

Apricot is a new host of *Apple scar skin viroid*

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Abstract. *Apple scar skin viroid* (ASSVd), a pathogenic RNA viroid, infects apple and pear trees. To determine if ASSVd can also infect apricot, we performed reverse transcription–polymerase chain reaction (RT-PCR), spot hybridisation and *in situ* RT-PCR and detected ASSVd-specific sequences from apricot. We cloned and sequenced the apricot-derived ASSVd and registered it in GenBank. *In situ* RT-PCR further confirmed the existence of ASSVd in apricot leaf tissues. Our data indicate that apricot is a novel host of ASSVd. These findings provide the basis for the rapid identification of ASSVd in apricot.

Viroids are the smallest pathogens discovered to date, consisting of a single-stranded RNA of 246–574 nucleotides in an unencapsulated, covalently closed-circular conformation. Viroids do not encode any proteins and they depend on the host for replication (Guo 2006). Viroids are classified into two families, *Pospiviroidae* and *Avsunviroidae*, based on homology in primary sequences, secondary structures and replication characteristics. *Apple scar skin viroid* (ASSVd), a member of the *Pospiviroidae* that replicates in the nucleus through an asymmetrical rolling circle mechanism, usually comprises 330 nucleotides, and possesses no nuclease activity.

Koganezawa *et al.* (1983) first reported the detection of ASSVd RNA in diseased apple fruits. Subsequently, Chen *et al.* (1986) isolated ASSVd from diseased branches, determined the sequence of the RNA, and proved that apple scar skin was a viroid disease. Hashimoto and Koganezawa (1987) first reported the entire sequence of ASSVd. Subsequently, Guo *et al.* (2005) and Zhao and Niu (2006) have cloned and analysed its sequence. ASSVd can infect apple and pear (Hadidi *et al.* 1991; Yang *et al.* 1992; Koganezawa *et al.* 2003; Kyriakopoulou *et al.* 2003), but previously it was not known if ASSVd could also infect apricot. In this study, we detected ASSVd by reverse transcription–polymerase chain reaction (RT-PCR), spot hybridisation and *in situ* RT-PCR in apricot trees in Xinjiang, China. Thus, for the first time, our study showed that ASSVd is also a pathogen of apricot trees, suggesting that ASSVd may have a wider impact in agricultural disease.

Tender leaves and shoots used for RNA extraction were collected from a 50-year-old orchard in Yanqi County, Xinjiang, China, growing many species of fruit trees, including apple, pear, peach and apricot. The infected apricot tree showed the symptoms of leaf epinasty and distortion. Biologically characterised ASSVd samples numbered AP1 to AP10 were thus obtained from symptomatic trees. Samples AP1 to AP10 were collected from 10 different trees that had

symptomatic leaves. Spot hybridisation confirmed the presence of ASSVd in each of the samples. RNA extraction was performed using ~250 mg of fresh or frozen tender leaf tissue, which was ground to a fine powder in liquid nitrogen and extracted using the method of Zhao and Niu (2006). The isolated total RNA (0.2 µg) from each sample was used as template for RT. Primers were designed based on the reported sequences by The National Center for Biotechnology Information of USA (NCBI, GenBank accession number X17696) (Puchta *et al.* 1990): ASSVd₁: 5'-CAGCACCACAGGAACCTCACGG-3' (antisense, 10–32); ASSVd₂: 5'-CTCGTCGTCGACGAAGG-3' (sense, 80–97) and were synthesised by Shanghai Biological Engineering Technology and Service Corp. The antisense primer ASSVd₁ (20 µM) was used as a primer for RT by Moloney murine leukemia virus (M-MLV) reverse transcriptase (Shanghai Biological Engineering Technology & Service Corp.) at 42°C for 1 h in 50 mM Tris-Cl pH 8.3, 75 mM KCl, 3 mM MgCl₂, and 10 mM DDT. PCR was performed in 10 mM dNTPs, 2 mM Mg²⁺, antisense and sense primers (ASSVd₁ and ASSVd₂ at 20 µM), cDNA (3 µg) and 2.5 U Taq DNA polymerase (Shanghai Biological Engineering Technology & Service Corp.). Samples were amplified for 32 cycles as follows: denaturation at 95°C for 3 min, 62°C for 45 s and 72°C for 60 s, with the final extensions at 72°C for 7 min. The amplified products were separated by electrophoresis on 2% (w/v) non-denaturing agarose gel in 1 × TBE buffer at 120 V for 40 min, and visualised by staining with Gold Viewer Dye (Beijing Liuyi Factory, China). PCR markers (Hua Mei, China) were used to determine the sizes of the amplified fragments. Expected fragments of ~330 bp were obtained through RT-PCR using specific primers for ASSVd (Fig. 1).

To identify the sequence of each amplified product from RNA isolated from each sample of AP1 to AP10, the amplified products were separated by electrophoresis on a 2% agarose gel in 1 × TAE buffer, excised from the gel under ultraviolet light and recovered using UNIQ-10 Gel Extraction Kit (Sangon) according to

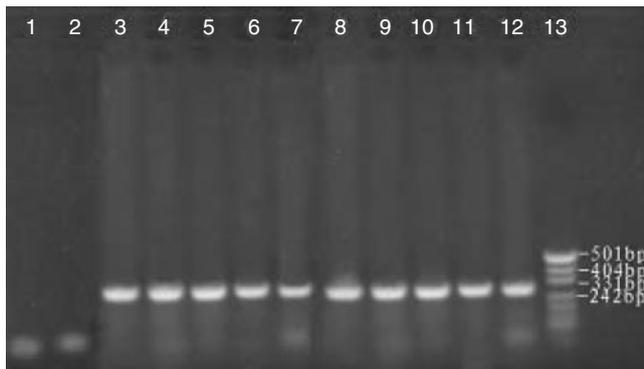


Fig. 1. RT-PCR products from ASSVd. Lane 1, products amplified with primers ASSVd1 and ASSVd2 primers from RNA from healthy leaf tissues; lane 2, products amplified from RNA isolated from sample AP1 using the antisense primer ASSVd1 only; lanes 3–12, fragments amplified using both ASSVd1 and ASSVd2 primers from RNA isolated from apricot samples AP1–AP10, respectively; lane 13, DNA marker.

manufacturer's protocol. The recovered fragments were cloned into pUCm-T vector (Shanghai Biological Engineering Technology and Service Corp.). A representative clone from each sample was submitted to Shanghai Biological Engineering Technology and Service Corp for sequencing. The 10 sequences obtained in this study showed at least 98% sequence identity to each other and were submitted to GenBank (accession numbers EU031487 to EU031496). Sequence analysis of the selected clones revealed that the full-length of viroid in apricot was 330 nt and exhibited the highest homology with the ASSVd (accession number X17696) using Basic Local Alignment Search Tool (BLAST).

Three full length apple isolates of ASSVd from different regions of China (Liaoning isolate (accession number AY972082, Guo *et al.* 2005), China isolate (accession numbers NC_001340 and X17696, Puchta *et al.* 1990) and Xinjiang isolate, Zhao *et al.* 2006)) showed no less than 99.7% homology with each other demonstrating low sequence variation across geographical regions. The ASSVd sequences obtained in this study from an apricot tree grown in Yanqi County, Xinjiang are 98% identical to these apple isolates. This finding suggests that the apricot isolate of ASSVd has been transmitted from an infected apple tree in this region.

The distribution of ASSVd within apricot leaf tissues was investigated by *in situ* RT-PCR. Paraffin sections of apricot leaves were prepared according to the method of Niu *et al.* (2007). Complementary DNA synthesis was performed according to the method of Zhao and Niu (2006) and *in situ* RT-PCR amplification and immunological detection used the methods of Niu *et al.* (2007). Positive signals (Fig. 2A) were detected in palisade cells while it was not detected when no RT was included in the protocol (Fig. 2B). These data demonstrate that ASSVd does indeed infect apricot cells.

Previously it has been shown that ASSVd can infect apple and pear trees (Puchta *et al.* 1990; Zhu *et al.* 1995; Guo *et al.* 2005; Zhao and Niu 2006, 2007) but there was no report that ASSVd has been discovered in trees that bear drupes. We describe the amplification, cloning and sequencing of full-length ASSVd

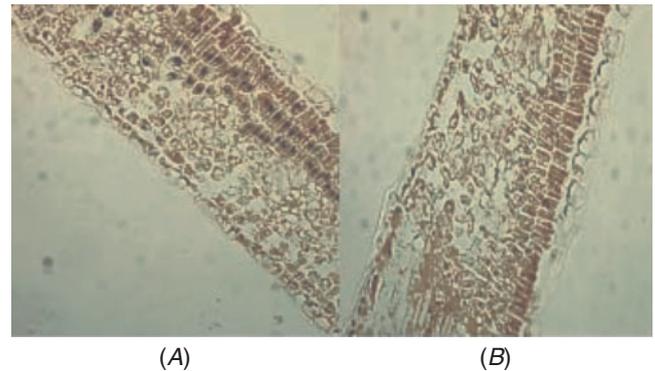


Fig. 2. *In situ* reverse transcription-polymerase chain reaction of ASSVd. (A): positive staining of ASSVd by reverse transcription-polymerase chain reaction (RT-PCR); (B): negative control with RT step omitted.

from apricot grown in China (GenBank accessions EU031477 to EU031486) and the existence of ASSVd in apricots was reconfirmed by *in situ* RT-PCR. Our study showed that ASSVd was detectable in apricot and provides the basis for the rapid identification of ASSVd in apricot.

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