa crus-galli, Panicum miliaceum, Setaria italica, Sorghum halepense* and *Cynodon dactylon*. The identity of plant species was confirmed by Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan, Iran.

In this study, MIMV was detected in *Zea mays*, *O. sativa*, *E. crus-galli, P. miliaceum* and *C. dactylon* by RT-PCR.

Maize isolates of MIMV showed different symptoms and were grouped in three symptom types. Isolates in group one showed fine chlorotic stripes with mild intensity (Fig. 1 A). The symptoms in group two were fine chlorotic stripes with sever intensity (Fig. 1 B). In group three the symptoms were wide chlorotic stripes with severe intensity (Fig. 1 C). Symptoms of *O. sativa* and *E. crus-galli* consisted of chlorotic stripes and mosaic on

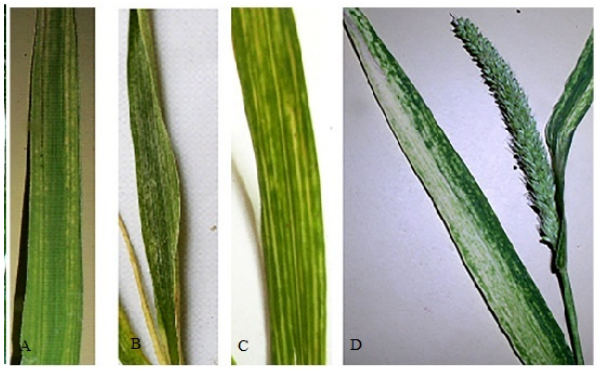

Fig. 2 Chlorotic stripes and mosaic symptoms on A) *Oryza sativa*, B) *Echinochloa crus-galli*, C) *Cynodon dactylon* and D) *Panicum miliaceum*.

leaves (Fig. 2 A and B respectively). In *C. dactylon* and *P. miliaceum* the symptoms were chrolotic stripes on leaves (Fig. 2 C and D respectively). *C. dactylon* is reported as MIMV host for the first time.

Genetic diversity of MIMV

The full-length N and G genes and a partial fragment of L gene as well as the non-coding regions of MIMV genome of different isolates were sequenced and compared by pairwise alignment.

All isolates characterized in this study had an additional A at the position 253 in N gene region compared to a MIMV isolate have been already deposited in GenBank with the (accession number NC 011542), confirming this change by an earlier report (Ghorbani et al. 2017). This modification made the length of MIMV nucleocapsid gene 141 ntcorresponding to 47 amino acids shorter than MIMV sequence available in GenBank. Multiple alignment of N gene sequences of MIMV isolates characterized in this study showed 98.7-100% and 99.3-100% nucleotide and amino acid sequence identities, respectively. Based on glycoprotein gene sequence alignment analysis, 98.7-100% and 99.2-100%, nucleotide and amino acid sequence identities, respectively were observed among MIMV isolates. Different isolates of MIMV shared 99-100% nucleotide sequence identity in 500 bp fragment of L gene. MIMV isolates from non-maize hosts showed 98.8-99.8%, 98.8-99.8% and 99-100% nucleotide sequence identities with MIMV maize isolates in N, G and L genes, respectively. Nucleotide sequence identity ranged from 97.2 to 100% for the non-coding regions between

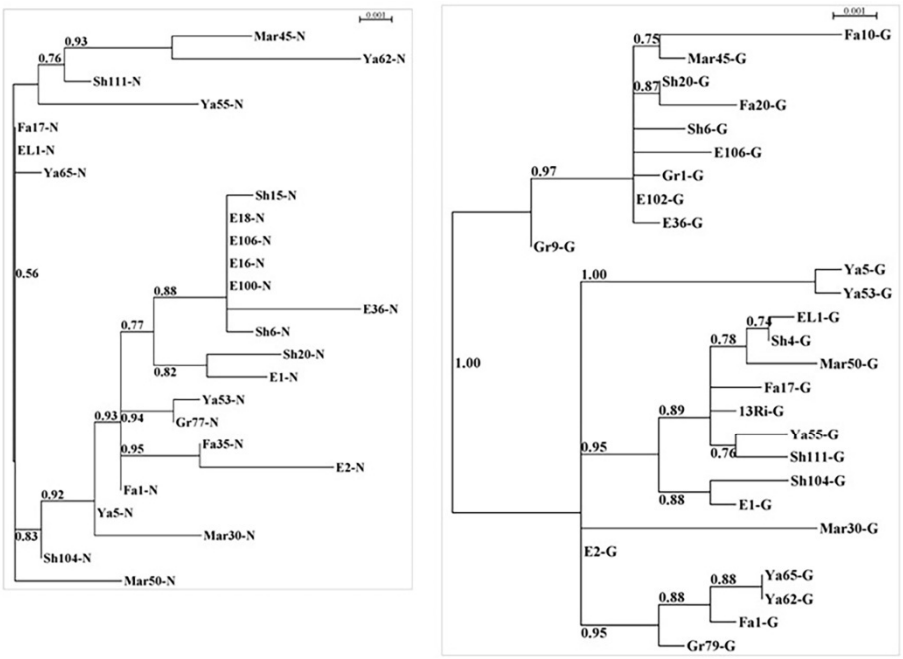

Fig. 3 Phylogenetic trees constructed by the maximum-likelihood method based on nucleotide sequence of N (right) and G (left) ORFs of MIMV isolates. All trees are midpoint-rooted. The numbers above each branch are the non-parametric bootstrap percentages (1000 replicates).

Fig. 3 Phylogenetic trees constructed by the maximum-likelihood method based on nucleotide sequence of N (right) and G (left) ORFs of MIMV isolates. All trees are midpoint-rooted. The numbers above each branch are the non-parametric bootstrap percentages (1000 replicates). Isolates were described in Table 1.

N and P genes, 85.3-100% between P and ORF 3, 94.4-100% between M and G genes and 96.2-100% between G and L genes.

Phylogenetic analysis

Phylogenetic trees were constructed from nucleotide sequences of N and G genes. There was no correlation between these sequences and geographical regions, hosts or symptom types (Fig. 3).

dN/dS ratio and selection pressure analysis

In nucleocapsid gene, MIMV isolates showed 32 modified positions, eight of which resulted in amino acid changes (non-synonymous). In glycoprotein gene, only eight of 52 modified positions, were recognized as non-synonymous substitutions (Table 3)

Mean values of dN/dS ratio for N and G genes based on SLAC method were calculated to be 0.2 and 0.1, respectively (Table 4). The SLAC method did not recognize any codons under positive selection in the nucleocapsid protein and glycoprotein genes but 2 and 4 putative codons in N and G genes, respectively, were found to be under negative selection. The FEL and REL recognized codon positions which are potentially under positive selection in glycoprotein gene. The FEL identified only one codon at position 582 and the REL recognized one additional codon at position 552 under positive selection, i.e., nucleotide substitutions caused amino acid changes. Furthermore, 13 and 18 putative codons were evaluated for the negative selection in N and G genes, respectively, by FEL method. Twenty three and 43 putative codons were detected under negative selection for N and G genes, respectively, by REL method (Table 4).

Discussion

To expand our knowledge of MIMV genetic variability, the full-length fragments of N and G genes and a 500bp fragment of L gene as well as

Table 3. Nucleotide and amino acid substitutions and modified nucleotide positions in N and G genes of MIMV

Nucleotide Position	390	844	1057	1090	1111	1120	1147	1162
Nucleotide substitution in N gene	GAG	CGG	GCT	ATG	CAC	ACG	GAT	GAT
	AAG	TGG	GTT	ATT	CAA	CTG	TAT	GCT
Amino acid substitution in N gene	Glu	Arg	Ala	Met	His	Thr	Asp	Asp
	Lys	Trp	Val	Ils	Gln	Leu	Tyr	Ala
Nucleotide Position	313	691	964	1099	1654	1735	1744	1777
Nucleotide substitution in G gene	A**A**G	T**A**C	AGT	AGC	GTA	TCA	GCG	GCA
	AGG	T**T**C	GGT	CGC	ATA	CCA	ACG	TCA
Amino acid substitution in G gene	Lys	Tyr	Ser	Ser	Val	Ser	Ala	Ala
	Arg	Phe	Gly	Arg	Ile	Pro	Thr	Ser

Table 4. Positive selection analysis of MIMV N and G genes using the SLAC, FEL and REL methods.

Gene	Number of codon	SLAC		FEL		REL		dN/dS
		PS	NS	PS	NS	PS	NS	
N	445	0	2	0	13	0	23	0.211
G	594	0	4	1(P=0.01)	18	2	43	0.106

PS, positively selected sites; NS, negatively selected sites; dN/dS, average ratio between non-synonymous and synonymous substitutions for each pair of comparisons, where dN/dS >1 gives an indication of positive selection pressure on a gene

the non-coding regions (approximately one third of the genomic sequence) of MIMV isolates collected from main regions of maize cultivation in Iran were sequenced. Results revealed that the genetic variability of N, G and a 500 bp fragment of L genes in maize and non-maize isolates is less than 1.3, 1.3 and 1%, respectively. However, non-coding sequences between genes were more variable (ranging from 2.8 to 14.7%). The non-coding sequence between ORF 3 and P genes was more variable (up to 14.7%) than other non-coding regions. Despite that, the non-coding motif 3'-AAUUCUUUUUGGGUUU/G-5' was conserved in all isolates. Other rhabdoviruses have shown more variability in nucleotide sequences. Different isolates of LNYV from Australia and New Zealand had less than 20% diversity in N gene. Research on genetic diversity of EMDV isolates from Greece and Cyprus showed variability of 14-22% in coding and 9-41% in non-coding regions (Pappi et al. 2015). In TaVCV, nucleotide diversity of N and L genes were less than 19.3% and 27.4%, respectively. Different isolates of SCV from Europe shared less than 11% diversity in nucleotide sequence of L gene. Our results showed that MIMV had less diversity (less than 1.3%) compared to other plant rhabdoviruses. Although LNYV, EMDV and TaVCV showed diversity more than MIMV, their high nucleotide sequence diversity is not reflected in the amino acid sequence diversity which is mostly due to the changes of the third nucleotide position in the codons (Callaghan and Dietzgen 2005; Klerks et al. 2004; Revill et al. 2005).

In RNA viruses, high mutation and recombination rates, large populations and short replication cycles are known as factors causing high genetic variation (Domingo and Holland 1997). However, molecular comparisons of sequences available in GenBank suggest that genetic stability is the rule in natural populations of plant viruses (Garcia-Arenal et al. 2001).

Additionally genetic diversity is related to ecological factors such as virus-vector relationship, host range and geographic distribution (Sanchez-Campos et al. 2002; Vives et al. 2002). The interaction of the virus with host plants and vectors reduce genetic diversity of populations. (Garcia-Arenal et al. 2001).

The epidemiological factors, such as the means of spread, mode of transmission and the host type (annual or perennial) could determine the structure of the virus population (Garcia-Arenal et al. 2001). Different hosts may increase or decrease the rate of viral evolution by allowing or hindering the diversity in viral populations (Feuer et al. 1999; Schneider and Roossinck 2001). Plant viruses depend on specific vectors to spread from one plant to another (Ng and Falk 2006). Considering the fact that maize has been imported to Iran and increased its cultivation in recent years it appears that MIMV has been transmitted from weeds or other field crops to maize by its planthopper vec-

tors. Plant rhabdoviruses are not transmitted through seed and based on their host type could be transmitted through vegetative plant materials. Most MIMV hosts are annual crops such as maize, wheat, barley and rice, in which viruses spread among plants rapidly and the life of the plant host is relatively short. In contrast, the perennial crops provide opportunity for maintenance of chronic infection and efficient transfer of viruses through vegetative organs. This situation is ideal for long term evolution of virus in host plants (Walia et al. 2014). In this study, among 95 non-maize isolates, MIMV was detected in only five samples (Table 1) which most of them are annual plants. MIMV was detected only in *C. dactylon* and not in *Sorghum halepense* that previously reported as the MIMV host (Izadpanah 1989).

The role of vector transmission is important not only in the host range determination but also in genetic diversity of RNA viruses (Garcia-Arenal et al. 2001; Hogenhout et al. 2003). Population bottlenecks during transmission are regarded as limiting factors in population diversity (Li and Roossinck 2004).

For maintenance of protein function, negative selection plays an important role by elimination of deleterious variants (Garcia-Arenal et al. 2001; Power 2000; Roossinck and Palukaitis 1990). Based on the values of dN/dS<1, N and G genes were under negative selection pressure. Two other factors which influence genetic diversity are the natural selection and random genetic drift. For most viruses, the negative selection acts on virus genes.

In phylogenetic analysis no correlation was found between MIMV isolates and geographical regions, type of symptoms and host differences (Fig. 2). Most molecular studies and genogroup definition were based on sequences of the N and L genes. In SCV the grouping of eight isolates based on partial L gene sequence were independent of symptom severity or geographical regions (Klerks et al. 2004; Kuzmin et al. 2009).

The information on the low genetic diversity of MIMV will be useful in plant breeding strategies and producing transgenic plants based on pathogen-derived resistance to obtain maize varieties with durable resistance. In addition, new insights on genetic diversity mechanisms can play an important role to prevent the development of emerging viruses.

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