Occurrence and Molecular Variability of Kiwifruit Viruses in *Actinidia deliciosa* 'Xuxiang' in the Shaanxi Province of China

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Abstract

Kiwifruit (*Actinidia* spp.) is an economically substantial fruit crop with China the main producer. China is the primary source of wild kiwifruit and the largest producer of kiwifruit in terms of both production and planting area, and Shaanxi province is the largest kiwifruit producer in China. Previous studies reported presence of kiwifruit viruses in *Actinidia chinensis*. In this study, six viruses were identified in kiwifruit 'Xuxiang' (*A. deliciosa*) in Shaanxi, China. The incidence, distribution, and genetic diversity of these viruses were studied. The results showed that *Actinidia* virus A (AcVA), *Actinidia* virus B (AcVB), *Actinidia* chlorotic ringspot-associated virus (AcCRaV), cucumber mosaic virus (CMV), apple stem grooving virus (ASGV), and potato virus X (PVX) were the main viruses infecting Xuxiang kiwifruit in Shaanxi, China. Incidence of the various viruses with both single and multiple infection varied with different kiwifruit-growing counties. For single virus infection, the highest and the lowest numbers of samples infected were about 22 for AcCRaV and

0 for AcVB in Meixian out of 170 samples, 12 for AcVA and 0 for CMV in Zhouzhi out of 120 samples, 10 for AcVA and 0 for AcVB, AcCRaV, ASGV, PVX, and CMV in Yangling out of 70 samples, and 8 for AcCRaV and CMV and 0 for AcVA, AcVB, ASGV, and PVX in Hanzhong out of 80 samples, respectively. Samples which were multiply infected with two or more viruses were also detected. Analysis of the phylogenetic tree of these viruses showed some genetic variability in the AcVA, AcVB, and AcCRaV isolates of Shaanxi kiwifruit. There was no obvious molecular variation in the coat protein genes of ASGV, CMV, and PVX virus isolates from Shaanxi kiwifruit. The present study is the first large-scale survey of kiwifruit viruses in Shaanxi, China. To our knowledge, this is the first report of PVX infecting kiwifruit and the first report of molecular variability of AcVA, AcVB, and AcCRaV. These results provide important data for studying the genetic evolution of AcVA, AcVB, AcCRaV, ASGV, CMV, and PVX.

Kiwifruit (*Actinidia* spp.) is an economically important fruit crop in the world (Belrose Inc. 2016). China is the primary source of wild kiwifruit (Ferguson and Huang 2007) and the largest producer of kiwifruit in terms of both production and planting area (Belrose Inc. 2016). By 2015, the cultivation area and fruit production reached 2,300,000 metric tons and 250,000 ha (Belrose Inc. 2016). The major kiwifruit-producing provinces include Shaanxi, Sichuan, Henan, Hunan, Hebei, Guangxi, and Guangdong, and Shaanxi province is the largest kiwifruit producer in China, accounting for approximately 84% of kiwifruit production in China. In Shaanxi province, kiwifruit is mainly grown in Zhouzhi (40%), Meixian (30%), Hanzhong (10%), and Yangling (5%). *A. deliciosa* 'Xuxiang' is the most popular kiwifruit cultivar and has the largest cultivation area in Shaanxi province.

To date, about 16 kiwifruit viruses have been reported throughout the world, including *Actinidia* virus X, alfalfa mosaic virus (AMV), cucumber mosaic virus (CMV), ribgrass mosaic virus (RMV), turnip vein clearing virus (TVCV), cucumber necrosis virus (CNV), apple stem grooving virus (ASGV), *Actinidia* virus A (AcVA),

Actinidia virus B (AcVB), citrus leaf blotch virus (CLBV), cherry leaf roll virus (CLRV), pelargonium zonate spot virus (PZSV), tomato necrotic spot associated virus (TNSaV), *Actinidia* chlorotic ringspot-associated virus (AcCRaV), *Actinidia* virus 1 (AcV-1), and *Actinidia* seed-borne latent virus (ASbLV; Blouin et al. 2012, 2013, 2018; Chavan et al. 2012, 2013; Clover et al. 2003; Pearson et al. 2011; Veerakone et al. 2018; Wang et al. 2016; Zheng et al. 2017). At least seven kiwifruit viruses have previously been reported from China, including ASGV (Clover et al. 2003; Wang et al. 2018b), TNSaV (Wang et al. 2016), AcCRaV (Zheng et al. 2017), CMV (Wang et al. 2018a), CLBV (Zhu et al. 2016), AcVA, and AcVB (Zheng et al. 2014). Symptoms induced by the various viruses include ringspots, vein yellowing, and chlorotic spots in *A. chinensis* infected by AcCRaV (Zheng et al. 2017), chlorosis and mosaic in *A. chinensis* infected with ASGV (Wang et al. 2018b), vein chlorosis in *A. chinensis* infected with CLBV (Chavan et al. 2013), and mottle, vein chlorosis, and chlorotic ringspots in *A. chinensis* infected with AcVA and AcVB (Blouin et al. 2012).

Although influences of virus infection on growth, development, and yield of kiwifruit have not been quantified, control of virus movement by quarantine between countries is necessary because interests are increasing in introducing genetic material from other countries in the form of woody cuttings for breeding purposes (Clover et al. 2003). Exchange of plant genetic material between countries creates risks of spreading virus diseases from one to another. For example, a virus was detected in cuttings of *A. chinensis* that were exported from China, in postentry quarantine in New Zealand in 2001 (Clover et al. 2003).

Availability of information on types and incidence of viruses, symptoms induced by viruses, and virus genetic diversity is a fundamental requirement in all types of virus-related research and applied applications. However, to date studies of kiwifruit viruses have been far fewer than those on viruses of pome and stone fruits (Clover et al. 2003; Hadidi and Barba 2011). Therefore, the objectives of the present study were first to investigate the types of virus and their incidence and then to analyze genetic diversity of these viruses in the

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most popular kiwifruit cultivar, Xuxiang, in Shaanxi province of China.

Materials and Methods

Plant material. Leaves showing symptoms that were suspected to be virus-infected, including mosaic, vein yellowing, leafroll, ring-spots, chlorotic spots, and mottles, were collected during the growing season of April to May 2017 and 2018 from Xuxiang kiwifruit plants grown in the commercial orchards in Shaanxi province, China. A total of 440 samples were collected, including 170 from Meixian, 120 from Zhouzhi, 70 from Yangling, and 80 from Hanzhong. All samples were stored at -80°C until use.

Virus detection by reverse transcription polymerase chain reaction (RT-PCR). Total RNA was extracted from leaf tissue (0.5 g) of the collected samples using TRIzol reagent, following the manufacturer's recommendations (Invitrogen, Carlsbad, CA). The RNA was diluted in 35 μl of DNase- and RNase-free water, and its quality and concentration were detected by a spectrophotometer (DU Series 500UV-Vis; Beckman Coulter, Fullerton, CA). cDNA was synthesized from 2 μg of total RNA using M-MLV

reverse transcription and random primers (TaKaRa Bio, Dalian Inc., Dalian, China). The reaction process and conditions were conducted as described by Zhao et al. (2012). Primers used for amplifying sequences of specific viruses are listed in Table 1. These included primers for apple stem pitting virus (ASPV), apple chlorotic leaf spot virus (ACLSV), potato virus X (PVX), AMV, CMV, RMV, TVCV, CNV, ASGV, AcVA, AcVB, CLBV, CLRV, PZSV, TNSaV, AcCRaV, AcV-1 and ASbLV. The cDNAs were then used as templates in PCR, with the following programs for all the viruses detected: 94°C for 3 min for pre-denaturation and 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min. The PCR products were analyzed by gel electrophoresis and were purified using a DNA gel extraction kit (Bio Tech Corporation, Beijing, China) and confirmed by sequencing.

Cloning and sequencing analysis. Purified PCR products of six viruses (AcVA, AcVB, AcCRaV, ASGV, CMV, and PVX) were cloned and analyzed to study the molecular variability of the viruses. Twenty-eight samples that were RT-PCR positive to AcVA, 25 to AcCRaV, 16 to AcVB, 16 to ASGV, 16 to CMV, and 11 to PVX were selected and used for amplification of the complete coat protein

Table 1. All the primers used in this study^a

Name of primer	Sequence (5'–3')	Product size (bp)	Usage	Reference
AcVA F	CATGGCAAAGAATATCTCAAG	471	Virus detection for AcVA	This study
AcVA R	AGATCCAACCCAGAGTTGAAA			
AcVB5F	GTTTGCGAGGAGACGTAGGGC	342	Virus detection for AcVB	Blouin et al. (2012); Zheng et al. (2015)
AcVB5R	AGTTAAGTGCTCTYGGRRGTGTG			
AcCRaV3F	ATCCAAGAATTCCTTAACAGCA	477	Virus detection for AcCRaV	Zheng et al. (2017)
AcCRaV3R	TGTGCAATCATGGCTTATCAGA			
ASGV-U	CCCGCTGTTGGATTGATACACCTC	499	Virus detection for ASGV	James et al. (1997)
ASGV-2	GGAATTTACACGACTCCTAACCTCC			
CTLV-AP	CCTGAATTGAAAACCTTTGCTGCC	456	Virus detection for CTLV	Ito et al. (2002)
CTLV-AM	TAGAAAAACCACACTAACCCGGAAATGC			
AMV F	GCTGGTAAACCTACTAAAC	399	Virus detection for AMV	This study
AMV R	AAACCCGAACCTTCTCATT			
ACLSV-s	TTCATGGAAAGACAGGGGCAA	677	Virus detection for ACLSV	Menzel et al. (2002)
ACLSV-a	AAGTCTACAGGCTATTTATTATAAGTCTAA			
ASPV-s	ATGTCTGGAACCTCATGCTGCAA	370	Virus detection for ASPV	Meijneke et al. (1983)
ASPV-a	TTGGGATCACTTTACTAAAAAGCATAA			
CMV F	GAGTCGTGCGTTTCTTTGTGTTTT	183	Virus detection for CMV	This study
CMV R	AATGGCGAGGGTTTATTGAGTC			
RW1	GTCGGAAGATTACGTAAAAGG	416	Virus detection for CLRV	Werner et al. (1997)
RW2	TGGCGACCGTGTAAACGGCA			
CNV-F791	CCTCGCAGAAGACCTTATGC	215	Virus detection for CNV	Blouin et al. (2013)
CNV-R1002	GCCGACTCCTCCACTCCA			
PZSV2F	GATAAATTCAGAGCTCTCGG	997	Virus detection for PZSV	Blouin et al. (2013)
PZSV2R	ATCTCTGCAGATTGTGTTC			
AT2F	AGACAGCAATTCTCAAACCTGT	223	Virus detection for TVCV and RMV	Blouin et al. (2013)
AT4R	CGGTGCGCATCATCAACAC			
NGS CLO2_F	AGGAATATTGGATCGGTTGTCG	208	Virus detection for AcV-1	Blouin et al. (2018)
NGS CLO1_R	GATGGTCAGTCTTAATCTCC			
AVC 1F	GAAAAGCAACGAAAGCAACCTA	328	Virus detection for CLBV	Chavan et al. (2013)
AVC 1R	CGAACAAGATGTGATTCTCG			
TNSaVF	ATGATTTGTTTGGGGTTATG	350	Virus detection for TNSaV	This study
TNSaVR	GAATGGTTCTCCCTCTATGG			
dPR1	GCDAAARGCNGNCARACHHTVGCBTGYTT	363	Virus detection for ASbLV	Saldarelli et al. (1998)
dPR2	RAAYTCNCCNSWRAANCKCAT			
PVX F	AGTGCGCGAGGTTTACCAATC	790	Virus detection and CP gene clone for PVX	This study
PVX R	GTGGTTTGGCGGCAACGATTC			
AcVA CPF	TGCCTAGCGTGTATGAAGC	644	CP gene clone for AcVA	This study
AcVA CPR	CCGTGAGAAATGATGGGTC			
AcVB CPF	CTTCCCTGGTTACTTTGTG	1331	CP gene clone for AcVB	This study
AcVB CPR	GTTTATTCACGCCTGTCTC			
AcCRaV CP-F	CGAGCTCAGTGAAGAACCACAATATT	945	CP gene clone for AcCRaV	Zheng et al. (2017)
AcCRaV CP-R	CGGGATCCATGCCAAAGCCTATGCAAGG			
ASGV CPF	ATGAGTTTGGGAAGACGTGCTT	714	CP gene clone for ASGV	This study
ASGV CPR	CTAACCTCCAGTTCCAGA			
CMV CPF	TTGTGTTTTCGCTCTTAGTGTAT	725	CP gene clone for CMV	This study
CMV CPR	GAGAGTGTAGTTTAACGTCTTCG			

^a CP = coat protein. Virus abbreviations defined in text.

(CP) gene using the CP gene primers (Table 1) and the PCR programs described above. All the amplified CP genes were cloned into the PMD19-T vector, according to the manufacturer's protocols (TaKaRa Bio). The recombinant vectors were transformed into *Escherichia coli* strain JM109 and sequenced using an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). For each CP gene, three clones were sequenced in both orientations to confirm the consensus sequence.

The nucleotide sequences were aligned using Clustal X (Thompson et al. 1997) with default parameters. Phylogenetic analyses were conducted using the molecular evolutionary genetics analysis tool (MEGA version 5.0; Tamura et al. 2011) with 1,000 bootstrap replicates. Phylogenetic trees were constructed with the neighbor-joining method using MEGA software (MEGA version 5.0; Tamura et al. 2011).

PVX detection by dot enzyme-linked immunosorbent assay (dot-ELISA). Because PVX was for the first time detected by RT-PCR in *Actinidia*, dot-ELISA was used to confirm PVX infection. Five samples that were PVX-positive responses to RT-PCR were used for dot-ELISA. Dot-ELISA was conducted as described by Wu and Zhou (2005) using the monoclonal antibodies to the PVX (a generous gift from Yan Xie, Zhejiang University, Hangzhou, China) and goat anti-mouse immunoglobulin G conjugated with alkaline phosphatase (Sigma), and BCIP/NBT (Sigma) was used as the substrate for color development.

Results

Viruses infecting kiwifruit detected by RT-PCR. Twenty-three sets of primers were used to detect the presence of 18 different viruses. Only six viruses (AcVA, AcVB, AcCRaV, ASGV, CMV, and PVX) were detected in *A. deliciosa* 'Xuxiang' in Shaanxi. Virus-specific RT-PCR detected AcVA in samples that showed mild chlorosis along the veins (Fig. 1A); AcVB in samples showing

chlorotic fleck produced a specific band of 342 bp (Fig. 1B); AcCRaV in samples showing chlorosis along the veins and mosaic produced a specific band of 477 bp (Fig. 1C); ASGV was detected in samples showing leaf shrinkage (Fig. 1D); PVX in samples showing mild yellowing and mild shrinkage produced a specific band of 790 bp (Fig. 1E); and CMV was detected in samples showing yellow spots and shrinkage (Fig. 1F). CLBV and AcV-1 were detected in low frequency in some samples of *A. deliciosa* 'Hayward' showing chlorosis and yellow spot symptoms collected from Meixian, with low frequencies. Because the present study focused on kiwifruit Xuxiang, detailed data on these two viruses were not included here, and that work on these two viruses is continuing and will be the subject of a later publication.

PVX detection by dot-ELISA. The results of dot-ELISA for PVX detection are shown in Figure 2. Five samples that were detected positive for PVX by RT-PCR were also detected positive by dot-ELISA, and the negative control of kiwifruit not showing symptoms typical of virus infection was not detected positive for PVX by dot-ELISA.

Virus incidence and distribution. Incidence of the various viruses with both single and multiple infection varied with different kiwifruit-growing counties. For single virus infection, the highest and the lowest numbers of samples infected were about 22 for AcCRaV and 0 for AcVB in Meixian out of 170 samples, 12 for AcVA and 0 for CMV in Zhouzhi out of 120 samples, 10 for AcVA and 0 for AcVB, AcCRaV, ASGV, PVX, and CMV in Yangling out of 70 samples, and 8 for AcCRaV and CMV and 0 for AcVA, AcVB, ASGV, and PVX in Hanzhong out of 80 samples, respectively. Samples that were multiply infected with two or more viruses were also detected, and the results are shown in Supplementary Tables S1, S2, S3, and S4.

Genetic diversity of the viruses. The complete CP gene sequences of the six kiwifruit viruses detected in the present study

Fig. 1. Symptoms on leaves of *Actinidia* infected with a single different virus: **A**, *Actinidia* virus A; **B**, *Actinidia* virus B; **C**, *Actinidia* chlorotic ringspot-associated virus; **D**, apple stem grooving virus; **E**, potato virus X; and **F**, cucumber mosaic virus.

Fig. 2. Results of dot-ELISA for potato virus X (PVX) detection. P = positive control (*Nicotiana benthamiana* leaves that were infected by PVX); N = negative control (virus-free kiwifruit leaves); and 1 to 5 = five samples that were detected positive for PVX by RT-PCR, which were also detected positive by dot-ELISA.

were deposited in GenBank. For AcVA there were two AcVA complete CP gene sequences in GenBank, for Haenam and TP7-93A, from South Korea and New Zealand, respectively (Supplementary Table S5). The phylogenetic tree of the 30 complete CP gene sequences of AcVA (Fig. 3) shows that isolates are divided into two groups. The majority of Meixian, Zhouzhi, Hanzhong, and Yangling isolates were clustered into group I and group II, whereas Yangling 4, Meixian 6, and TP7-93A were outliers.

Fig. 3. Unrooted neighbor-joining phylogenetic tree based on nucleotide sequences of the *Actinidia virus A* complete coat protein gene. Black dots mark the sequences obtained in this study. Meixian, Zhouzhi, and Yangling denote location in Shaanxi province, China. Bootstrap values below 60% are not shown.

For AcVB there were two AcVB complete CP gene sequences in GenBank, Haenam and TP7-93B, from South Korea and New Zealand, respectively (Supplementary Table S6). The phylogenetic tree of the 18 complete CP sequences shows that AcVB isolates were divided into two groups (Fig. 4). Group I was further divided into two subgroups (IA and IB). The majority of Meixian, Zhouzhi, and Yangling isolates were clustered into group IA, whereas Haenam and TP7-93B isolates were placed in group IB. Meixian 6 was an outlier, and Meixian 7 and Meixian 8 were placed in group II.

For AcCRaV there was only one AcCRaV complete CP gene sequence in GenBank, HN-6, from China (Supplementary Table S7). The phylogenetic tree of the 26 complete CP sequences showed that AcCRaV isolates were divided into two groups (Fig. 5). Group I was divided into two subgroups (IA and IB). The majority of Meixian, Zhouzhi, Hanzhong, and Yangling isolates were clustered into groups IA and IB; HN-6 clustered into group IA, whereas Zhouzhi 1, Hanzhong 5, and Meixian 10 isolates were clustered into group II.

For ASGV, 20 complete CP gene sequences were downloaded from GenBank (Supplementary Table S8). The phylogenetic tree of the 36 complete CP sequences showed that ASGV isolates were divided into two groups (Fig. 6). Group I was divided into two subgroups (IA and IB). The majority of Meixian, Zhouzhi, Hanzhong, and Yangling isolates were clustered into group IA with a closest relationship to isolate ASGV-CHN from apple in China. Hanzhong 1 and Meixian 5 isolates were clustered into group IB with a closest

relationship to isolate CTLV-MTH from citrus in China. ASGV-ki-1 and CTLV-ML isolates were placed into group II.

For PVX, 16 complete CP gene sequences were downloaded from GenBank (Supplementary Table S9). The phylogenetic tree of the 27 complete CP sequences showed that PVX isolates were divided into two groups (Fig. 7). All of the Meixian, Zhouzhi, Hanzhong, and Yangling isolates were clustered into group I with a closest relationship to isolates Beijing 2 and Chinese, identified in China from *Sanicula chinensis* and *Orychophragmus violaceus*, respectively. Group I also contained QLD1974, QLD249, QLD1970, TAS3, WADel, Hebei, and Fujian isolates identified from potato from various countries. Other potato isolates HB, DY, B, BL4, EX, and EX2 were clustered into group II.

For CMV, 14 complete CP gene sequences were downloaded from GenBank (Supplementary Table S10). The phylogenetic tree of 30 complete CP sequences shows that CMV isolates were divided into two groups (Fig. 8). Group I was divided into two subgroups. All of the Meixian, Zhouzhi, Hanzhong, and Yangling isolates were clustered into group II. Isolates S, Trk7, LS, LY, and Q from various countries were also placed in group II, whereas other isolates from different hosts in various countries were clustered into group I.

Discussion

The present study is the first large-scale survey of kiwifruit viruses in Shaanxi, the largest kiwifruit-growing province in China. Six

Fig. 4. Unrooted neighbor-joining phylogenetic tree based on nucleotide sequences of the *Actinidia* virus B complete coat protein gene. Black dots mark the sequences obtained in this study. Meixian, Zhouzhi, and Yangling denote location in Shaanxi province, China. Bootstrap values below 60% are not shown.

viruses (AcVA, AcVB, AcCRaV, CMV, ASGV, and PVX), were surveyed either as individual virus infections or as coinfections with two or more other viruses. Symptoms induced by these viruses were easily observed and varied with the type of viruses in *A. deliciosa* 'Xuxiang'. These data suggest this cultivar may be used as a biological indicator for diagnosing kiwifruit viruses. Because these symptoms were observed mainly in plants that had been co- or mix-infected with the viruses, further studies are needed to obtain symptoms induced in plants infected with a single virus. These studies are under investigation.

Previous studies reported six viruses from different kiwifruit-growing provinces in China, for example, AcVA, AcVB, and AcCRaV from Hubei (Zheng et al. 2014, 2017), CMV from Sichuan (Wang et al. 2018a), and ASGV from Fujian (Wang et al. 2018b). The present study identified for the first time the occurrence of these six kiwifruit viruses in Shaanxi province of China. Qinling Mountain, which is located only about 5 to 10 km away from the major

kiwifruit-producing regions surveyed in the present study, is the major site of origin of *Actinidia* spp. in China (Ferguson and Bollard 1990; Ferguson and Huang 2007; Huang 2010). Kiwifruit has been cultivated in Shaanxi province for many years, along with other major agricultural crops such as apple and potato. A wide diverse of plants including both cultivated and wild species may act as a reservoir harboring diverse types of kiwifruit viruses.

The phylogenetic trees for AcVA, AcVB, and AcCRaV isolates from Shaanxi kiwifruit showed a substantial sequence variation with the isolates forming three distinct clades. In contrast, the CP genes of ASGV, CMV, and PVX isolates from Shaanxi kiwifruit showed a high degree of sequence conservation. ASGV was first detected in kiwifruit by ELISA and RT-PCR in 2003 (Clover et al. 2003). The virus was then reported to infect kiwifruit in India in 2014 (Bhardwaj et al. 2014). In 2018, the virus was reported to infect kiwifruit in China (Wang et al. 2018b). Previous reports usually indicated that the phylogenetic relationships among ASGV isolates were not

Fig. 5. Unrooted neighbor-joining phylogenetic tree based on nucleotide sequences of the *Actinidia* chlorotic ringspot-associated virus complete coat protein gene. Black dots mark the sequences obtained in this study. Meixian, Zhouzhi, and Yangling denote location in Shaanxi province, China. Bootstrap values below 60% are not shown.

related to their host and geographic origins (Magome et al. 1997; Pūpola et al. 2011). ASGV isolates from Shaanxi kiwifruit formed a highly conserved clade and were distinct from other ASGV isolates, except for two isolates (Hanzhong 1 and Meixian 5) that clustered with citrus tatter leaf virus (CTLV) isolates.

CMV has a broad host range, with over 1,200 plant host species in over 100 families (Douine et al. 1979). Previous research found that CMV virus isolates on different crops could be divided mainly into two groups, with no significant correlation to hosts or locality (Rabie

et al. 2017). CP genes of the CMV isolates from Shaanxi kiwifruit all clustered with group II. PVX mainly infects solanaceous plants and is transmitted mechanically via infected sap and infected seed potato tubers but not by insect vectors (Stevenson et al. 2001). The PVX of kiwifruit isolates in Shaanxi province were highly conserved and were placed in group I, their closest relatives being Beijing 2 and Chinese.

In conclusion, this study identified the main viral species infecting Xuxiang kiwifruit in Shaanxi, China, and determined the incidence, distribution, and genetic diversity of these viruses. To

Fig. 6. Unrooted neighbor-joining phylogenetic tree based on nucleotide sequences of the apple stem grooving virus complete coat protein gene. Black dots mark the sequences obtained in this study. Meixian, Zhouzhi, and Yangling denote location in Shaanxi province, China. Bootstrap values below 60% are not shown.

our knowledge, this is the first report of PVX to infect kiwifruit and the first report of the molecular variability of AcVA, AcVB, and AcCRaV. These results provide important data for the study of kiwifruit viruses, especially the genetic evolution of the six viruses. Our study will provide important data for virus detection of kiwifruit seedlings and a basis for guidelines for the prevention of the spread of kiwifruit viruses in Shaanxi province of China. This study only tested for the presence of selected known viruses, and consequently other viruses may have gone undetected. Next-generation sequencing methods can be used in subsequent studies to detect new viruses

of kiwifruit. At present, the effect of viruses on kiwifruit production in China is unclear, because few studies have been conducted. Consequently, there is an urgent need for further studies to determine the impact of viruses and recommend appropriate management practices.

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Fig. 7. Unrooted neighbor-joining phylogenetic tree based on nucleotide sequences of the potato virus X complete coat protein gene. Black dots mark the sequences obtained in this study. Meixian, Zhouzhi, and Yangling denote location in Shaanxi province, China. Bootstrap values below 60% are not shown.

Fig. 8. Unrooted neighbor-joining phylogenetic tree based on nucleotide sequences of the cucumber mosaic virus complete coat protein gene. Black dots mark the sequences obtained in this study. Meixian, Zhouzhi, and Yangling denote location in Shaanxi province, China. Bootstrap values below 60% are not shown.

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