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Incidence, genomic diversity, and evolution of strawberry mottle virus in China

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Abstract: Strawberry mottle virus (SMoV) is one of the most common viruses infecting strawberries, causing losses to fruit yield and quality. In this study, 165 strawberry leaf samples were collected from six provinces of China, 46 of which tested positive for SMoV. The complete genome sequences of 11 SMoV isolates were obtained from Liaoning (DGHY3, DGHY16-2, DGHY17, DGHY20-2, DGHY21, DGHY26-2), Shandong (SDHY1, SDHY5, SDHY31-2, SDHY33-2), and Beijing (BJMX7). The RNA1 and RNA2 nucleotide identities between the 11 Chinese isolates were 95.4–99.3% and 96.3–99.6%, respectively, and they shared 78.4–96.6% and 84.8–93.5% identities with the available SMoV isolates in GenBank. Recombination analysis revealed that Chinese isolate SDHY33-2 and Canadian isolates Ontario and Simcoe were recombinants, and recombination events frequently occurred in the 3' UTR of SMoV. Phylogenetic analysis showed that in an RNA1 tree, most Chinese isolates clustered into the same group while isolate DGHY17 clustered into another group together with Czech isolate C and three Canadian isolates. In an RNA2 tree, all Chinese isolates clustered into a single group. The phylogenetic analysis based on nucleotide sequences was consistent with the results based on coat protein (CP) and RNA-dependent RNA polymerase (RdRp). Further evolutionary analysis indicated that negative selection drives SMoV evolution, and gene flow plays a major role in genetic differentiation. Additionally, reassortment and recombination also influence the evolution of SMoV. To our knowledge, this is the first report of the complete genome of SMoV isolates from China and a detailed analysis of the SMoV population structure.

Introduction

Strawberry mottle virus (SMoV) was first described as a distinct virus in 1946 (Prentice and Harris, 1946; Martin and Tzanetakis, 2006). It is the most economically important and common virus infecting strawberry (*Fragaria* spp.) in Europe and North America (Tzanetakis and Martin, 2013; Cieślińska, 2019). The virus is found in all areas where its vectors (*Apbis gossypii* and *Cbaetosipbon* species) are present, and it can infect all species of strawberry (Thompson and Jelkmann, 2003). SMoV causes up to 30% losses in fruit yield and runner production, even though most modern cultivars do not exhibit any obvious symptoms upon a single infection (Thompson *et al.*, 2002; Yang *et al.*, 2009; Tzanetakis and Martin, 2013). When co-infecting with other strawberry viruses such as SVBV, SMYEV, and/or SCV, SMoV can reduce the vigor and yield of strawberry plants by up to 80% (Thompson and Jelkmann, 2003). SMoV contains two positive-sense RNA genome segments, and each encodes a polyprotein (Thompson *et al.*, 2002). The RNA1 polyprotein (P1) is cleaved by 3CL-Pro into a putative helicase (Hel), a 3C-like protease (3CL-Pro), a viral genome-linked protein (VPg), and an RNA-dependent RNA polymerase (RdRp) at its C-terminus, and two unknown proteins (X1 and X2) at its N-terminus (Mann *et al.*, 2017). The 3CL-Pro enzyme also cuts the RNA2 polyprotein (P2) at a single site to release the predicted movement protein (MP). The RNA2-encoded glutamic protease then cleaves P2 at two sites to release the putative coat protein (CP), glutamic protease (Pro2Glu), and an unknown protein (Mann *et al.*, 2017; Mann *et al.*, 2019). In 2004 SMoV was reported to be a member of the *Sadwavirus* genus (Martin and Tzanetakis, 2006; Sanfaçon *et al.*, 2020). However, it was demoted from the genus *Sadwavirus* in 2009 because the number of CPs was unclear (Sanfaçon *et al.*, 2009). Recently, analysis of cleavage sites in P2 of SMoV revealed that this virus encodes one putative

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large CP (Mann *et al*., 2019), indicating that SMoV is not a typical member of the *Sadwavirus* genus, which possess two CPs. Additionally, SMoV also encodes a novel type of viral glutamic protease that is not present in the *Secoviridae* family, apart from the black raspberry necrosis virus (BRNV) (Mann *et al*., 2019; Sanfaçon *et al*., 2020). Therefore, the International Committee on the Taxonomy of Viruses (ICTV) *Secoviridae* Study Group proposed to create a subgenus called '*Stramovirus*' within the genus *Sadwavirus*. SMoV and BRNV were subsequently classified into the subgenus *Stramovirus* (Sanfaçon *et al*., 2020).

Based on data from the Food and Agriculture Organization of the United Nations (FAO), China is the world's largest producer of strawberries in terms of area harvested. Some viruses, including SMoV, strawberry vein banding virus (SVBV), strawberry mild yellow edge virus (SMYEV), strawberry crinkle virus (SCV) (Wang *et al*., 1991), cucumber mosaic virus (CMV) (Chen *et al*., 2014), strawberry necrotic shock virus (SNSV) (Li and Yang, 2011) and various others have been found to infect strawberry in the major production areas of China. Among these viruses, SMoV and SVBV occur frequently in most strawberry production areas of China (Xi, 2017; Wang *et al*., 2020). In recent years, SMoV was detected by transmission to susceptible indicator plants and reverse transcription-polymerase chain reaction (RT-PCR; Thompson and Jelkmann, 2003), but more convenient, sensitive, and specific detection methods such as real-time quantitative RT-PCR (RT-qPCR) and enzyme-linked immunosorbent assay are unestablished. Up to December 2020, seven complete genome sequences (one from the Netherland and six from Canada) of SMoV are available in the GenBank database; however, the complete genome sequence from China is still unreported, and the evolutionary characteristics between them remain unknown.

Herein, the complete genome sequences of 11 SMoV isolates from China were determined and annotated, and the sequence, recombination, phylogenetic, and population structure analyses were also performed. Our study would lay a foundation for developing molecular diagnosis and effective disease control strategies for this damaging pathogen.

Materials and Methods

Sampling and detection

In this study, 165 strawberry leaf samples were collected from the main strawberry production areas of China, including Beijing (68 samples), Anhui (20 samples), Shandong (22 samples), Liaoning (35 samples), Xinjiang (17 samples), and Sichuan (3 samples). The presence of SMoV, SVBV, SMYEV, SCV, CMV, and SNSV was investigated. Total RNA was extracted from leaf tissues using an E.Z.N.A. Plant RNA Kit (Omega Bio-tek, Norcross, USA) following the manufacturer's instructions. For reverse transcription (RT), total 10 μL of reaction mixture containing 2 μL M-MLV 5× reaction buffer, 2 μL dNTPs (10 mM), 0.5 μL random hexamer primer (10 mM), 0.5 μL oligo dT (18) primer (10 mM), 0.25 μL recombinant RNasin® ribonuclease inhibitor (50 U/μL), 0.25 μL M-MLV Reverse Transcriptase (200 U/μL ; Promega, Madison, USA), 1 μL total RNA (1 μg/μL) and 3.5 μL nuclease-free water was prepared and incubated at 42°C for 1 h. PCR was performed using *Taq* polymerase (Tiangen, Beijing, China) with specific primers (Tab. S1). The reaction mixture (20 μL) consisted of 10 μL 2× Taq PCR Mix, 0.8 μL sense and antisense primers, respectively, 1 μL cDNA and 7.4 μL nuclease-free water, and then was denatured at 95°C for 3 min and followed by 35 cycles of PCR amplification at 95°C for 30 s, 50°C (SMoV, SVBV and SMYEV; 54°C for SCV; 58°C for SNSV; 56°C for CMV) for 30 s, 72°C for 30 s and a final elongation step of 5 min at 72°C. The 11 SMoV-positive samples (DGHY3, DGHY16-2, DGHY17, DGHY20-2, DGHY21, and DGHY26-2 are 'Benihope' cultivars from Donggang in Liaoning province; SDHY1, SDHY5, SDHY31-2, and SDHY33-2 are 'Benihope' cultivars from Shandong, and BJMX7 is a 'Miaoxiang 7' cultivar from Beijing) were subjected to full-length amplification of the SMoV genome.

Genome cloning and sequencing

For determination of the 5' and 3' cDNA ends of the genomic RNA, a SMARTer RACE 5'/3' Kit (Clontech, California, USA) was used according to the manufacturer's instructions. For genomic sequences, reverse transcription was performed using a PrimeScript II 1st Strand cDNA Synthesis Kit (TaKaRa, Kusatsu, Japan), and Q5 High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, USA) was subsequently employed for PCR. Three overlapping PCR fragments were amplified for each RNA1 and RNA2 to obtain the SMoV isolates DGHY3, DGHY16-2, DGHY17, DGHY20-2, DGHY26-2, SDHY1, SDHY5, SDHY31-2, SDHY33-2, and BJMX7. The isolate DGHY21 was amplified successfully using primers of 5' and 3' terminal sequences. The primers used in this study were listed in Tab. S1. The PCR products were gel-purified (Axygen, Union, USA), cloned into the pTOPO-Blunt vector (Aidlab, Beijing, China), and sequenced by Sangon Biotech (Shanghai) Co., Ltd. The resulting sequences were assembled with default parameters using Seqman within DNASTAR Lasergene v7.1.0 (DNASTAR Inc., Madison, USA).

The complete genome sequences of 11 SMoV Chinese isolates have been deposited in GenBank with the following accession numbers: for SMoV RNA1 sequence, DGHY3, DGHY21, SDHY1, SDHY5, and BJMX7 is MT070747–MT070751, respectively; DGHY16-2, DGHY17, DGHY20-2, DGHY26-2, SDHY31-2, and SDHY33-2 is MT991093–MT991098, respectively. For SMoV RNA2 sequence, DGHY3, DGHY21, SDHY1, SDHY5, and BJMX7 is MT070752–MT070756, respectively; DGHY16-2, DGHY17, DGHY20-2, DGHY26-2, SDHY31-2, and SDHY33-2 is MT991099–MT991104, respectively (https://www.ncbi.nlm.nih.gov/nuccore/?term=strawberry+mottle+virus).

Sequence analysis

Nucleotide (nt) and deduced amino acid (aa) sequences were aligned using ClustalW in MegAlign of DNASTAR Lasergene v7.1.0. The percentage of homology was then calculated according to the Martinez-NW method using MegAlign v7.1.0. Phylogenetic trees were constructed with MEGA 6.06 software (Tamura *et al*., 2013) using maximum likelihood (ML; Tamura and Nei, 1993) and neighbor-joining (NJ;

Saitou and Nei, 1987) methods with 1000 bootstrap replications (Felsenstein, 1985). SMoV RNA, CP, and RdRp full coding sequences of 11 Chinese isolates and ten (one from the Netherland, six from Canada, and three from the Czech Republic) available isolates in GenBank by December 2020 were analyzed. Recombination events were examined based on SMoV complete genome sequences using a suite of seven prediction programs implemented in the RDP4 software package (Martin *et al.*, 2015). Only events detected by six or more detection methods with default parameters (highest acceptable probability value = 0.05) were considered. DnaSP v5.1 (Librado and Rozas, 2009) was used to estimate Tajima's *D*, Fu and Li's D^* and F^* statistical tests, nonsynonymous (d_N) and synonymous (d_S) substitutions, nucleotide and haplotype diversity, genetic differentiation, and gene flow for SMoV coding regions.

Results

The incidence and distribution of strawberry viruses

To investigate the incidence and distribution of six viruses infecting strawberry plants, a total of 165 strawberry leaf samples were collected randomly in six provinces of China. Among these samples, 46 (27.9%) were positive for SMoV, 70 (42.4%) for SVBV, while negative for the other four viruses (Tab. S2). All SMoV-positive samples which were collected from Liaoning, Shandong, and Beijing tested positive for SVBV. Liaoning province suffered the worst viral disease rate, with single infection rates for SMoV and SVBV of 77.1% (27/35) and 82.9% (29/35), respectively, and a mixed infection rate of 77.1% (27/35; Tab. S2 and Fig. S1). But no significant correlation was found between the viral presence and symptom. Asymptomatic samples from Beijing (Fig. 1H) and Liaoning (Fig. 1J) showed SMoV and SVBV positive while symptomatic samples with mottled (Figs. 1D, 1E, 1G and 1L), distorted (Figs. 1A and 1D), crinkled (Figs. 1C and 1E), deformed (Fig. 1G) and/or purplish red (Fig. 1K) on the leaves were negative for the six tested viruses.

Genomic characteristics of the Chinese SMoV isolates

The genomic RNA1 sequences of the 11 Chinese isolates ranged from 7017 to 7027 nt, excluding the poly (A) tail (Tab. S3). All of these 11 isolates encoded polyprotein P1. Previously identified P1 cleavage sites (Q^{146}/G, Q^{348}/S,

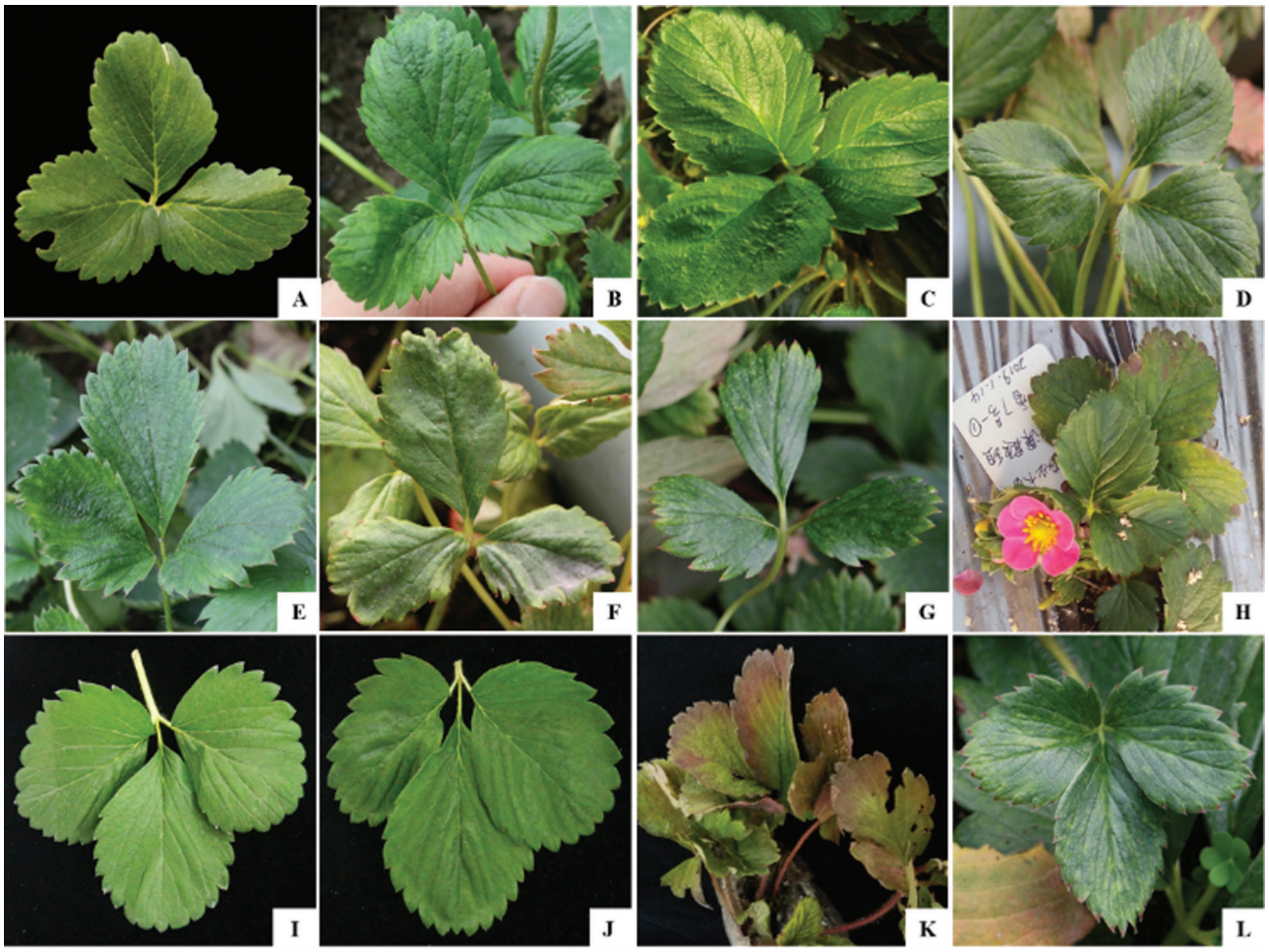

FIGURE 1. The pictures of representative samples, collected from different regions of China.
(A, B) From Xinjiang, A, negative for the six tested viruses, B, positive for SVBV; (C, D) From Sichuan, negative for the six tested viruses; (E, F) From Shandong, E, negative for the six tested viruses, F, positive for SMoV and SVBV; (G, H) From Beijing, G, negative for the six tested viruses, H, positive for SMoV and SVBV; (I, J) From Liaoning, I, negative for the six tested viruses, J, positive for SMoV and SVBV; (K, L) From Anhui, negative for the six tested viruses.

Q^{964}/G, Q^{989}/G, and Q^{1220}/G) were conserved in all Chinese isolates. Subsequent pair-wise comparisons of the RNA1 and polyprotein sequences were performed, and the nt and aa identities were 95.4–99.3% and 98.1–99.7%, respectively, among the 11 Chinese isolates. Collectively, the 11 Chinese isolates shared the highest sequence identities (95.5–96.6% nt and 98.3–99.3% aa) with Canadian isolate NSper3 and the lowest sequence identities (78.4–79.5% nt and 88.3–89.2% aa) with Netherlandish isolate 1134.

The genomic RNA2 sequences of the 11 Chinese isolates ranged from 6298 to 6324 nt, excluding the poly (A) tail (Tab. S3). These 11 isolates all encoded polyprotein P2. Previously identified P2 cleavage sites (E^{452}/G, P^{1101}/AFP, and P^{1444}/KFP) were conserved in all Chinese isolates. Pair-wise comparisons of the 11 Chinese isolates revealed nt and aa identifies of 96.3–99.6% and 97.7–99.9%, respectively. Additionally, the 11 Chinese isolates shared similar sequence identities (92.6–93.5% nt and 96.9–97.7% aa) with NB926, Ontario, and NSper17 isolates from Canada and isolate 1134 from the Netherlands (92.6–93.1% nt and 96.2–96.8% aa). The lowest sequence identities were shared with Canadian isolate NSper3 (84.8–85.2% nt and 94.4–94.7% aa). Overall, the genomic RNA1 region of the 11 Chinese isolates shared a higher sequence identity with Canadian isolates than Netherlandish isolate 1134, but there were no obvious geographic differences between RNA2 sequences.

Phylogenetic analysis

Phylogenetic trees were constructed based on SMoV RNA1, RNA2, CP, and RdRp full coding regions using the ML and NJ methods. Only ML trees are shown in this study because both ML and NJ trees displayed almost identical topologies. In the RNA1 tree, most Chinese isolates clustered into the same group while DGHY17 clustered into another group together with Czech isolate C and Canadian isolate NSper3, NSper17, and NB926, and shared a close relationship with Czech C. The Netherlandish isolate 1134 and the Canadian isolate NSper51 and Simcoe formed a different branch furthest from all Chinese isolates. Czech isolates A and B formed two separate groups, respectively (Fig. 2A). Similar results were obtained when the analysis was conducted with the RdRp coding region within RNA1 (Fig. S2A). These results indicated that Czech and Canadian isolates showed greater molecular variation than Chinese isolates.

In the RNA2 tree, all of the 11 Chinese isolates formed a single group, characterized by the close relationship with Czech isolate C and the furthest distance from Czech isolate A. Canadian isolate NB926 and NSper17 were grouped into another branch with Netherlandish isolate 1134. Meanwhile, Canadian isolates NSper51 and NSper3 formed a single group. Czech isolate B also formed a separate group (Fig. 2B). In the CP tree based on the full CP coding region within RNA2, isolates from China, Canada, the Netherlands, and the Czech Republic displayed phylogenetic relationships that were consistent with those based on the RNA2 whole coding sequences (Fig. S2B). Concurrently, phylogenetic analysis revealed no obvious tendency for isolates to group according to geographical origin among different countries, but a clear tendency among Liaoning, Shandong, and Beijing of China.

Recombination analysis

A recombination event was detected by all seven methods with *p*-values ranging from 3.708×10^{-159} to 3.891×10^{-36}, and the recombination junction located at the RNA1 422–1884 nt region of the Ontario isolate (Tab. S4). We also identified two recombination events that were supported with a high degree of confidence in the RNA2 of the SDHY33-2 isolate (4023–5081 nt) and the Simcoe isolate (2780–6306 nt), respectively (Tab. S4). Additionally, in the 3' UTR of SMoV RNA1 and RNA2, most SMoV isolates were detected recombination events with a moderate degree

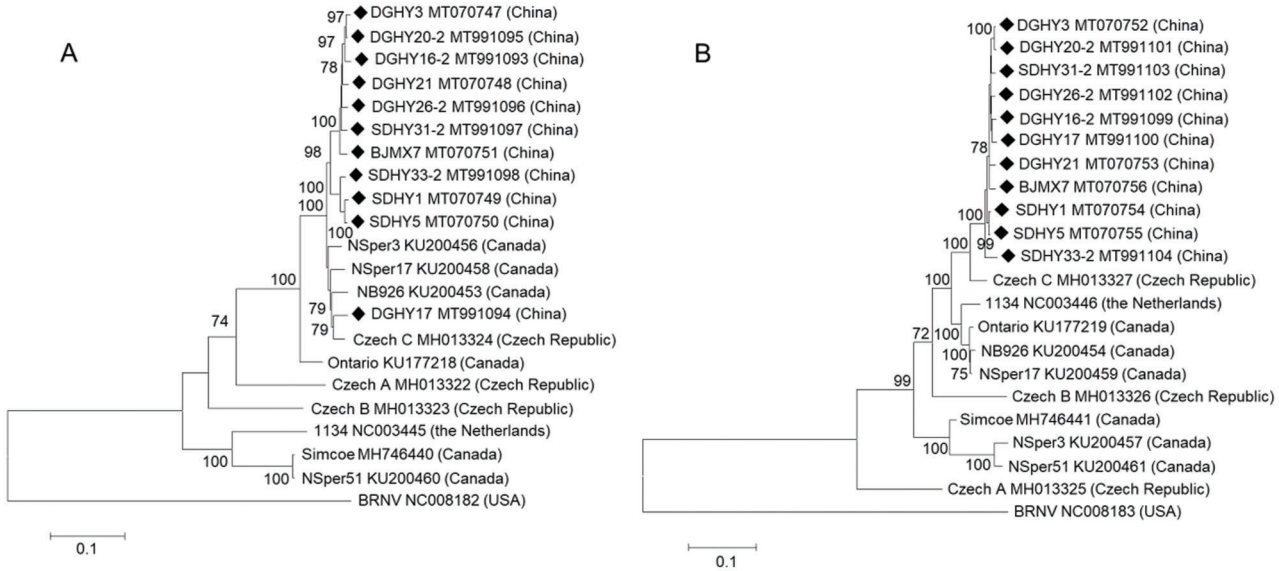

FIGURE 2. Phylogenetic analysis of strawberry mottle virus (SMoV) Chinese isolates and available isolates from GenBank based on whole coding sequences of RNA1 (A) and RNA2 (B) using the maximum likelihood (ML) method with 1000 bootstrap replicates.
Only values above 70% are shown. SMoV Chinese isolates are indicated by black diamonds. Black raspberry necrosis virus (BRNV) was used as the outgroup.

of confidence (Tab. S4). These results suggest that recombination events frequently occurred in the 3' UTR of SMoV.

Evolutionary analysis

Evolutionary analysis, including selection pressure, neutrality tests, population demography, genetic differentiation, and gene flow, were carried out on the SMoV RNA1 coding region (including X1, X2, Hel, Vpg, 3CL-Pro, and RdRp coding regions) and the RNA2 coding region (including MP, CP, Pro2Glu, and unknown protein-coding regions) based on geographic populations. For selection pressure, the ratios of non-synonymous (d_N) and synonymous (d_S) sites were calculated. The results revealed that the d_N/d_S ratios for each protein-coding region of SMoV isolates from China, Canada, and the Czech Republic were <1, suggesting that SMoV populations were under negative selection (Tab. 1).

For neutrality tests and population demography, we evaluated the values of Tajima's *D*, Fu and Li's *D** and Fu, and Li's *F** statistical tests, as well as haplotype and nucleotide diversities. Except for Canadian isolates, all protein-coding regions from different countries were negative, indicating that most SMoV populations were in a state of expansion. However, their *p*-values were not significant (Tab. 2). Haplotype and nucleotide diversities for all coding regions were estimated, and high haplotype diversity and low nucleotide diversity were presented for each coding region within individual geographic groups (Tab. 2).

For genetic differentiation, the *p*-values of *Ks**, *Z*, and *Snn* were calculated. The *P-Ks** and *Z* values for each protein-coding region between populations from China and populations from Canada and the Czech Republic were between 0.001 and 0.01, and <0.001, respectively, indicating

TABLE 1

Selection pressure analysis of SMoV protein coding regions based on geographical population

Protein encoded	Group	Number of sequences	dN[A]	dS[B]	dN/dS[C]
X1	All	21	0.02914	0.16898	0.172
	China	11	0.00728	0.01762	0.413
	Canada	6	0.04126	0.21909	0.188
	Czech Republic	3	0.03591	0.30753	0.117
	The Netherlands	1	——	——	——
X2	All	21	0.02398	0.39690	0.060
	China	11	0.00289	0.11080	0.026
	Canada	6	0.03314	0.46046	0.072
	Czech Republic	3	0.02464	0.62107	0.040
	The Netherlands	1	——	——	——
Hel	All	21	0.02152	0.44200	0.049
	China	11	0.00359	0.13655	0.026
	Canada	6	0.03071	0.50083	0.061
	Czech Republic	3	0.02698	0.78496	0.034
	The Netherlands	1	——	——	——
Vpg-Pro	All	21	0.03558	0.41771	0.085
	China	11	0.00508	0.13311	0.038
	Canada	6	0.05221	0.47971	0.109
	Czech Republic	3	0.04842	0.80314	0.060
	The Netherlands	1	——	——	——
RdRp	All	21	0.03280	0.43544	0.075
	China	11	0.00460	0.12647	0.036
	Canada	6	0.04784	0.50771	0.094
	Czech Republic	3	0.05280	0.81998	0.064
	The Netherlands	1	——	——	——
MP	All	21	0.01350	0.30184	0.045
	China	11	0.00372	0.05359	0.069
	Canada	6	0.01209	0.35553	0.034
	Czech Republic	3	0.03619	0.62280	0.058
	The Netherlands	1	——	——	——

(Continued)

Table 1 (continued).

Protein encoded	Group	Number of sequences	dN[A]	dS[B]	dN/dS[C]
CP	All	21	0.01416	0.38427	0.037
	China	11	0.00355	0.06054	0.059
	Canada	6	0.01141	0.39993	0.029
	Czech Republic	3	0.03601	0.73957	0.049
	The Netherlands	1	——	——	——
Pro2Glu	All	21	0.03012	0.37752	0.080
	China	11	0.00880	0.07256	0.121
	Canada	6	0.03470	0.39735	0.087
	Czech Republic	3	0.06327	0.74208	0.085
	The Netherlands	1	——	——	——
Unknown protein	All	20	0.03531	0.41987	0.084
	China	11	0.01285	0.15638	0.082
	Canada	6	0.03195	0.39564	0.081
	Czech Republic	3	0.05866	0.74142	0.079

Note: [A]average number of nonsynonymous substitutions per nonsynonymous site. [B]average number of synonymous substitutions per synonymous site. [C]d_N/d_S ratios represent estimated selection pressures; values of $d_N/d_S < 1.0$ indicate negative selection; $d_N/d_S = 1.0$ indicate neutral selection, and $d_N/d_S > 1.0$ indicate positive selection.

TABLE 2

Haplotype diversity, nucleotide diversity, and neutrality testing of SMoV protein coding regions based on geographical population

Protein encoded	Group	Number of sequences	Number of Haplotypes	Haplotype diversity[A]	Nucleotide diversity[B]	Tajima's D[C]	Fu and Li' D^*[D]	Fu and Li' F^*[E]
X1	All	21	19	0.990 ± 0.018	0.06200 ± 0.00659	−1.06268	−0.58534	−0.85566
	China	11	10	0.982 ± 0.046	0.00971 ± 0.00281	−0.18133	−0.34109	−0.34012
	Canada	6	5	0.933 ± 0.122	0.08326 ± 0.00818	1.22025	1.32127	1.42669
	Czech Republic	3	3	1.000 ± 0.272	0.09893 ± 0.01208	——	——	——
	The Netherlands	1	——	——	——	——	——	——
X2	All	21	21	1.000 ± 0.015	0.10937 ± 0.00663	−0.52194	−0.12248	−0.28709
	China	11	11	1.000 ± 0.039	0.02778 ± 0.00422	−0.56566	−0.77617	−0.81975
	Canada	6	6	1.000 ± 0.096	0.13069 ± 0.00846	1.83089	1.40418	1.64414
	Czech Republic	3	3	1.000 ± 0.272	0.16172 ± 0.01311	——	——	——
	The Netherlands	1	——	——	——	——	——	——
Hel	All	21	21	1.000 ± 0.015	0.11463 ± 0.00396	−0.88247	−0.31101	−0.57032
	China	11	11	1.000 ± 0.039	0.03292 ± 0.00259	−0.61100	−0.75231	−0.81339
	Canada	6	6	1.000 ± 0.096	0.13525 ± 0.00507	0.90532	1.13728	1.19737
	Czech Republic	3	3	1.000 ± 0.272	0.19462 ± 0.00807	——	——	——
	The Netherlands	1	——	——	——	——	——	——

(Continued)

Table 2 (continued).

Protein encoded	Group	Number of sequences	Number of Haplotypes	Haplotype diversity[A]	Nucleotide diversity[B]	Tajima's D[C]	Fu and Li' D^*[D]	Fu and Li' F^*[E]
Vpg-Pro	All	21	21	1.000 ± 0.015	0.12484 ± 0.00662	−1.02861	−0.26189	−0.58290
	China	11	11	1.000 ± 0.039	0.03499 ± 0.00412	−0.40528	−0.44611	−0.49471
	Canada	6	6	1.000 ± 0.096	0.15165 ± 0.00852	0.76243	1.11601	1.14476
	Czech Republic	3	3	1.000 ± 0.272	0.22569 ± 0.01348	——	——	——
	The Netherlands	1	——	——	——	——	——	——
RdRp	All	21	21	1.000 ± 0.015	0.12476 ± 0.00395	−1.03185	−0.32786	−0.63796
	China	11	11	1.000 ± 0.039	0.03238 ± 0.00249	−0.84009	−1.01427	−1.10144
	Canada	6	6	1.000 ± 0.096	0.15284 ± 0.00514	0.71941	1.10022	1.12169
	Czech Republic	3	3	1.000 ± 0.272	0.22943 ± 0.00828	——	——	——
	The Netherlands	1	——	——	——	——	——	——
MP	All	21	21	1.000 ± 0.015	0.08136 ± 0.00417	−0.84727	−0.71359	−0.88337
	China	11	11	1.000 ± 0.039	0.01546 ± 0.00224	−1.15546	−1.00999	−1.18946
	Canada	6	6	1.000 ± 0.096	0.09302 ± 0.00482	1.56807	1.27282	1.46978
	Czech Republic	3	3	1.000 ± 0.272	0.17994 ± 0.00908	——	——	——
	The Netherlands	1	——	——	——	——	——	——
CP	All	21	21	1.000 ± 0.015	0.10350 ± 0.00376	−0.80672	−0.72064	−0.87460
	China	11	11	1.000 ± 0.039	0.01731 ± 0.00206	−1.39407	−1.49631	−1.66991
	Canada	6	6	1.000 ± 0.096	0.10491 ± 0.00433	1.31472	1.16094	1.31396
	Czech Republic	3	3	1.000 ± 0.272	0.20647 ± 0.00815	——	——	——
	The Netherlands	1	——	——	——	——	——	——
Pro2Glu	All	21	20	0.995 ± 0.016	0.10659 ± 0.00546	−1.05229	−0.68826	−0.93682
	China	11	10	0.982 ± 0.046	0.02286 ± 0.00337	−1.67819	−1.97058	−2.15279
	Canada	6	6	1.000 ± 0.096	0.11416 ± 0.00655	0.55807	0.82702	0.84673
	Czech Republic	3	3	1.000 ± 0.272	0.21315 ± 0.01139	——	——	——
	The Netherlands	1	——	——	——	——	——	——

(Continued)

Table 2 (continued).

Protein encoded	Group	Number of sequences	Number of Haplotypes	Haplotype diversity[A]	Nucleotide diversity[B]	Tajima's D[C]	Fu and Li' D^*[D]	Fu and Li' F^*[E]
Unknown protein	All	20	20	1.000 ± 0.016	0.12087 ± 0.00659	−0.68377	−0.37590	−0.54838
	China	11	11	1.000 ± 0.039	0.04495 ± 0.00529	−1.45887	−1.56909	−1.75026
	Canada	6	6	1.000 ± 0.096	0.11228 ± 0.00755	0.67788	0.94364	0.97539
	Czech Republic	3	3	1.000 ± 0.272	0.21008 ± 0.01341	——	——	——

Note: [A, B]were estimated using average pairwise differences at all sites in the coding sequence of each protein (standard deviations are shown after '±'). [C]Tajima's D test compares the nucleotide diversity with the proportion of polymorphic sites, which are expected to be equal under neutral selection. [D]Fu and Li's D^* test is based on the differences between the number of singletons and the total number of mutations. [E]Fu and Li's F^* test is based on the differences between the number of singletons and the average number of nucleotide differences between pairs of sequence.

significant genetic differentiation between them. The Ks^* and Z values for all coding regions between Canadian and Czech populations were not significantly different, indicating no significant genetic differentiation. Regarding *Snn* values, most coding regions were significantly different between Chinese and Canadian populations, but other geographical groups showed no significant differences (Tab. 3).

Regarding gene flow, the absolute values of *Fst* for most coding regions between Chinese, Canadian, and Czech populations were <0.33 (Tab. 3), suggesting frequent gene flow between these populations. The corresponding *Nm* absolute values for these populations were >1, also indicating that there were pathways for gene flow between them. However, the absolute values of *Fst* were >0.33 and *Nm* <1 between Chinese and Canadian RNA2 coding regions (Tab. 3), suggesting infrequent gene flow, hence genetic drift might be the main factor shaping genetic differentiation in RNA2 between Chinese and Canadian populations. Furthermore, the most frequent gene flow occurred between Canadian and Czech populations, based on the lowest *Fst* and highest *Nm* absolute values (Tab. 3).

Discussion

In recent years, the detection rates reported for SMoV, SVBV, SMYEV, SCV, and CMV were 18.9%, 21.7%, 4.2%, 37.3%, and 21%, respectively (Xi, 2017; Wang *et al.*, 2020), in major strawberry production areas of China. SNSV has only been reported in Heilongjiang province. In the present study, only SMoV (27.9%) and SVBV (42.4%) tested positive, and SMoV was detected in three provinces (Liaoning, Shandong, and Beijing) while SVBV was also detected in another two provinces (Xinjiang and Anhui), indicating higher SVBV and SMoV infection rates than reported in the previous studies (Xi, 2017; Wang *et al.*, 2020) and SVBV was widespread in China. The samples from Sichuan were negative for six viruses, which may be because of too few samples. The symptoms of SVBV may be masked in combination with SMoV or by high levels of nitrogen (Martin and Tzanetakis, 2006). Symptomless strawberry samples from Liaoning province exhibited the highest virus infection rates among the six provinces; some symptomatic samples were negative for viruses, suggesting that it is more

TABLE 3

Gene flow and genetic differentiation of SMoV protein coding regions based on geographical population

Protein encoded	Population	*Fst* [A]	*Nm* [B]	Ks^*[C]	Z [D]	*Snn* [E]
X1	China-Canada	0.25634	1.45	2.07238 (0.0010**)	55.53450 (0.0010**)	0.70588 (0.1110)
	China-Czech Republic	0.24717	1.52	1.80196 (0.0080**)	35.61485 (0.0080**)	0.88095 (0.0370*)
	Canada-Czech Republic	0.08742	5.22	3.29849 (0.2220)	15.52667 (0.1620)	0.77778 (0.2000)
X2	China-Canada	0.32685	1.03	3.10145 (0.0000***)	53.33753 (0.0000***)	0.94118 (0.0000***)
	China-Czech Republic	0.22089	1.76	2.99374 (0.0010**)	32.98667 (0.0010**)	0.75000 (0.1740)
	Canada-Czech Republic	0.08397	5.45	3.91425 (0.1270)	17.19333 (0.3800)	0.77778 (0.0500)
Hel	China-Canada	0.24742	1.52	4.37426 (0.0000***)	51.37925 (0.0000***)	0.94118 (0.0010**)
	China-Czech Republic	0.21857	1.79	4.19667 (0.0010**)	32.78152 (0.0030**)	0.78571 (0.1960)
	Canada-Czech Republic	0.03118	15.53	5.33487 (0.1560)	16.91333 (0.2820)	0.50000 (0.5940)
Vpg-Pro	China-Canada	0.17578	2.34	3.57382 (0.0010**)	56.41422 (0.0000***)	1.00000 (0.0000***)
	China-Czech Republic	0.18455	2.21	3.42490 (0.0010**)	36.41818 (0.0100**)	0.78571 (0.1790)
	Canada-Czech Republic	0.01503	32.77	4.50537 (0.1330)	17.13333 (0.2210)	0.55556 (0.4480)

(Continued)

Table 3 (continued).

Protein encoded	Population	*Fst* [A]	*Nm* [B]	*Ks** [C]	*Z* [D]	*Snn* [E]
RdRp	China-Canada	0.19782	2.03	4.45589 (0.0000***)	55.50490 (0.0000***)	0.82353 (0.0190*)
	China-Czech Republic	0.21044	1.88	4.26806 (0.0040**)	35.31879 (0.0050**)	0.78571 (0.1540)
	Canada-Czech Republic	0.00506	98.37	5.51685 (0.1580)	18.30667 (0.6560)	0.33333 (0.8850)
MP	China-Canada	0.44606	0.62	3.46226 (0.0000***)	45.48438 (0.0000***)	1.00000 (0.0000***)
	China-Czech Republic	0.18554	2.19	3.29020 (0.0000***)	32.58333 (0.0000***)	0.78571 (0.2030)
	Canada-Czech Republic	0.03406	14.18	4.59443 (0.0500)	17.10000 (0.4680)	0.66667 (0.2360)
CP	China-Canada	0.52609	0.45	3.94323 (0.0000***)	43.97972 (0.0000***)	1.00000 (0.0000***)
	China-Czech Republic	0.24197	1.57	3.76325 (0.0020**)	32.36667 (0.0020**)	0.85714 (0.0470*)
	Canada-Czech Republic	0.08969	5.07	5.11801 (0.0430*)	14.33333 (0.0580)	0.66667 (0.2940)
Pro2Glu	China-Canada	0.46532	0.57	3.29028 (0.0000***)	44.03473 (0.0000***)	1.00000 (0.0000***)
	China-Czech Republic	0.21704	1.80	3.13192 (0.0040**)	34.01364 (0.0050**)	0.78571 (0.2080)
	Canada-Czech Republic	0.10320	4.35	4.44135 (0.0720)	15.14667 (0.0920)	0.77778 (0.0670)
Unknown protein	China-Canada	0.46249	0.58	3.38471 (0.0010**)	47.68671 (0.0010**)	1.00000 (0.0020**)
	China-Czech Republic	0.20069	1.99	3.37178 (0.0020**)	33.80121 (0.0020**)	0.85714 (0.0660)
	Canada-Czech Republic	0.11018	4.04	4.07513 (0.0530)	14.68000 (0.0540)	0.77778 (0.0980)

Note: [A, B]are parameters for gene flow; the absolute values of *Fst* <0.33 or *Nm* >1 indicate frequent gene flow between populations. [C, D, E]are parameters for genetic differentiation; *p*-values obtained by permutation test with 1000 replicates are shown in parentheses; $p < 0.05$ was used as the criterion for rejecting the null hypothesis that there is no genetic differentiation between populations (*$0.01 < p < 0.05$; **$0.001 < p < 0.01$; ***$p < 0.001$).

difficult to identify viral plants by apparent symptoms. Thus, molecular diagnosis is still indispensable for strawberry viruses. Notably, strawberry plants with SVBV and SMoV were more susceptible to strawberry fusarium wilt, powdery mildew, *Botrytis cinerea*, and red spider than virus-free plants (data not shown). Therefore, developing effective molecular diagnostic technology for SMoV and SVBV remains urgent. The complete genome sequence of SMoV Chinese isolates will be conducive to designing newly specific primers and probes for RT-qPCR and preparing antibodies against SMoV.

The research about the SMoV genome sequence was relatively slow. Only one complete genome sequence (isolate 1134 from the Netherlands) was obtained by 2002 (Thompson *et al.*, 2002). In 2016, five complete genome sequences of SMoV from Canada were reported (Bhagwat *et al.*, 2016), but none have yet been reported from China. In our present study, we obtained the complete genome sequences of 11 Chinese isolates. All Chinese isolates shared high sequence identity and clustered in the same clade in the RNA2 tree, but the DGHY17 isolate was grouped into the same branch with Czech isolate C and three Canadian isolates in the RNA1 tree, suggesting that recombination or reassortment events occurred during the evolution of SMoV isolates. This was also reported previously for Canadian isolate NSper3 (Bhagwat *et al.*, 2016). Further recombination analysis was performed, but no recombination event was identified in the coding sequences of the DGHY17 isolate, indicating that reassortment occurred in the DGHY17 isolate. Additionally, recombination events occurred frequently in the 3′UTRs of both RNA1 and RNA2, suggesting that recombination is an important driving force during the evolution of SMoV. The phylogenetic results reveal that the Chinese isolates kept low molecular variation, but Czech and Canadian isolates happened high molecular variation. This may be related to vectors and strawberry transplants. Recent research demonstrated that SMoV can be transmitted by *Chaetosiphon fragaefolii*. *C. fragaefolii* is the most important vectors of viruses in strawberry fields and presumed to originate from North America (Converse, 1987; Fránová *et al.*, 2019). *C. fragaefolii* has also been found in the South Bohemia area of the Czech Republic (Fránová *et al.*, 2019). Thus, we believe that *C. fragaefolii* may make a significant contribution to the high variation in the Czech SMoV isolate. In addition, some strawberry transplants used in Canadian production are from the USA (Bonneau *et al.*, 2019), which may assist in virus spreading.

During the evolution of plant viruses, genetic selection and drift are the two main processes (Garcia-Arenal *et al.*, 2003). Previous studies reported that negative selection operates on most animal and plant viruses (Garcia-Arenal *et al.*, 2001; Yin *et al.*, 2013; He *et al.*, 2013). In our current study, SMoV protein populations from China, Canada, and the Czech Republic were subjected to negative selection, indicating strong purifying selection against SMoV mutation as a driving force for SMoV evolution. Among these proteins, the d_N/d_S ratios for X1 were much greater than for other proteins, indicating that constraints on X1 were higher than for other proteins; hence, the X1 sequence may be more highly conserved.

Genetic differentiation and gene flow analysis revealed no genetic differentiation between Canadian and Czech SMoV populations, and consistent with this observation, the most frequent gene flow was found between these groups. This may be due to the expansion of the *C. fragaefolii* vector from Canada to the Czech Republic. According to previous studies (Fránová *et al.*, 2019; CABI, 2019), the *C. fragaefolii* may originate from North America and spread widely in Europe. Strawberry or other plant material imports and exports may assist the *C. fragaefolii* in spreading by carrying them. There was no gene flow between Chinese RNA2 and

Canadian RNA2 populations, and genetic differentiation was significant. Interestingly, there was genetic differentiation between Chinese and Czech SMoV populations, although gene flow was identified between them. These results indicate that gene flow was the main element influencing SMoV genetic differentiation, but some other factors such as gene drift, recombination, or reassortment may also drive SMoV evolution.

In conclusion, our study provides the first complete genome sequences of SMoV isolates from China. The Chinese isolates shared high sequence identity, but Czech isolates occurred high molecular variation and existed frequent gene flow with Canadian isolates. Negative selection drove SMoV population evolution, and gene flow played a major role in SMoV genetic differentiation. In addition, reassortment and recombination also influence the structure of SMoV populations. To our knowledge, this is the first detailed analysis of SMoV population structure.

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Ethics Approval: This work did not involve any studies on human participants or animals.

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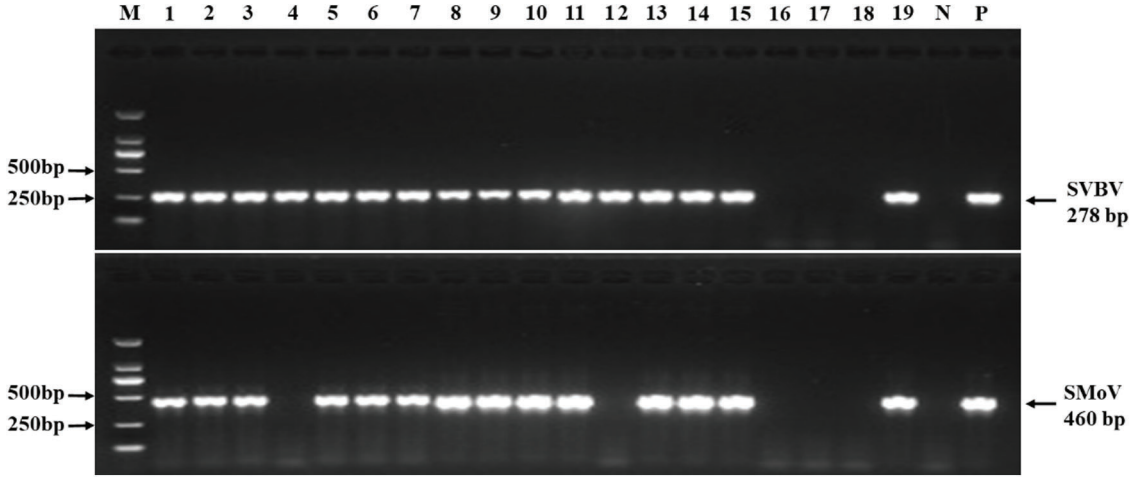
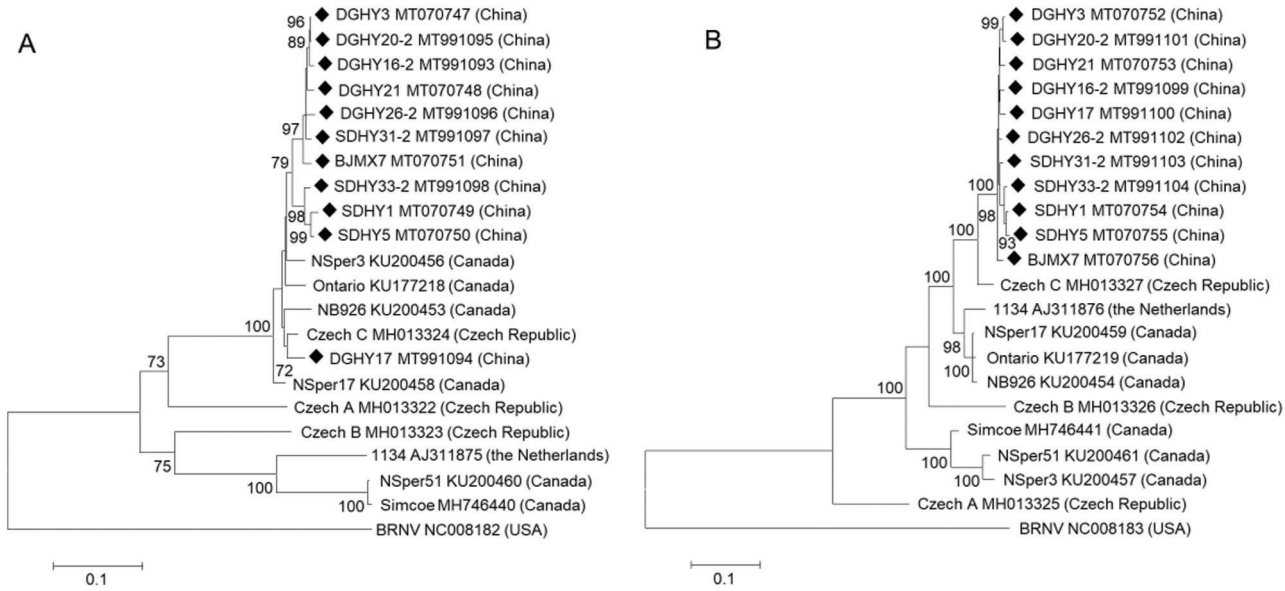
Supplementary Materials



SUPPLEMENTARY FIGURE 1. Incidence of strawberry vein banding virus (SVBV) and strawberry mottle virus (SMoV) in the strawberry samples from Liaoning of China by RT-PCR.
M: DNA marker 2000; lanes 1 to 19, partial strawberry samples from Liaoning of China; N: double-distilled water as a negative control; P: SVBV or SMoV plasmid as a positive control.



SUPPLEMENTARY FIGURE 2. Phylogenetic analysis of SMoV Chinese isolates and available isolates from GenBank based on RNA-dependent RNA polymerase (A) and coat protein (B) full coding regions using the ML method with 1000 bootstrap replicates. Only values above 70% are shown.
SMoV Chinese isolates are indicated by black diamonds. Black raspberry necrosis virus (BRNV) was used as the outgroup.

SUPPLEMENTARY TABLE 1

Primers used in this study

Clones	Primers	Sequence (5'-3')	Position	Size (bp)	Reference
For detection	SMoV-F	TAAGCGACCACGACTGTGACAAAG	6167–6190	461	Thompson and Jelkmann, 2003[1]
	SMoV-R	ATTCGGTTCACATCCTAGTCTCAC	6604–6627		
	SVBV-F	GAATGGGACAATGAAATGAG	2274–2293	278	Petrzik *et al.*, 1998[2]
	SVBV-R	AACCTGTTTCTAGCTTCTTG	2532–2551		
	SMYEV-F	GTGTGCTCAATCCAGCCAG	5639–5657	271	Thompson *et al.*, 2003[3]
	SMYEV-R	CATGGCACTCATTGGAGCTGGG	5889–5910		
	SCV-F	ACTGTAATGTCACCAGAGAAG	58–78	612	Posthuma *et al.*, 2002[4]
	SCV-R	TTCTGACACTAGTAGATCTCC	610–670		
	CMV-F	TGATTCTACCGTGTGGGTGA	455–474	431	Chen *et al.*, 2014[5] (L36525)
	CMV-R	CCGTAAGCTGGATGGACAAC	866–885		
	SNSV-F	AACAACTCCAATGGTTGCCCAACT	1321–1344	372	Veetil *et al.*, 2016[6]
	SNSV-R	ACCAAATGTCCCATCGGACACGGCA	1668–1692		
5'-RACE	R1-GSP1	ACAACCTTCCCAACCCATCCAAGTG	645–669	714	KU200453
	R1-GSP2	CTCAGTAGGCACATAATCGTCAT	345–367	390	KU200453
	R2-GSP1	GGAATTGGTGGTATTGACAGCGGGAAC	636–662	707	KU200454
	R2-GSP2	CAACAGTGACGAAGGACAAT	297–316	339	KU200454
3'-RACE	GSP	CCCAAAGAGGCTGGTGGTGTATTC	6382–6405	681	KU200453
DGHY21 complete genome	fullR1-F	CACTGGACCCAGCTTACTGAAAAAATACT	1–29	7052	RNA1-5'-RACE
	fullR1-R	TTTTTTTTTTTTTTTTTTTTTTTTTACATCT			RNA1-3'-RACE
	fullR2-F	CACTGGACCCAGCTCACTGAAAAAATTTG	1–29	6349	RNA2-5'-RACE
	fullR2-R	TTTTTTTTTTTTTTTTTTTTTTTTTACATCT			RNA2-3'-RACE
SMoV-RNA1-fragment-1	SMR1-F1	GATGAGTTACCTCTGCGATGAC	151–172	2659	KU200453
	SMR1-R1	GTAAGAGTTGCGTGCCGATT	2790–2809		
SMoV-RNA1-fragment-2	SMR1-F2	ACACCTGGCGAACGAACAT	2416–2434	2514	KU200453
	SMR1-R2	TCAATGCGGCTCATAATCTTCC	4908–4929		
SMoV-RNA1-fragment-3	SMR1-F3	CTTCAATGGCGATTACACAGGAT	4648–4670	1901	KU200453
	SMR1-R3	GGCACCACAGAACCTATTCCA	6528–6548		
SMoV-RNA2-fragment-1	SMR2-F1	CGCTTGCTTGATCCTCTACACTCTC	122–146	2076	KU200454
	SMR2-R1	ATCCTATCATCAGTAACTGCTCCAACAC	2170–2197		

(Continued)

Supplementary Table 1 (continued).

Clones	Primers	Sequence (5'-3')	Position	Size (bp)	Reference
SMoV-RNA2-fragment-2	SMR2-F2	CCGAGATGGTTGGCGAAGCAT	1942–1962	2049	KU200454
	SMR2-R2	CCGAAGGGTTGAAAGAGTAAGGTTGA	3965–3990		
SMoV-RNA2-fragment-2	SMR2-F3	ATGGCAACTACGAGGGCTGGAA	3736–3757	2188	KU200454
	SMR2-R3	CGGTTCACATCCTAGTCTCACTTATGG	5897–5923		

Note: [1]Thompson JR, Jelkmann W (2003). The detection and variation of strawberry mottle virus. *Plant Disease* **87**: 385–390. [2]Petrzik K, Benes V, Mraz I, Honetslegrova FJ, Ansorge W, Spak J (1998). Strawberry vein banding virus-definitive member of the genus Caulimovirus. *Virus Genes* **16**: 303–305. [3]Thompson JR, Wetzel S, Klerks MM, Vašková D, Schoen CD, Špak J, Jelkmann W (2003). Multiplex RT-PCR detection of four aphid-borne strawberry viruses in *Fragaria* spp. in combination with a plant mRNA specific internal control. *Journal of Virological Methods* **111**: 85–93. [4]Posthuma KI, Adams AN, Hong Y, Kirby MJ (2002). Detection of strawberry crinkle virus in plants and aphids by RT-PCR using conserved L gene sequences. *Plant Pathology* **51**: 266–274. [5]Chen L, Shang QX, Chen XY, Xing DM, Yang R, Ran C, Wei YM, Zhao XY, Liu ZP (2014). First report on the occurrence of *Cucumber mosaic virus* on *Fragaria ananassa* in China. *Plant Disease* **98**: 1015. [6]Veetil TT, Ho T, Moyer C, Whitaker VM, Tzanetakis IE (2016). Detection of *Strawberry necrotic shock virus* using conventional and TaqMan® quantitative RT-PCR. *Journal of Virological Methods* **235**: 176–181.

SUPPLEMENTARY TABLE 2

Detection of viruses in the main strawberry production areas of China

Area	Sample No.	SMoV	SVBV	SMYEV	SCV	SNSV	CMV	SMoV and SVBV
Beijing	68	8/68	20/68	0/68	0/68	0/68	0/68	8/68
Anhui	20	0/20	1/20	0/20	0/20	0/20	0/20	0/20
Shandong	22	11/22	13/22	0/22	0/22	0/22	0/22	11/22
Liaoning	35	27/35	29/35	0/35	0/35	0/35	0/35	27/35
Xinjiang	17	0/17	7/17	0/17	0/17	0/17	0/17	0/17
Sichuan	3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Total	165	46/165	70/165	0/165	0/165	0/165	0/165	46/165

SUPPLEMENTARY TABLE 3

Genomic characteristics of eleven strawberry mottle virus (SMoV) isolates from China

Isolate	RNA1 (nt)									RNA2 (nt)						
	Length	5' UTR	X1	X2	Hel	VPg	Pro	RdRp	3' UTR	Length	5' UTR	MP	CP	Pro2Glu	Unknown protein	3' UTR
DGHY3	7026	140	438	606	1848	75	693	2085	1141	6319	89	1356	1947	1029	744	1154
DGHY21	7027	143	438	606	1848	75	693	2085	1139	6324	93	1356	1947	1029	744	1155
SDHY1	7022	139	438	606	1848	75	693	2085	1138	6319	89	1356	1947	1029	744	1154
SDHY5	7022	139	438	606	1848	75	693	2085	1138	6319	89	1356	1947	1029	744	1154
BJMX7	7027	143	438	606	1848	75	693	2085	1139	6320	89	1356	1947	1029	744	1155
DGHY16-2	7017	138	438	606	1848	75	693	2085	1134	6298	72	1356	1947	1029	744	1150
DGHY17	7017	138	438	606	1848	75	693	2085	1134	6298	72	1356	1947	1029	744	1150
DGHY20-2	7017	138	438	606	1848	75	693	2085	1134	6298	72	1356	1947	1029	744	1150
DGHY26-2	7017	138	438	606	1848	75	693	2085	1134	6298	72	1356	1947	1029	744	1150
SDHY31-2	7017	138	438	606	1848	75	693	2085	1134	6298	72	1356	1947	1029	744	1150
SDHY33-2	7017	138	438	606	1848	75	693	2085	1134	6298	72	1356	1947	1029	744	1150

SUPPLEMENTARY TABLE 4

Prediction of recombination events

Recombinant		Minor parent	Major parent	Region (nt)	RDP	GENECONV	BootScan	MaxChi	Chimaera	SiScan	3Seq
RNA1	Ontario	NSper51	DGHY17	422–1884	7.090×10^{-133}	1.147×10^{-138}	3.108×10^{-139}	3.891×10^{-36}	1.491×10^{-36}	2.648×10^{-44}	3.708×10^{-159}
	Ontario	NSper51	DGHY17	6664–7002	4.398×10^{-16}	3.037×10^{-14}	8.641×10^{-17}	5.916×10^{-06}	1.902×10^{-05}	3.005×10^{-06}	——
	NB926	NSper51	DGHY17	6549–7004	4.398×10^{-16}	3.037×10^{-14}	8.641×10^{-17}	5.916×10^{-06}	1.902×10^{-05}	3.005×10^{-06}	——
	NSper51	NB926	DGHY16-2	5925–6411	2.386×10^{-44}	4.359×10^{-30}	4.970×10^{-45}	1.647×10^{-12}	1.729×10^{-09}	8.240×10^{-15}	1.221×10^{-06}
	NSper3	Simcoe	DGHY17	5947–6525	3.842×10^{-26}	7.172×10^{-15}	2.656×10^{-23}	5.419×10^{-08}	2.915×10^{-08}	2.336×10^{-07}	8.428×10^{-10}
	NSper3	NSper51	DGHY17	6561–7001	4.398×10^{-16}	3.037×10^{-14}	8.641×10^{-17}	5.916×10^{-06}	1.902×10^{-05}	3.005×10^{-06}	——
	1134	NB926	DGHY16-2	5925–6545	2.386×10^{-44}	4.359×10^{-30}	4.970×10^{-45}	1.647×10^{-12}	1.729×10^{-09}	8.240×10^{-15}	1.221×10^{-06}
	DGHY3	NSper51	DGHY17	6552–7016	4.398×10^{-16}	3.037×10^{-14}	8.641×10^{-17}	5.916×10^{-06}	1.902×10^{-05}	3.005×10^{-06}	——
	DGHY21	Simcoe	DGHY17	5948–6542	3.842×10^{-26}	7.172×10^{-15}	2.656×10^{-23}	5.419×10^{-08}	2.915×10^{-08}	2.336×10^{-07}	8.428×10^{-10}
	DGHY21	NSper51	NSper17	6549–7013	4.398×10^{-16}	3.037×10^{-14}	8.641×10^{-17}	5.916×10^{-06}	1.902×10^{-05}	3.005×10^{-06}	——
	SDHY1	Simcoe	DGHY17	5948–6541	3.842×10^{-26}	7.172×10^{-15}	2.656×10^{-23}	5.419×10^{-08}	2.915×10^{-08}	2.336×10^{-07}	8.428×10^{-10}
	SDHY1	NSper51	NSper17	6548–7012	4.398×10^{-16}	3.037×10^{-14}	8.641×10^{-17}	5.916×10^{-06}	1.902×10^{-05}	3.005×10^{-06}	——
	SDHY5	Simcoe	DGHY17	5948–6541	3.842×10^{-26}	7.172×10^{-15}	2.656×10^{-23}	5.419×10^{-08}	2.915×10^{-08}	2.336×10^{-07}	8.428×10^{-10}
	SDHY5	NSper51	NSper17	6548–7012	4.398×10^{-16}	3.037×10^{-14}	8.641×10^{-17}	5.916×10^{-06}	1.902×10^{-05}	3.005×10^{-06}	——
	BJMX7	Simcoe	DGHY17	5948–6542	3.842×10^{-26}	7.172×10^{-15}	2.656×10^{-23}	5.419×10^{-08}	2.915×10^{-08}	2.336×10^{-07}	8.428×10^{-10}
	BJMX7	NSper51	NSper17	6549–7013	4.398×10^{-16}	3.037×10^{-14}	8.641×10^{-17}	5.916×10^{-06}	1.902×10^{-05}	3.005×10^{-06}	——
	DGHY16-2	Simcoe	DGHY17	5929–6542	3.842×10^{-26}	7.172×10^{-15}	2.656×10^{-23}	5.419×10^{-08}	2.915×10^{-08}	2.336×10^{-07}	8.428×10^{-10}
	DGHY16-2	NSper51	NSper17	6549–7013	4.398×10^{-16}	3.037×10^{-14}	8.641×10^{-17}	5.916×10^{-06}	1.902×10^{-05}	3.005×10^{-06}	——
	DGHY17	NSper51	NSper17	6549–7013	4.398×10^{-16}	3.037×10^{-14}	8.641×10^{-17}	5.916×10^{-06}	1.902×10^{-05}	3.005×10^{-06}	——
	DGHY20-2	Simcoe	DGHY17	5948–6542	3.842×10^{-26}	7.172×10^{-15}	2.656×10^{-23}	5.419×10^{-08}	2.915×10^{-08}	2.336×10^{-07}	8.428×10^{-10}
	DGHY20-2	NSper51	NSper17	6549–7013	4.398×10^{-16}	3.037×10^{-14}	8.641×10^{-17}	5.916×10^{-06}	1.902×10^{-05}	3.005×10^{-06}	——
	DGHY26-2	Simcoe	DGHY17	5948–6542	3.842×10^{-26}	7.172×10^{-15}	2.656×10^{-23}	5.419×10^{-08}	2.915×10^{-08}	2.336×10^{-07}	8.428×10^{-10}
	DGHY26-2	NSper51	NSper17	6549–7013	4.398×10^{-16}	3.037×10^{-14}	8.641×10^{-17}	5.916×10^{-06}	1.902×10^{-05}	3.005×10^{-06}	——
	SDHY31-2	Simcoe	DGHY17	5948–6542	3.842×10^{-26}	7.172×10^{-15}	2.656×10^{-23}	5.419×10^{-08}	2.915×10^{-08}	2.336×10^{-07}	8.428×10^{-10}
	SDHY31-2	NSper51	NSper17	6549–7013	4.398×10^{-16}	3.037×10^{-14}	8.641×10^{-17}	5.916×10^{-06}	1.902×10^{-05}	3.005×10^{-06}	——
	SDHY33-2	Simcoe	DGHY17	5948–6542	3.842×10^{-26}	7.172×10^{-15}	2.656×10^{-23}	5.419×10^{-08}	2.915×10^{-08}	2.336×10^{-07}	8.428×10^{-10}
	SDHY33-2	NSper51	NSper17	6549–7013	4.398×10^{-16}	3.037×10^{-14}	8.641×10^{-17}	5.916×10^{-06}	1.902×10^{-05}	3.005×10^{-06}	——
	Simcoe	Ontario	DGHY3	5921–6540	1.257×10^{-32}	1.626×10^{-21}	9.748×10^{-33}	1.084×10^{-09}	4.067×10^{-10}	1.070×10^{-10}	5.395×10^{-07}
	Simcoe	NSper3	NSper51	6558–7001	3.591×10^{-29}	2.423×10^{-32}	1.455×10^{-28}	3.129×10^{-05}	3.077×10^{-05}	9.619×10^{-07}	——

(Continued)

Supplementary Table 4 (continued).

Recombinant		Minor parent	Major parent	Region (nt)	RDP	GENECONV	BootScan	MaxChi	Chimaera	SiScan	3Seq
RNA2	NSper51	DGHY16-2	SDHY5	5202–6125	1.075×10^{-22}	6.292×10^{-37}	4.228×10^{-32}	8.344×10^{-08}	9.778×10^{-07}	3.161×10^{-08}	2.019×10^{-06}
	NSper3	SDHY5	DGHY16-2	5200–6255	1.075×10^{-22}	6.292×10^{-37}	4.228×10^{-32}	8.344×10^{-08}	9.778×10^{-07}	3.161×10^{-08}	2.019×10^{-06}
	DGHY3	1134	SDHY31-2	5236–5946	4.765×10^{-15}	2.300×10^{-10}	4.724×10^{-15}	1.289×10^{-10}	4.627×10^{-10}	1.899×10^{-04}	6.646×10^{-11}
	DGHY21	1134	SDHY31-2	5236–5947	4.765×10^{-15}	2.300×10^{-10}	4.724×10^{-15}	1.289×10^{-10}	4.627×10^{-10}	1.899×10^{-04}	6.646×10^{-11}
	BJMX7	1134	SDHY31-2	5189–5828	4.765×10^{-15}	2.300×10^{-10}	4.724×10^{-15}	1.289×10^{-10}	4.627×10^{-10}	1.899×10^{-04}	6.646×10^{-11}
	DGHY16-2	1134	SDHY31-2	5189–5828	4.765×10^{-15}	2.300×10^{-10}	4.724×10^{-15}	1.289×10^{-10}	4.627×10^{-10}	1.899×10^{-04}	6.646×10^{-11}
	DGHY17	1134	SDHY31-2	5236–5947	4.765×10^{-15}	2.300×10^{-10}	4.724×10^{-15}	1.289×10^{-10}	4.627×10^{-10}	1.899×10^{-04}	6.646×10^{-11}
	DGHY20-2	1134	SDHY31-2	5236–5947	4.765×10^{-15}	2.300×10^{-10}	4.724×10^{-15}	1.289×10^{-10}	4.627×10^{-10}	1.899×10^{-04}	6.646×10^{-11}
	DGHY26-2	1134	SDHY31-2	5236–5947	4.765×10^{-15}	2.300×10^{-10}	4.724×10^{-15}	1.289×10^{-10}	4.627×10^{-10}	1.899×10^{-04}	6.646×10^{-11}
	SDHY33-2	DGHY17	SDHY1	4023–5081	1.998×10^{-21}	7.813×10^{-20}	1.262×10^{-21}	3.075×10^{-18}	4.078×10^{-18}	1.992×10^{-37}	4.227×10^{-37}
	Simcoe	NSper17	NSper51	2780–6306	7.050×10^{-96}	1.271×10^{-90}	3.842×10^{-95}	5.544×10^{-42}	3.022×10^{-43}	1.639×10^{-64}	2.972×10^{-193}