

Nucleotide Sequence of Hop Stunt Viroid Kh Strain (HSVd-Kh)

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호프 왜화 바이로이드 Kh 계통(HSVd-Kh)의 염기서열

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ABSTRACT: The nucleotide sequence of hop stunt viroid (HSVd) Kh strain was sequenced by the reverse transcription and polymerase chain reaction. It consists of 296 nucleotides, and differs by one nucleotide deletion of cytosine at the position of 295 from the HSVd-K strain which consists of 297 nucleotides.

Key words: Hop stunt viroid Kh strain, nucleotide sequence.

Viroids are known to be infectious low molecular weight RNA species which cause serious diseases of higher plants. Hop stunt viroid RNA (HSVd) has been detected by molecular hybridization from the stunted plants of the Japanese hop (*Humulus lupulus* L. cultivar Kirin II) cultivated in Korea (2, 3). Viroid RNA molecules were purified from the viroid infected hop plants by Qiagen column chromatography and electroelution. Reverse transcription and polymerase chain reaction (RT-PCR) with two pairs of reverse and forward viroid-speci-

fic primers, and sequence analysis of the corresponding cDNA fragments according to the method of Sanger were performed as previously described (3, 5).

The complete nucleotide sequence of HSVd type strain that consists of 297 nucleotides has been established in Japan (4). The Korean strain of HSVd (HSVd-K) consists of the same number of nucleotides with the type strain. But HSVd-K has three nucleotides differences with HSVd type strain (3, 4).

The full sequence of Hop stunt viroid (HSVd) Kh

Table 1. Synthetic primer sequences for reverse transcription and PCR

Primer name	Sequence	Size	Restriction site	Direction
pHS1	5'-GCCGAATTC CCCAGAGGGGCTCAAG-3'	25 mer	<i>EcoRI</i>	forward
pHS3	5'-GGGAATTCTCGATTGCCGCAAAAGGC-3'	27 mer	<i>EcoRI</i>	reverse
pHS4	5'-GCCCCGGGGCTCCTTTCTCAG-3'	21 mer	<i>SmaI</i>	forward
pHS2	5'-GCCCCGGGGCAACTCTTCTCAGAATC-3'	26 mer	<i>SmaI</i>	reverse

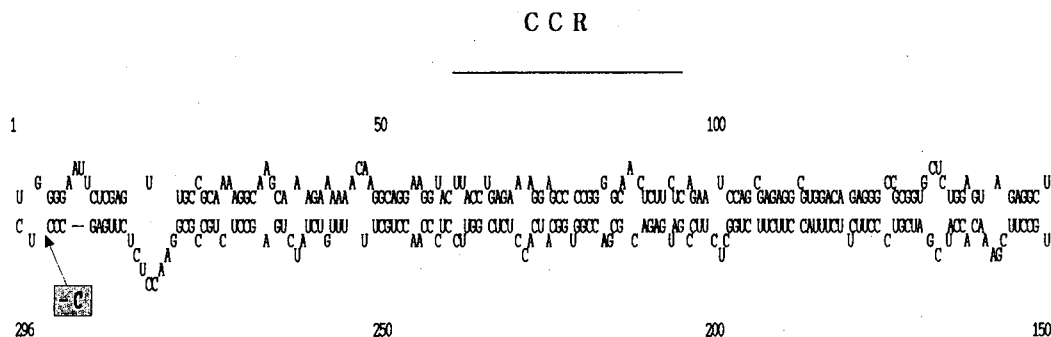


Fig. 1. Secondary structure of the hop stunt viroid (HSVd) Kh strain. CCR means central conserved region.

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strain has been sequenced directly from the hop plants cultivated in Korea. It consists of 296 nucleotides (BankIt 231904, AF 100641) and differs from HSVd-K by one nucleotide deletion at the position of 295. HSVd-K strain has nucleotide differences from Japanese type strain of HSVd by three nucleotide substitutions. All these nucleotide variations are located in the left hand part of the secondary structure of viroid molecule.

Several strains of HSVd have been isolated in different host plants of cucumber, grapevine, citrus, peach and plum. The nucleotide sequences of HSVd type strain and HSVd-K could be expected to be same, because most of hop plants cultivated in Korea are imported from Japan and are propagated widely for a long time. But the two strains are differed three nucleotides in their genetic structures. Even the nucleotide sequence of HSVd-Kh purified directly from the Korean hop plants expressed one nucleotide difference comparing to that of HSVd-K purified from the prokaryotic plants of wild curcubit.

The variations of the nucleotide sequences among the three different strains of the same viroid RNA molecule could be induced from the environmental factors including the geographic differences and the different host plants.

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요 약

호프 왜화 바이로이드(HSVd) Kh 계통의 염기서열을 역전사와 중합효소 연쇄반응을 이용하여 결정하였다. 296개의 염기로 구성된 HSVd-Kh의 염기서열은 295번째 시토신 염기 하나가 없어지면서 297개의 염기로 구성된 HSVd-K 계통과 또 다른 차이를 보였다.

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