



Association of Satellite RNA with *Grapevine fanleaf virus* in its Geographical Origin and Sequence Characteristics

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ABSTRACT

The region between The Caspian Sea and Black Sea has been hypothesized as the origin of grapevine. Likewise, an extensive study on *Grapevine fanleaf virus* (GFLV) from this region suggests this region as a possible origin of the virus. However, as yet there is no information as to whether or not the virus variants from this region accompanied with the virus satellite RNA (satRNA) and if so, how diverse these satRNAs can be. To answer these questions, *Grapevine fanleaf virus* isolates were collected from different vineyards in the northwest region of Iran to study occurrence of the satellite RNA. A total of 421 samples including *Vitis vinifera*, *Chenopodium quinoa*, *C. amaranticolor*, *Cynodon dactylis*, *Medicago sativa* were initially screened against GFLV by RT-PCR with the coat protein primers (Cp433, Cp912). When the GFLV-infected plants

(36 samples) were screened by RT-PCR with the newly designed satRNA primers Gf750 and Gr750, three samples appeared to possess the satRNA. The satellite cDNA fragments were cloned and sequenced and when the resulting data were compared with sequences of previously-reported satRNA of GFLV and *Arabis mosaic virus* (ArMV), 70-98% and 69-71% similarities were found, respectively. Phylogenetic studies revealed distinctness of the satRNAs from Iran. This may suggest coevolution of the satRNA with the helper virus because GFLV isolates from this region also form a distinct branch. Among previously reported corresponding satRNA sequences, those from Slovenia and South Central Europe were the closest. This study also reports *C. amaranticolor* as the natural host for GFLV, which was previously reported only as an experimental host.

Keywords: *Arabis mosaic virus*, Double-stranded RNA, *Grapevine fanleaf virus*, Satellite RNA

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1. Introduction

Grapevine fanleaf virus (GFLV) is responsible for one of the important diseases of grapevine (*Vitis vinifera* L.), fan leaf, worldwide and causes economic losses (Martelli & Boudon-Padieu 2006). This virus has two genomic RNA molecules each coding for a viral polyprotein (Andret-Link et al. 2004; Sokhandan Bashir et al. 2009). Some GFLV isolates have an extra RNA molecule known as satellite RNA (satRNA). SatRNAs are thought to be originated from similar helper viruses through mutation events such as deletion, insertion, recombination or reassortment (Cepin et al. 2016). GFLV satRNA was initially reported in GFLV isolate F13 which caused severe symptoms on *Chenopodium quinoa* (Pinck et al. 1988). Later on, satRNA was also detected in other nepoviruses such as *Arabis mosaic virus* and *Tomato black ring virus* (Cepin et al. 2016).

SatRNAs associated with nepoviruses are divided into three groups: large linear, small linear and circular satRNAs (Roossinck et al. 1992; Palukaitis 2016). The large satRNAs usually encode nonstructural proteins. They all encode a protein which is necessary for the replication of satRNA associated with nepoviruses (Hu et al. 2009; Cepin et al. 2016). In contrast, the small satRNAs are highly structural and have internal base pairing. Small satRNAs of nepoviruses are packaged as linear RNAs; however, during replication in plants they form a circular RNA (Roossinck et al. 1992; Palukaitis 2016).

SatRNAs of nepoviruses vary in size from 1.1 to 1.5 kb and have end structures (5'-Vpg and 3'-poly A) like that of the helper virus genome. These satRNAs encode a 37-48 kDa non-structural protein which is necessary, but not sufficient, for the satRNA replication (Roossinck et al. 1992). SatRNAs might affect the symptoms incurred by the helper virus (Simon et al. 2004); which could be due to satRNA's potential in multiplication and accumulation level in host plant (Betancourt et al. 2011). This role of satRNAs involves some aspects of RNA silencing and its suppression as described by Palukaitis (2016). A comparative analysis

of GFLV isolates containing satRNA or not, showed no obvious effect on virus accumulation and symptom expression in *C. quinoa* (Gottula et al. 2013).

This study was carried out for the first time to determine presence of satRNA in the GFLV isolates from vineyards in the northwest region of Iran. Vineyards in this region have previously been shown to be widely infected with GFLV. Also, phylogenetic analyses have shown that GFLV variants from this region form a distinct clade. This region is located between The Caspian Sea and Black Sea and hypothesized to be the origin of the grapevine and GFLV (Raski et al. 1983). Therefore, we were interested to find out if the GFLV isolates from this region are accompanied with the satellite and, if so, what would be the differences between satRNA of these isolates compared to that of GFLVs reported from elsewhere. Association of satRNA with viruses may have an important implication as to the biology of helper virus and its potential use in the virus control.

2. Material and Methods

2.1. Primer design

Initially, previously-published (Fuchs et al. 1989; Cepin et al. 2016) primer pairs, Gsat191f/Gsat1005r and Fp3/Rp, were used for detection of GFLV satRNA in RT-PCR before designing new primers. Previously reported GFLV satRNA sequences were retrieved from GenBank (Sequences which reported by Fuchs et al. 1989; Liu et al. 1990; Gottula et al. 2013; Cepin et al. 2016; Chiumenti et al. 2016) and incorporated in the design of new primers (Table 1) by the use of Primer 3 software (Primer3web version 4.1.0, <http://primer3.ut.ee/>).

Table 1- Features of the GFLV coat protein and satellite RNA specific primers tried in this study

Primer name	Primer sequence	Expected product size (bp)	The amplified fragment	Reference
Cp433	F: 5'-GAACTGGCAAGCTGTCGTAGAA-3'	350	GFLV coat protein	Izadpanah et al. 2003
Cp912	R: 5'-GCTCATGTCTCTCTGACTTTGACC-3'			
Gf750	F: 5'-ACACAAACAGCAGTCTGATGGA-3'	700-750	GFLV satellite	Newly designed Primers
Gr750	R: 5'-GCTGAGGAAAACTGTTCCGA-3'			
Rp	R: 5'-TAAWGAGCAACCAAAATCCCA-3'	870-900	GFLV satellite	Cepin et al. 2016
Fp3	F: 5'-GTGGSCCCGCRAGTGT-3'			
Gf1000	F: 5'-GCTTGTATTGTCGTGTAAGCAC-3'	1000	GFLV satellite	Newly designed Primers
Gr1000	R: 5'-AGTTGGCTAATGAGCAACC-3'			
Gsat191f	F: 5'-CCGAGACCGAAATGGGAGTAAAACA-3'	850-900	GFLV satellite	Fuchs et al. 1989
Gsat1005r	R: 5'-ACAGAAGCAACCGTGGGGATACAC-3'			

2.2. Virus sources, extraction of total RNA and RT-PCR

First, 421 samples including grapevine and several weeds were subjected to total RNA extraction according to Rowhani et al. (1993) with modifications as described elsewhere (Sokhandan et al. 2007; Khabbazi et al. 2017) and then screened by RT-PCR with GFLV coat protein primers to determine the virus infection. Then, 36 GFLV-infected plants (Table 2) including grapevine (30 samples), *Chenopodium quinoa* (4 samples), *C. amaranticolor* (1 sample) and *C. dactylis* (1 sample) were subjected to RT-PCR with the satellite primers.

RT-PCR was initially conducted to confirm the GFLV entity of the extracted total RNA by the use of Cp433/Cp912 primers (Table 1). Accordingly, cDNA was synthesized with the reverse primer, Cp912 following the RevertAid First Strand cDNA Synthesis Kit instructions (Thermo Fisher Scientific Inc., Waltham, USA). Subsequently, PCR was carried out using the Cp433 and Cp912 primer pairs under a thermal profile of an initial denaturation at 94 °C for 1 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 30 s, and a final polymerization step at 72 °C for 7 min.

cDNA synthesis was also performed with Gsat1005r, GR1000, Rp or Gr750 primer to detect the virus satRNA, respectively. Then, the generated cDNA was used as the template to amplify the expected fragment by PCR using Gsat191f/Gsat1005r, Gf1000/Gr1000 or Fp3/Rp primer pairs, respectively. PCR amplification was performed by using one Unit of *Taq* DNA Polymerase (SinaClon Co., Tehran, Iran) in a total reaction mixture of 20 µl containing 1× reaction buffer, 1.5 mM MgCl₂, 1 mM dNTPs and 0.25 pmol of each primer. A gradient PCR (for all the satRNA primer pairs) was initiated with an initial denaturation at 94 °C for 1 min and the reaction was progressed in 30 cycles of denaturation at 94 °C for 30 secs, annealing at 52, 54, 56, 58 and 60 °C for 30 sec and extension at 72 °C for 1 min. The amplification was ended with the elongation step of 72 °C for 7 min. In PCR with the new primer set Gf750/Gr750, an annealing temperature at 60 °C for 30 secs was applied. A 5 µl- aliquot of the products were electrophoresed on 1% agarose.

Table 2- List of samples tested for GFLV and its satRNA

Sample code	Host	Symptoms	Origin	GFLV	satRNA
M3	C. quinoa	Local lesions	Khelejan, E. Azar.1	+	+
A2	V. vinifera cv. Keshmeshi	Mosaic	Urmia, W. Azar.2	+	+
G3,G4,G1,Kh2	V. vinifera cv. Keshmeshi	Vein banding	Khalatpoushan, E. Azar.	+	-
G2	V. vinifera cv. Keshmeshi	Vein banding and leaf deformation	Khalatpoushan, E. Azar.	+	-
Kh3, Kh6	V. vinifera cv. Shahani	Vein banding	Khalatpoushan, E. Azar.	+	-
Kh4	C. quinoa	Local lesions	Khalatpoushan, E. Azar.	+	-
Kh7, Kh8	V. vinifera cv. Shahani	Mosaic	Khalatpoushan, E. Azar.	+	-
S2	V. vinifera cv. Asgari	Mosaic	Sardrud, E. Azar.	+	-
S3	V. vinifera cv. Asgari	Vein banding	Sardrud, E. Azar.	+	-
S4, S6, S10	V. vinifera cv. Keshmeshi	Vein banding	Sardrud, E. Azar.	+	-
A6	V. vinifera cv. Hoseini	Mosaic and yellowing	Ajabshir, E. Azar.	+	-
A9	V. vinifera cv. Hoseini	No symptoms	Ajabshir, E. Azar.	+	-
A2, A11	V. vinifera cv. Asgari	No symptoms	Ajabshir, E. Azar.	+	-
A14	C. quinoa	Local lesions and yellowing	Ajabshir, E. Azar.	+	-
T.H	V. vinifera cv. Keshmeshi	Mosaic	Tabriz, E. Azar.	+	-
L2, L3	V. vinifera cv. Keshmeshi	Vein banding	Lahroud, Ardebil	+	-
U3, U4	V. vinifera cv. Sahebi	No symptoms	Urmia, W. Azar.	+	-
U6, U7	V. vinifera cv. Keshmeshi	No symptoms	Urmia, W. Azar.	+	-
M.ch	C. quinoa	Local lesions	Mayan, E. Azar.	+	-
M.cy	C. dactylis	Mosaic	Mayan, E. Azar.	+	-
B2, B10	V. vinifera cv. Qizil uzum	Vein banding	Bonab, E. Azar.	+	-
N1, N5	V. vinifera cv. Keshmeshi	No symptoms	Khelejan, E. Azar.	+	-
T1	C.amaranticolor	Local lesions	Khelejan, E. Azar.	+	+

1: East Azarbaijan, 2: West Azarbaijan

2. 3. Cloning and sequence analysis

DNA fragments amplified by the use of the satellite primers (Gf750, Gr750) were excised from agarose gel and purified using Thermo Fisher DNA Gel Extraction kit (Waltham, USA). The purified fragment (150 ng) from each sample was ligated into pTG19-T cloning vector (50 ng) according to the manufacturer's protocol (SinaClon Corp., Tehran, Iran) and transformed into 100 µl home-prepared competent *Escherichia coli* DH5α cells through heat-shock method (Chung et al. 1989). The transformed bacteria were grown on LB medium containing ampicillin (100 mg/L), X-GaL (20 mg/ml) and IPTG (0.1 M). Three desired colonies each from an isolate (RT-PCR product species) were selected by colony- PCR screening (Sokhandan et al. 1997) and the plasmids were extracted following the alkaline lysis method (Green & Sambrook 2012). To confirm that the satellite fragment is cloned into the vector (pTG19-satRNA), *Bam*HI restriction analysis was conducted. Subsequently, recombinant plasmids from the desired colonies were sequenced (Refgen Corp., Ltd., Ankara, Turkey) by the use of satRNA specific primers, Gf 750 and Gr 750. Analysis of the sequences was performed to assess variation between the satRNA sequences. Nucleotide (nt) alignment and phylogenetic trees were constructed using GeneDoc (Nicholas & Nicholas, 1997) and CLC sequence viewer 7.6.1 program (CLC Bio-Qiagen, Aarhus, Denmark), respectively. The SIAS online tool (Immunomedicine Group, UCM, Spain) was used for calculation of pairwise identity percentages. A 700 nt data from each of the three satRNA isolates were aligned with satellite sequences of GFLV and ArMV which were retrieved from GenBank. The alignment numbering was relative to the satRNA strain R2 (accession number: KC162000.1) to find out the position of highly conserved regions.

2. 4. Glasshouse studies

Glasshouse studies were performed to gather further evidence for association of the satellite with GFLV. It was hypothesized that if the GFLV possesses the satRNA, it may still be accompanied with the virus after seed transmission. Accordingly, *C. quinoa* (50 pots) was used in this study. One healthy control was included for every five inoculated samples. Three-week old *C. quinoa* plants were subjected to mechanical inoculation. The leaves were cleaned with a piece of wet cloth before being dusted with carborundum and rubbed with sap of GFLV-infected grapevine leaves ground in 0.1 M phosphate buffer (pH 7.2) containing 2.5% (v/v) nicotine. The inoculated leaves were rinsed with water after 10 minutes of the inoculation and the plants were kept at 24 ± 2 °C for 10 to 14 days in glasshouse until appearance of the symptoms. The infected plants were tested by total RNA extraction and RT-PCR for infection with GFLV and presence of the satRNA.

The infected *C. quinoa* plants were grown to flowering and allowed to produce seeds. The resultant seeds were germinated and maintained under glasshouse conditions for the appearance of any viral symptom before being checked by total RNA extraction and RT-PCR for the presence of the virus and satRNA.

3. Results and Discussion

3. 1. Detection of GFLV and satRNA

When total RNA samples were tested by RT-PCR, only 36 samples (8.6%) appeared to have an infection with GFLV. The expected 350 bp and 700 bp fragments were amplified with the coat protein (Cp912, 433) and satRNA (gf 750, gr 750) primers, respectively (Figure 1). Among the GFLV-infected plants, satRNA was detected only in three plant samples (Table 2) including *V. vinifera*, *C. quinoa*, and *C. amaranticolor*. However, no satisfying result was achieved using the primers already reported in the literature.

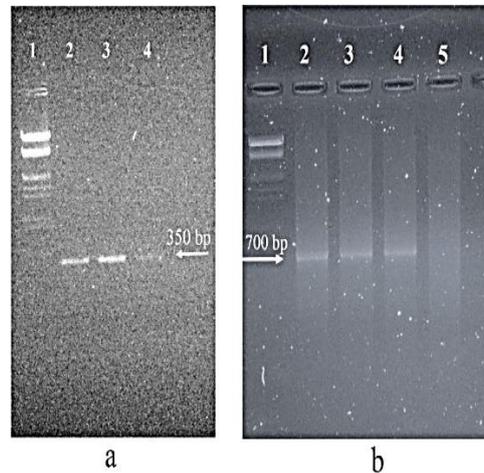


Figure 1- Electrophoresis of products from RT-PCR with GFLV primers (Cp912, Cp433) [a] and GFLV satellite (gf750 and gr750) primers [b] on 1% agarose gel. Lane 1(a, b): Lambda DNA *EcoRI* + *HindIII*; (a) Lanes 2, 3 and 4: infected *C. quinoa*, *V. vinifera*, and *C. amaranticolor*, respectively. (b) Lanes 2, 3 and 4: infected *C. quinoa*, *V. vinifera*, and *C. amaranticolor* containing satRNA, respectively; Lane 5: GFLV-infected sample lacking satRNA

3. 2. Sequencing and phylogenetic analysis

When RT-PCR-amplified satRNA fragment (700 bp) was ligated into pTG19-T and the resulting recombinant plasmid was used for transformation of *E. coli*, the desired colonies were found among which three colonies (A2, M3, and T1) were randomly selected for further analyses. A subsequent *Bam*HI restriction digestion also confirmed cloning of the 700 bp satRNA cDNA into the pTG19-T vector. When the recombinant plasmids were subjected to sequencing and the resultant sequence data were aligned, homologies of 91-97% were observed between the three satRNA isolates. Maximum (97%) homology was between the isolates A2 and M3 and the least homology (91%) between T1 and M3. By Comparison of these sequences to previously recorded GFLV satRNA sequences, homologies of 70-98% were revealed between them. In addition, comparison of the newly-generated GFLV satRNA isolates with ArMV satRNA sequences (accession number D00664.1 and NC_003523.1) revealed homologies of 59-61% (Table 3). Further, there were 80-85% homologies between the new isolates and that reported from Slovenia and South Central Europe (Cepin et al. 2016). This considerably big difference (15-20%) between these satRNAs could be due to the differences in lineages of the helper virus or independent events in evolutionary history such as deletion or insertion of nucleotides.

Table 3- Similarity analysis of the isolated satRNA sequences performed by SIAS program; A2, M3 and T1 are satRNAs isolated in this study, L1, L2 and C1-C22 are codes for the sequences reported earlier. Numbers represent the similarity percentage between the isolates

Code of isolates	A2	M3	T1
A2	-	97%	94%
M3	97%	-	91%
T1	94%	91%	-
L1, L2		59-61%	
C1-C22		80-85%	

In the cladogram constructed on the basis of the satRNA sequences (Table 4), there were two clades so that the newly-generated GFLV satRNA sequences (A2, T1, and M3) were clustered in a distinct position together with previously-reported GFLV and ArMV satRNAs (Figure 2). Thus, the isolates from Iran were clustered in the same clade together with the isolates

3. 3. Glasshouse studies

Among the 50 plants of *C. quinoa* 40 plants (80%) exhibited symptoms including local lesions and leaf deformation. However, when total RNA from all the inoculated plants were subjected to RT-PCR to verify the results. 350 bp and 700 bp fragments were obtained by the use of GFLV coat protein (Cp 433/Cp 912) and satellite primers (Gf750/Gr750), respectively.

Based on the assessments of GFLV+satRNA transmission to *C. quinoa*, it could be concluded that the plants infected with satRNA-containing viruses indicate normal growth with local lesions only, whereas the satRNA-free GFLV infection causes stunting, chlorosis, and local lesions as well (Figure 3). When the seeds of *C. quinoa* from the plants infected with satRNA-possessing GFLV were germinated and the progeny plants were compared to the healthy plants, obvious difference in plant height was evident, validating the virus transition to the next progeny. Subsequent RT-PCR assays confirmed the presence of GFLV and satRNA in the progeny (Figure 4).



Figure 3- A (a1): Grapevine leaves infected with satRNA-free GFLV, showing leaf deformation and growth reduction; A (a2): Grapevine leaves infected with GFLV isolate containing satRNA (T1) showing no obvious symptoms; B: Healthy plant; C (c1): *C. quinoa* inoculated with GFLV isolate containing satRNA (T1) only shows local lesions on leaves with normal growth; C (c2): *C. quinoa* inoculated with GFLV isolate lacking satRNA shows stunting, chlorosis in lower leaves and local lesions in upper leaves

In this study, samples were collected in late spring, early summer and early autumn. This timing was because of the symptom disappearance in hot summer days (Francki et al. 1985). In addition to different samples with various GFLV symptoms, asymptomatic samples from *V. vinifera* and weeds were also collected, because it has been documented that some *V. vinifera* varieties are tolerant to GFLV (Martelli & Boudon-Padieu 2006). Thus, they could be infected with GFLV although asymptotically, as 9 out of 71 asymptomatic samples appeared to be carrying GFLV although none of them had the satRNA.

The distribution pattern of the satRNA among the GFLV-infected vines suggests that the satRNA could be absent in a GFLV-infected plant adjacent to others which contain the satRNA (Gottula et al. 2013). Likewise, in the present study, satRNA-containing GFLV was detected in *C. amaranticolor* grown in vineyards, but not in the surrounding grapevines in the same vineyard.

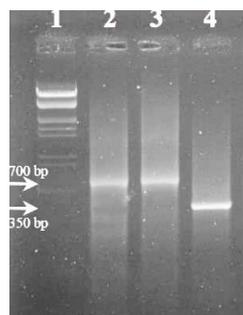


Figure 4- Surveying seed transmission of the satellite RNA in *C. quinoa*; electrophoresis of the RT-PCR products on 1.2% agarose gel. Lane 1: Lambda DNA *EcoRI*+*HindIII*; Lane 2: RT-PCR product from satRNA in the progenies; Lane 3: RT-PCR product from satRNA in the parental plant; Lane 4: RT-PCR product from the GFLV coat protein gene in the progenies as the control

To confirm the detection of GFLV and its satRNA, RT-PCR with GFLV coat protein and satRNA primers were applied in this study. SatRNA detection was carried out using different primer pairs, of which three pairs had earlier been utilized in similar researches elsewhere in the world; however, detection of satRNA was not successful with the previously published primers (Gsat191f/Gsat1005r, Fp3/Rp) which might be due to the satellite genetic variation. The genetic variation in the flanking sequences of satRNA and failure in the primer annealing process might be the reason for unsuccessful RT-PCR amplification

once other primers were used. Also, previous studies on Iran isolates of GFLV have revealed that they possess distinct genotypes, consequently the primers which were designed based on exotic GFLV isolates could not be efficient in annealing the local isolates (Sokhandan et al. 2012). Among the newly designed primers, Gf750/Gr750 had a better amplification thus were utilized in the study. The other newly designed primers (Gf1000/Gr1000) were not useful in the detection and the expected amplified fragment (1000 bp) was very faint and the PCR conditions could not be optimized further.

Comparison of results from the two different primer sets (Gf1000/Gr1000 and Gf750/Gr750) suggested that the satRNA may possess some conserved regions internally rather than at the ends because only Gf750/Gr750 satisfactorily gave the expected band.

The main objective of this study was to determine presence of the satRNA and investigate its diversity in GFLV isolates from Iran. To confirm further that the GFLV is accompanied with the satRNA, seeds from GFLV+satRNA infected *C. quinoa* plants were germinated and a subsequent analysis of the progeny plants revealed that the transmitted GFLV contained the satellite (Figure 4). This gave a further evidence as to the association of the satRNA with the GFLV isolates. The results also indicated that the satRNA affected severity of symptoms so that normal growth together with only local lesions were seen on the inoculated plants compared to the satRNA-free isolates which caused severe damage. This is in agreement with the previous report that satRNAs impact on their helper viruses and the induced symptoms (Roossinck et al. 1992). In general, the effect of satRNA on virus symptoms is influenced by helper virus, host plant and features of satRNA (Roossinck et al. 1992). As reported by Lamprecht et al. (2013), helper virus was the determining factor in the infection of *C. quinoa* plants when they were mechanically inoculated with satRNA-containing GFLV isolates. Accordingly, the GFLV satellite RNA was supported only when co-inoculated with GFLV as the helper virus but not when co-inoculated with ArMV-NW.

Seeds from GFLV+satRNA infected *C. quinoa* plants germinated one month later than that from the control plants and also showed reduction in plantlet growth in 50% of the replicates when compared to the control plants which were in agreement with the results reported by Saldarelli et al. (1993) and Gottula et al. (2013). Accordingly, the effect of satRNA on infection and symptom appearance might be dependent on the host species as well.

Among 36 infected grapevine samples, GFLV isolates harboring the satRNA (8.33%) were detected in three cases which demonstrates the low frequency of the satRNA in the studied region. In a similar study conducted by Saldarelli et al. (1993) only five isolates from among 34 GFLV-infected samples were determined to contain the satRNA (14.70%). Likewise, in another study more than 100 plants were screened but the satRNA was found in two samples only. Therefore, it appears that the occurrence of satRNA is a rare event (Lamprecht et al. 2013) and it is speculated that helper virus and host plant are the factors involved in satRNA origination (Roossinck et al. 1992; Saldarelli et al. 1993; Lamprecht et al. 2013).

This study also dealt with the variation of the satRNA compared to the previously reported GFLV satRNAs. Accordingly, phylogenetic studies demonstrated that GFLV isolates M3 (*C. quinoa*) and T1 (*C. amaranticolor*) were placed on different branches in the same subclade although they were collected from the same region (Khelejan, East Azarbaijan, Iran). As such, there appears that geographical location is not the only determining factor for satRNA grouping in the phylogenetic trees. As this is the case for the helper virus as well (Lamprecht et al. 2013; Cepin et al. 2016). Comparison of the satRNA sequences from Iran with the previously reported satRNA sequences suggested that the former were phylogenetically close to that reported from Slovenia and Central Europe; however, the reported satRNA sequences in this study were placed in a distinct subclade on the phylogenetic tree. The previous studies on CP and MP genes of GFLV in Iran have shown the distinctness of the Iran isolates among all the reported GFLV sequences from around the world (Sokhandan et al. 2012). So, the distinct positions of satRNAs from Iran in the phylogenetic tree could be because of the distinctness of the helper virus. This could also strengthen the previous hypothesis that Iran is the origin of GFLV and from there it has spread to all over the world (Vuittenez 1970). The present study is the first study of GFLV satRNA in, Iran, which provides new insights into the occurrence and genetic diversity of GFLV satRNA. Results achieved pave the way for future studies of satRNA and perhaps development of resistant against GFLV.

4. Conclusions

In this study, GFLV satellite RNA was detected and characterized for the first time in the northwest region of Iran by RT-PCR. A heterogeneity of 2-30% was revealed between the three newly-reported satRNAs and the previously-reported isolates and it was also demonstrated that the GFLV satRNA sequences from Iran are phylogenetically close to that reported from Slovenia and central Europe. They have close phylogenetic relationship, but the Iran isolates formed a distinct subclade.

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