Genetic diversity and biological characterization of sugarcane streak mosaic virus isolates from Iran

Zohreh Moradi¹ · Mohsen Mehrvar¹ · Ehsan Nazifi²

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Abstract Sugarcane streak mosaic virus (SCSMV; genus *Poacevirus*, family *Potyviridae*) is a major causal agent of sugarcane mosaic disease in Asia. A survey of SCSMV was conducted in cultivated fields in Khuzestan province, southwestern Iran. Sixty-five sugarcane leaf samples showing mosaic symptoms were collected and investigated by RT-PCR. Almost one-fourth of the samples were found to be infected by SCSMV. To verify molecular variability, 12 SCSMV isolates were sequenced and analyzed by comparing partial NIB–CP gene sequences. The nucleotide identity among Iranian isolates was 83.1–99.8%, indicating high nucleotide variability, while amino acid identity was 95.2–100%, which suggesting selection for amino acid conservation. They shared nucleotide identities of 76.2–99.1% with those of other SCSMV isolates available in GenBank, the highest with isolates from Pakistan (PAK), India (IND671) and China (M117, KT257289). Further analysis was conducted based on complete CI coding region to gain more insight into the phylogenetic relationships of Iranian SCSMV compared to those from other Asian countries. Iranian isolates shared identities of 79.8–89.0% (nucleotide) and 94.8–98.6% (amino acid) with those from other geographical regions in the CI gene. The highest nucleotide identity of Iranian isolates was with

isolates PAK (Pakistan), M121 (JQ975096, China) and IND671 (India), respectively. The phylogenetic trees (based on CI and NIB–CP) revealed the segregation of SCSMV isolates into two major divergent evolutionary lineages that reflect geographical origin of the isolates (with minor exception). Phylogenetic analyses grouped Iranian SCSMV isolates together with isolates from Pakistan, India and just one Chinese isolate in group II. Biological results showed that Iranian SCSMV isolates infect sugarcane, sorghum, maize and some wild grasses, causing mosaic symptoms on the leaves.

Keywords Genetic diversity · Iran · Phylogenetic analysis · Sugarcane streak mosaic virus · Sugarcane

Introduction

Sugarcane is reported to be affected by several viral diseases, of which sugarcane mosaic disease (SMD) is most widely spread [9, 28]. This disease is commonly associated with strains of *Sugarcane mosaic virus* (SCMV, *Potyvirus*), *Sorghum mosaic virus* (SrMV, *Potyvirus*) and Sugarcane streak mosaic virus (SCSMV, *Poacevirus*) in the family *Potyviridae* [30, 31, 42, 43]. SCSMV is an important viral pathogen affecting sugarcane yield, widely prevalent in most of the Asian countries [2, 29, 40]. It can be transmitted mechanically, and no insect vector has yet been reported for the virus [15, 17, 43]. SCSMV has flexuous filamentous particles (890 × 15 nm) which encapsidate a single-stranded, positive-sense RNA genome of about 10 kb [13]. The genomic RNA of SCSMV contains single open reading frame (ORF) which encodes a large polyprotein that is autocatalytically cleaved into at least ten mature proteins: P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIB and CP

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✉ Mohsen Mehrvar
mehrvar@um.ac.ir

¹ Department of Plant Pathology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

² Department of Biology, Faculty of Basic Sciences, University of Mazandaran, Babolsar, Iran

[19, 36, 43]. Like other members of the family *Potyviridae*, an additional protein (pretty interesting *Potyviridae* ORF, PIPO) was also predicted to be expressed as a fusion protein with the N-terminal part of P3 (P3N-PIPO) via ribosomal frameshifting or transcriptional slippage [5]. SCSMV was detected for the first time in 1998 from quarantined sugarcane germplasm imported from Pakistan into the USA [10]. Subsequently it was detected in most of the sugarcane growing Asian countries including Iran [4, 6, 10, 12, 19, 22, 25, 29]. Sugarcane is one of the most important strategic industrial crops in Iran, covering approximately 94,000 ha of land areas with an average yield of 90 tonnes of cane per ha [7]. It is cultivated mainly in Khuzestan province, southwest of Iran. SCSMV is known to be one of the causative agents of sugarcane mosaic disease in Iran [22, 25]. Thus, understanding the genetic diversity of SCSMV is helpful to develop a reliable diagnosis and management strategies for SMD. The present study was carried out to investigate the partial prevalence and genetic diversity of SCSMV isolates in Iran. Therefore, the molecular variability in the partial Nib-CP region sequences of 17 Iranian isolates of SCSMV (12 isolates of this study plus five isolates deposited in the GenBank previously) were analyzed and compared with sequences from other parts of the world available in the GenBank. The nucleotide sequences of the genomic portion spanning the C-terminal part of Nib and N-terminal part of CP coding regions were used for genetic diversity and phylogenetic analysis as more sequence of this region was available in the GenBank. In addition to this, the N-terminal part of the CP is the most variable region, commonly used as a molecular marker for differentiating the isolates in members of the family *Potyviridae* and other plant viruses [12, 32, 38]. Consequently, sequence variability of the CP gene would increase the statistical power for analysis of the SCSMV population diversity. Further, the complete cylindrical inclusion (CI) nucleotide sequences of two isolates were obtained to understand more details of the exact phylogenetic position of Iranian SCSMV. Comparisons using the CI-coding region is most accurately reflected those for the complete ORF and this region would therefore be the best for diagnostic and taxonomic purposes if only a subportion of the genome were sequenced [1]. Furthermore, to have a clear view of the biological properties of Iranian SCSMV, the host range of the virus was also investigated.

Materials and methods

Virus isolates, RT-PCR, cloning and sequencing

During August to October 2014 and 2015, 65 sugarcane leaf samples exhibiting mosaic and streaking symptoms were collected from the major growing region of sugarcane in

Iran, Khuzestan province. Collected samples were chosen from common sugarcane cultivars: CP48-103, CP57-614, CP69-1062, CP63-588 and CP78-1628. Total RNA was extracted from the samples using RNeasy Mini Kit (Qiagen, Hilden, Germany) and used as template for reverse transcription test. RT-PCR analysis was performed by use of specific primer pair SCSMV-F (5'-GGCAAGTY-GAGTAYATGTCGCA-3') and SCSMV-R (5'-GTGGTGTGTAYCTCATCATCTGC-3') [22], which amplify 570 bp from the Nib and CP gene sequences. Synthesis of complementary DNA (cDNA) was performed using antisense primer and the Moloney murine leukemia virus (MMuLV) reverse transcriptase (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions. For this, 4 microliters (μl) of purified RNA was mixed with two μl of antisense primer (10 pmol), heated at 65 °C for 5 min and chilled on ice. The mixture was added to reverse transcription mix (50 mM Tris-HCl pH 8.3, 50 mM KCl, 4 mM MgCl₂, 10 mM dithiothritol, 1 mM of each dNTP, 200 units of MMuLV-RT) in a final volume of 20 μl and incubated at 42 °C for 60 min. PCR was carried out using Taq PCR Master Mix (Ampliqon, Denmark) according to the manufacturer's instructions. The PCR thermal profile was 94 °C for 3 min; followed by 35 cycles of 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 1 min; and 72 °C for 10 min as a final extension after the last cycle. Two positive samples were randomly selected and the CI coding regions of them were amplified using specific primers pairs described by Parameswari et al. [29] in RT-PCR method. PCR products were analyzed on 1% agarose gel and purified from the gel using the Qiaquick gel extraction kit (Qiagen). The purified PCR products were ligated into pTZ57R/T vector (Thermo Scientific) according to manufacturer's instructions. After the recombinant vectors were transformed into competent cells of *Escherichia coli* DH5α, the positive clones were screened by colony-PCR with M13-forward and reverse primers. Plasmid DNA from recombinant clones was purified using a Plasmid Miniprep Kit (Qiagen) and the purified recombinant plasmids were bi-directionally sequenced (Macrogen Inc., South Korea). Consensus sequences were verified using the BLAST program in NCBI database.

Sequence and phylogenetic analysis

To investigate phylogenetic relationships among SCSMV isolates, sequence subsets consisting of Nib-CP and CI gene sequences of the all SCSMV isolates (Table S1) were analyzed separately. The aligned sequences for CI and partial Nib-CP regions were 1968 and 570 nucleotides long, respectively. The pairwise nucleotide (nt) and amino acid (aa) sequence identity scores were represented as color-coded blocks using SDT v.1.2 software [26].

Phylogenetic trees were inferred by the maximum-likelihood (ML) method implemented in MEGA6 [35], with 1000 bootstrap replicates. The genetic distances of intra- and inter-clusters were computed using MEGA6 with 1000 bootstrap replicates.

Biological characteristics of the Iranian SCSMV

The partial host range of the one Iranian SCSMV isolate (IR-Khuz6a) was investigated by mechanical inoculation to a variety of hosts. For this purpose, the sugarcane leaf infected with IR-Khuz6a was homogenized in 50 mM phosphate buffer (pH 7.0) and then centrifuged at 8000 rpm/min for 2 min. The supernatant was mechanically inoculated onto carborandum-dusted leaves of 32 plant species/cultivars belong to five families: *Gramineae* (or *Poaceae*) (16), *Solanaceae* (7), *Leguminosae* (4) *Chenopodiaceae* (2) and *Cucurbitaceae* (3) (Table S2) at the stage of two to three leaves. At least, six plants of each species or cultivar were mechanically inoculated and two left uninoculated as controls. Inoculated plants were kept in an insect-proof greenhouse at 23–25 °C with supplementary lighting (18 h photoperiod). Observation of symptom was conducted at 3 weeks after inoculation and the presence of the virus was assayed using RT-PCR.

Results

Sequence determination and data analysis

17 samples (26.1%) were found to be infected with SCSMV by RT-PCR amplification using primers amplifying the partial NIB–CP coding region. The partial NIB–CP sequences of 12 SCSMV isolates were determined, analyzed and deposited in the GenBank database (accession numbers MF580141–MF580152). In addition, the full-length CI coding regions of two isolates were deposited to GenBank and allocated the accession numbers of MF579612 and MF579613 (Table 1).

Sequence comparisons

The pairwise sequence identity of partial NIB–CP genes among all 39 SCSMV isolates (12 from this study and 27 from GenBank) ranged from 76.2 to 99.8% at the nt sequence level (Fig. 1) and from 84.5 to 100% at the aa sequence level (data not shown). Comparative sequence analysis of Iranian isolates revealed 83.1–99.8% nt sequence identity among them, indicating a high nt variability. The nt sequence identity scores between Iranian and GenBank SCSMV isolates ranged between 76.2% (between isolates IR-Khuz8, Kh3 [KR868693] and MYA-

Formosa [KJ187049]) and 99.1% (between isolates IR-Khuz13 and M117 [KT257289]) (Fig. 1). In the deduced aa sequences, Iranian isolates shared 95.2–100% identity among themselves and 87.7–100% with the SCSMV isolates from other countries. High nt differences and low aa differences between the Iranian isolates indicate that selection for aa conservation. Compared with other isolates, the lowest aa identity (87.7%) was observed between isolates IR-Khuz6a, IR-Khuz30 and Andhra Pradesh (Y17738, India) and the highest (100%) was between IR-Khuz27 and PAK (NC_014037, Pakistan) as well as between IR-Khuz13 and M117 (KT257289, China). Isolate IR-Khuz45 exhibited the highest nt identity (98.2%) to that of Indian isolate IND671; Isolates IR-Khuz6a, IR-Khuz57a, IR-Khuz8, IR-Khuz30, IR-Khuz25, IR-Khuz27, Kh3 and Kh39 were more identical to the Pakistani isolate PAK (with 94.0–96.8% nt identity); while Isolates IR-Khuz15, IR-Khuz9, IR-Khuz65, IR-Khuz13, IR-Khuz12, Kh33, Kh34 and Kh36 were more resemble to Chinese isolate M117 (KT257289) (with 92.5–99.1% nt identity) (Fig. 1). The identities between the complete CI coding region of all SCSMV isolates were from 78.9 to 100% at nt level (Fig. 2) and from 94.8 to 100% at aa level (data not shown). SCSMV-IR-Khuz6 and IR-Khuz57 CI coding region sequences were 97.9 and 98.8% identical at nt and aa levels, respectively. The CI coding region of IR-Khuz6a and IR-Khuz57a shared nt identities of 79.8–89.0% with those of 46 other SCSMV isolates available in the GenBank, the highest with the isolate PAK (respectively 89.0 and 88.0%), and the lowest with isolate THA-NP3 (JN163911, Thailand) (respectively 79.8 and 80.4%) (Fig. 1). At aa level, IR-Khuz6a and IR-Khuz57a showed the highest identities (98.6 and 98.0%) with isolate M121 (JQ975096, China) and the lowest (95.7 and 94.8%) with isolate M111 (KT257238, China). Due to lack of availability of CI gene of M117 (KT257289) and NIB–CP gene sequences of M121 (JQ975096) in GenBank, they were not included in each of relevant analysis.

Phylogenetic analysis of SCSMV isolates

The SCSMV CI and partial NIB–CP region sequences were subjected to phylogenetic analyses, with those of *Triticum mosaic virus* (TriMV) (representing the nearest species) isolate (NC_012799) as outgroup. The NIB–CP phylogenetic tree revealed the segregation of SCSMV isolates into two divergent evolutionary lineages. These two major clades, designated as I and II clades, including 18 and 21 isolates, respectively (Table S1 and Fig. 1). Members of each group were further divided into two subgroups: A, B. Group I consisted of most of Chinese isolates plus isolates from Japan, Indonesia, Thailand, Myanmar and India. The TPT isolate from India, form a separate subgroup in this

Table 1 Sources and GenBank accession numbers of Iranian Sugarcane streak mosaic virus isolates reported in this study

Isolate	Source	Accession number	Genomic region
IR-Khuz6a	Sugarcane (Iran, Khuzestan)	MF580141	Partial Nib-CP
IR-Khuz8	Sugarcane (Iran, Khuzestan)	MF580142	Partial Nib-CP
IR-Khuz9	Sugarcane (Iran, Khuzestan)	MF580143	Partial Nib-CP
IR-Khuz12	Sugarcane (Iran, Khuzestan)	MF580144	Partial Nib-CP
IR-Khuz13	Sugarcane (Iran, Khuzestan)	MF580145	Partial Nib-CP
IR-Khuz15	Sugarcane (Iran, Khuzestan)	MF580146	Partial Nib-CP
IR-Khuz25	Sugarcane (Iran, Khuzestan)	MF580147	Partial Nib-CP
IR-Khuz27	Sugarcane (Iran, Khuzestan)	MF580148	Partial Nib-CP
IR-Khuz30	Sugarcane (Iran, Khuzestan)	MF580149	Partial Nib-CP
IR-Khuz45	Sugarcane (Iran, Khuzestan)	MF580150	Partial Nib-CP
IR-Khuz57a	Sugarcane (Iran, Khuzestan)	MF580151	Partial Nib-CP
IR-Khuz65	Sugarcane (Iran, Khuzestan)	MF580152	Partial Nib-CP
IR-Khuz6a	Sugarcane (Iran, Khuzestan)	MF579612	Complete CI
IR-Khuz57a	Sugarcane (Iran, Khuzestan)	MF579613	Complete CI

group. The isolates in group I shared within-group identities of 93.7–99.8 and 94.7–100% at nt and aa level, respectively (Fig. 1). All of the isolates from Iran together with two isolates from India (IND671, AP1), and one isolate from China (M117) and Pakistan (PAK) fell into group II, indicating that most isolates of this group has been distributed in South and Southwestern Asia (with the exception of a Chinese isolate M117). These isolates shared within-group identities of 79.9–99.8 and 88.3–100% at nt and aa level, respectively (Fig. 1). Of seventeen isolates from Iran, eight were clustered with members of the subgroup IIA, and nine were clustered with the members of subgroup IIB (Fig. 1). The genetic distance among 17 Iranian SCSMV isolates was 0.091 ± 0.008 , indicating a high nt variability in this region. The mean dN/dS ratio for Iranian isolates was 0.886 (dN/dS ratios < 1), indicating most of nt substitutions were silent, encoding the same amino acid (results not shown), demonstrating purifying selection. The overall mean value of nt sequence diversity was 0.172 ± 0.005 . The genetic distance within group II was high (0.101 ± 0.009), indicating that the isolates in this group were genetically divergent. On the contrary, the genetic distance within group I was low (0.033 ± 0.003). The between-group genetic distances of the two groups (0.268 ± 0.018) were significantly higher than the within-group ones, suggesting that the phylogenetic clustering results of these isolates were reasonable. A similar phylogenetic tree was obtained from an alignment of the translated aa sequences of the same isolates. Such results were consistent with similarity plot analysis (data not shown). Additionally, phylogenetic tree was constructed using the full-length CI nt sequences obtained in this study (Table 1) with representative sequences present in the GenBank (Table S1 and Fig. 2). Sequence analysis based on the CI region revealed the existence of two distinct phylogroups

similar to the Nib-CP phylogenetic tree. Group I consisted of 43 SCSMV isolates from sugarcane in China, Japan, India, Indonesia, Thailand and Myanmar in which MYA-Formosa isolate, form a separate sublineage (IB). The isolates in group I shared within-group identities of 96.1–100 and 98.2–100% at nt and aa level, respectively (Fig. 2). Group II included five isolates from Iran, Pakistan (PAK), India (IND671) and China (M121) among which Iranian isolates formed separate subgroup (IIA). These isolates shared within-group identities of 85.9–97.9 and 97.6–98.9% at nt and aa level, respectively (Fig. 2). The overall mean distance was 0.065 ± 0.013 for the CI gene sequences. The genetic distances within group II was high (0.129 ± 0.019), indicating that the isolates in this group were the most genetically divergent. On the contrary, the genetic distance within group I was low (0.021 ± 0.001). The between-group genetic distance of the two groups (0.249 ± 0.021) was significantly higher than the within-group ones, confirming the phylogenetic grouping. These results were further supported by the amino acid phylogenetic tree and pairwise sequence distances (data not shown). As shown in the phylogenetic trees (Figs. 1, 2), either in the Nib-CP-based tree or the CI-based tree, the majority of SCSMV isolates are arranged within the two clades according to their country of origin.

Biological characteristics

The results of inoculation of the tested plants by Iranian SCSMV are listed in Table S2. Biological results showed that Iranian SCSMV could infect sugarcane, sorghum, maize and some wild grasses, such as *Sorghum bicolor* ssp. *drummondii*, *Sorghum halepense*, *Pennisetum glaucum*, *Digitaria sanguinalis*, *Rottboellia exaltata* and *Dactyloctenium aegyptium*. Most of the infected plants showed

Fig. 1 Maximum-likelihood phylogenetic tree constructed based on partial NIB-CP aligned nucleotide sequences of SCSMV isolates from this study (marked) and their counterparts in the GenBank database from other parts of the world. The phylogenetic tree was generated in MEGA6. A *Triticum mosaic virus* (TriMV) isolate (accession no. NC_012799) was used as outgroup. Isolates are indicated in the trees

mosaic symptoms in the form of interveinal chlorotic streaks or stripes on the leaves. However, SCSMV was detected on *Dactyloctenium aegyptium* by RT-PCR without specific symptom (data not shown). The wild grasses infected by SCSMV are potentially the natural hosts of the virus in the field and thus could become the place for virus survival and reservoir.

Discussion

Sugarcane is one of the most economically important crops in Iran which has an important role in sugar industry. Mosaic is one of the most extensively distributed diseases

by accession number/isolate name/geographical origin of collection. Bootstrap values (1000 replicates) are shown at the branch internodes. Bootstrap support values equal or greater than 50% are indicated above the nodes. Two dimensional nucleotide diversity plot constructed based on SDT MUSCLE alignment

of sugarcane and has been reported in almost all of the major sugarcane-producing countries. It has been reported that three viruses, SCMV, SrMV and SCSMV naturally cause sugarcane mosaic in the field [30, 42, 43]. According to previous study, the sugarcane varieties under cultivation in Iran are widely infected with SCMV as evidenced by about 92% infection in surveyed samples [24]. However, according to results of this study, SCSMV is not widespread in sugarcane fields of Khuzestan province, the major sugarcane-growing region of Iran, probably due to lack of vector transmission or other unknown reasons. To evaluate molecular variability and phylogenetic relationships of Iranian SCSMV isolates to those from other Asian countries, two datasets were analyzed using the partial NIB-CP

Fig. 2 Maximum-likelihood phylogenetic tree calculated from the complete cylindrical inclusion (CI) gene nucleotide sequences of 48 SCSMV isolates (two from this study and 46 from the GenBank database), and graphical representation of pairwise nucleotide identity (with percentage identity scale). Tree was constructed by MEGA6. A *Triticum mosaic virus* (TriMV) isolate (accession no. NC_012799) was used as outgroup. The isolates obtained in this study are indicated

and CI coding regions individually. Pairwise comparisons and phylogenetic analysis clearly showed the existence of two groups in concordance with previously reports [11, 20]. According to genetic differentiation and phylogenetic analysis of full-length CI sequences, group I consisted of isolates that originated from East Asia (China and Japan) and Southeast Asia (Myanmar, Indonesia and Thailand), except the TPT which was isolated from India, while group II consisted of isolates that originated from Southwest Asia (Iran) and South Asia (India and Pakistan), except Chinese isolate M121 (JQ975096). This suggests that the majority of SCSMV isolates are divided into two major groups corresponding to their geographical situation,

by “black triangle”. The GenBank accession number, the name of each isolate and its country of origin are listed. Numbers at each node indicate bootstrap percentages based on 1000 replications. Values are shown only when the values are equal or greater than 50%. The graphical representation of pairwise identities is based on SDT MUSCLE alignment and the colour table on the right describes the percentage identity that each colour represents

in consistent with previous findings [20]. The CI gene nt sequences of Iranian isolates were more identical to the Pakistani isolate PAK followed by a Chinese isolate M121 (JQ975096) and an Indian isolate IND671 (JN941985), respectively. However, they shared the highest aa identities respectively with isolates M121 (JQ975096), IND671 and PAK in the CI gene. In the Nib-CP-based tree, the majority of SCSMV isolates were also arranged within the two clades according to their country of origin. Nevertheless, one of the Chinese isolates (M117, KT257289) and two of the Indian isolates (TPT and Andhra Pradesh) were clustered within the group II and group I clade, respectively. The possibility to explain similarities of geographically

distant SCSMV isolates is the movement and exchange of germplasm. SCSMV showed a spatial distribution pattern with a greater genetic distance in the Southwest (Iran) or South (India and Pakistan) Asian group than in the subpopulation composed of isolates from East (China and Japan) or Southeast (Thailand, Indonesia and Myanmar) Asia [12, 20]. The higher genetic diversity observed between groups than within groups of SCSMV populations suggests that there is more frequent gene flow within groups than between groups. Transmission characteristics of the virus and the existence of physical and quarantine barriers between Asian countries may explain the SCSMV population pattern [12, 20]. The value of nucleotide diversity (π) was 0.091 ± 0.0078 for partial NIB-CP regions of Iranian SCSMV isolates, implying important variability within isolates. Iranian isolates were more resemble to Pakistani (PAK), Indian (IND671) and Chinese (M117) isolates in partial NIB-CP. This suggests that there are different SCSMV strains or isolates infecting sugarcane in Iran. As SCSMV can be transmitted through vegetative sett, it seems that during commercial exchanges, Iranian isolates have been introduced into the country from sugarcane-producing Asian countries such as India, Pakistan and China via contaminated vegetative cuttings. Particularly from India as it has been proposed that SCSMV has probably been originated from northern India and spread to most of sugarcane growing Asian countries via infected sugarcane germplasm materials [12, 20]. The results presented here advance our understanding of the molecular variability of SCSMV Iranian isolates infecting sugarcane. SCSMV is frequently found in mixed infection along with SCMV and or SrMV [21, 39–42]. Sugarcane mosaic disease in Iran is reported to be caused by SCMV, SCSMV or IJMV (*Iranian johnsongrass mosaic virus*) lonely or in combinations [23, 24]. Mixed infection of sugarcane by two or more viruses leads to development of complex diseases and consequent difficulty in identifying the components of the disease complex. On the other hand, the possibility of recombination between the two species cannot be ruled out when a mixed infection takes place on the same sugarcane leaf [3, 8, 18, 34]. Recombination plays a significant role in increasing plant virus variability and adaptation to new hosts and may lead to emergence of new variants and resistance-breaking strains [27, 37]. The host range test using mechanical inoculation indicated that in addition to sugarcane SCSMV infects other cultivated plants like sorghum, maize and some weed grasses (Table S2). These observations are in agreement with the results reported by Damayanti and Putra [6], Hema et al. [14], Jensen [16], Singh and Rao [33] and Xu et al. [43]. In some plantations in Iran, maize is often planted close to sugarcane crops and weed grasses such as *Rottboellia exaltata*, *Dactyloctenium aegyptium* and *Sorghum*

halepense are commonly found in sugarcane fields. These plants could be as alternative hosts of SCSMV and thus serve as potential reservoirs of the virus in the field and contribute to its off-season survival. Therefore, avoidance of maize cultivation near sugarcane crops and control of those weeds are useful strategies for controlling of the disease [6]. This information provided an insight on the population structure and genetic diversity of SCSMV isolates from Iran that would be subsequently useful in epidemiological studies, breeding of resistant varieties and designing effective control strategies. Further studies are required to assess relative proportion of each viruses causing sugarcane mosaic as well as studying economic losses caused by mixed infection of either virus in sugarcane. Such additional information can help in the development and adoption of preventive control measures.

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