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Detection and Distribution of *Apple scar skin viroid*-Korean Strain (ASSVd-K) from Apples Cultivated in Korea

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***Apple scar skin viroid* (ASSVd) has been one of the most destructive diseases in Korean apple orchards. Symptoms of the scar skin viroid disease were detected in various apple cultivars, namely, Sansa, Fuji, Chukwang, Miki-Life, Hongro, and Songbongeum cultivated in the southern part of Korea. The RNA molecules were extracted from the apples bearing dapple apple symptoms with the application of CF-11 RNA extraction method. The purified RNAs were used for the synthesis of cDNA with RT-PCR. The PCR products were cloned and sequenced. The viroid RNA molecules from the six different cultivars bearing the dapple symptoms showed the same nucleotide sequences as that of the Korean strain of ASSVd (ASSVd-K). ASSVd-K was detected from apple orchards in Kunwi, Sangju, Uiseong, Yeongyang, Andong, and Youngduk in Gyeongbuk Province in 2001, and in Muju in Jeonbuk Province in 2002. As the viroid disease could be propagated vegetatively, it can be widely transmitted gradually in Korea.**

Keywords : *Apple scar skin viroid*-Korean strain, CF-11, RNA extraction, RT-PCR, viroid RNA molecules.

Viroids are the smallest plant pathogens distinguished from viruses by the absence of the coat protein and the protein coding capacity. They are circular, single-stranded RNA molecules with size ranging from 245-401 nucleotides. It is generally assumed that most reactions of viroid replication and pathogenesis depend on enzymes of the host plants (Diener, 1987; Sänger and Lee, 1993).

Viroids may also cause a significant damage to crops and fruit trees (Diener, 1987; Diserio et al., 1996; Hashimoto and Koganezawa, 1987; Sano et al., 1989). Currently, about 27 different viroids have been detected (Flores et al., 1998). *Apple scar skin viroid* (ASSVd) is one of the most destructive diseases in apples in Japan and China, although its occurrence is relatively rare in most apple producing countries.

It is known that the symptoms of ASSVd are restricted to apples. The disease causes fruit dappling, scarring, and cracking. The dapple apple symptom is similar to chlorosis. The symptoms are different in size and color depending on the fruit size and ripeness. This study investigated six cultivars of apples cultivated in the southern part of Korea.

The ASSVd was first detected in Uiseong, Gyeongbuk Province from the cultivar Miki-Life bearing dapple symptoms. The distribution of ASSVd was extended into neighboring places, namely, Kunwi, Sangju, Yeongyang, Andong,

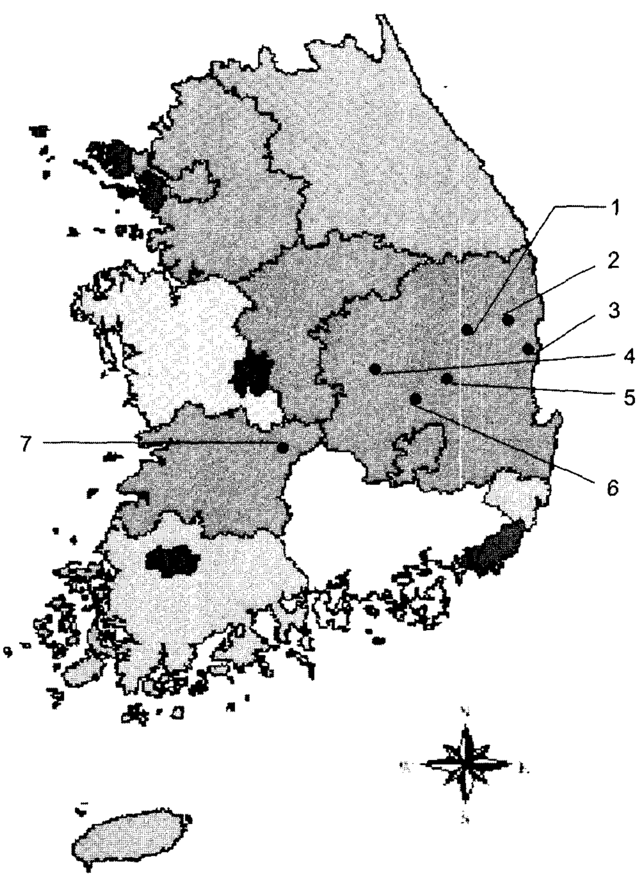

Fig. 1. ASSVd detected regions in Korea. Locations 1: Andong, 2: Yeongyang 3: Youngduk, 4: Sangju, 5: Uiseong, 6: Kunwi, and 7: Muju.

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and Youngduk in 2001, and Muju in 2002 (Fig. 1).

Viroid RNA molecules were extracted from six different cultivars with CF-11 cellulose (Whatman) and were used for the cDNA synthesis with the RT-PCR method. The PCR products were cloned into a pGEM-T Easy vector (Promega) and sequenced using dye terminators according to the manufacturer's protocol (ABI 377, Perkin Elmer). The ASSVd was detected from the five different cultivars of apple in 2001, and from one cultivar in 2002. The number of nucleotide sequences of ASSVd extracted from the five different cultivars was 331, except that of the newly detected Songbongeum cultivar, as shown in Fig. 2 (The nucleotide sequence of ASSVd extracted from the cultivar Songbongeum is still being determined).

The RNA molecules were extracted from the apples using CF-11 cellulose chromatography (Pallas et al., 1987). RT-PCR products were synthesized from purified RNAs, which were extracted from the various cultivars of apples, namely, Sansa, Fuji, Chukwang, Miki-Life, Songbonggeum, and Hongro.

The number of nucleotide sequences of the cloned viroid RNA molecules was 331, similar to the nucleotide sequences of *Apple scar skin viroid*-Korean strain (ASSVd-K) reported previously (Lee et al., 2001). ASSVd-K was first detected from Miki-Life cultivated in Gyeongbuk Province. It showed one nucleotide difference ("G" insertion between the position of 133 and 134) compared with the type strain of ASSVd reported in Japan (Hashimoto and Koganezawa, 1987; Puchta et al., 1990).

This report described the gradually wide distribution and detection of ASSVd in Korea. The viroid was detected from various apple cultivars distributed in several places in the southern part of Korea. The apple viroid is transmitted mainly by vegetative propagation including graft. On the basis of vegetative propagation and the symptom development on the fruit of the apple viroid-infected plants, the detection of the viroid would take at least 3-5 years. Therefore, it is very important to achieve viroid-free young plants including healthy scions and rootstocks. Recently known ASSVd caused significant damages on apple trees in several regions. In order to reduce the damage from the apple viroid disease, rapid and simple viroid detection method for the young plants is required urgently.

The polyacrylamide gel electrophoresis (PAGE) and RT-PCR methods are currently used for the detection of viroids (Hadidi et al., 1990; Hurtt et al., 1996; Yan et al., 1997). However, it is necessary to develop a more sensitive and economical method to detect the viroid from the young plants. In addition, the more the interactions between the viroid and the host plants are understood, the more there is a need to investigate the nature of the viroid RNAs *in vivo*.

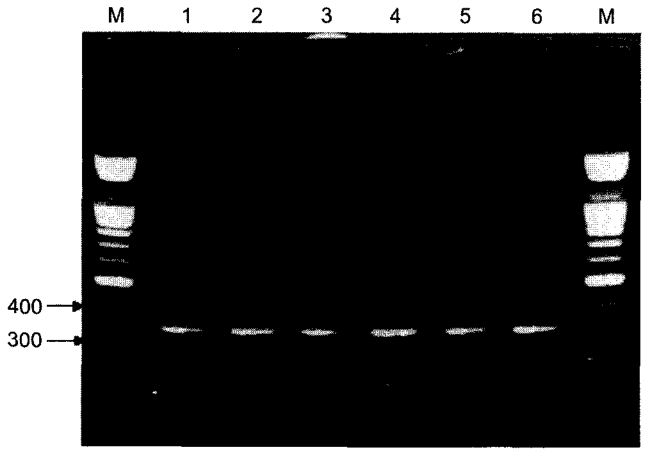

Fig. 2. Electrophoresis of the RT-PCR products from the different cultivars of apple showing ASSVd symptoms of apple. Lane M, 100bp ladder DNA marker; lanes 1-6 represent the different cultivars of Sansa, Fuji, Chukwang, Hongro, Miki-Life, and Songbongeum, respectively.

Acknowledgment

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