# Two novel variants of hop stunt viroid associated with yellow corky vein disease of sweet orange and split bark disorder of sweet lime

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## Abstract

Yellow corky vein was reported as a graft-transmissible disease of lime in India. It was attributed to infection by hop stunt viroid (HSVd) and citrus exocortis viroid (CEVd). Recently similar symptoms have been observed in Washington navel orange in Jahrom and Darab in the Fars province of Iran. It is characterized by yellowing and suberization of veins followed by tree decline. Sweet lime split bark is another disorder of increasing importance in the Fars province. It is characterized by cracks in the bark of the main stem which may spread to branches of the tree. Since these symptoms resembled those of certain viroids, a study was undertaken to determine possible association of viroids with the disorders. Reverse transcription polymerase chain reaction (RT-PCR) followed by cloning and sequencing of PCR products and dot-blot hybridization were used to identify the viroids associated with the diseases. Comparison of molecular properties (nucleotide composition, primary and secondary structures, molecular weights, phylogenetic relationships and percent nucleotide similarity and difference) of viroid variants were carried out. It was found that a novel variant of hop stunt viroid (HSVd-sycv) was associated with yellow corky vein disease of Washington navel and another new variant (HSVd-sb) with split bark disorder of sweet lime. No other viroids were constantly detected. HSVd-sycv was closely related to noncachexia variant of hop stunt viroid (HSVd-cit) but only with 93.7% homology with HSVd-lycv. It differed in a single nucleotide from HSVd-cit, in the variable domain in the so-called "cachexia expression motif". HSVd-sb had only 94.8% homology with a noncachexia variant of hop stunt viroid (CVd-IIa-117) which causes mild bark-cracking symptoms on Pomeroy trifoliate orange rootstocks. According to the performed molecular comparisons, HSVd-sb differed from CVd-IIa-117 in "cachexia expression motif" and probably severe cracks induced by HSVd-sb occurred because of variation in this motif.

Keyword: HSVd, Sweet lime split bark, Sweet orange yellow corky vein, Citrus viroids, Viroid phylogeny.

## Introduction

Citrus viroids are classified into four genera, all belonging to the family *Pospiviroidae.* (Flores et al., 2005; Ito et al., 2001). *Citrus exocortis viroid* (CEVd), *Hop stunt viroid* (HSVd, formerly called CVd-II) and *Citrus viroid IV* (CVd-IV) are assigned to the genera *Pospiviroid*, *Hostuviroid*, and *Cocadviroid*, respectively (Ito et al., 2000; Ito et al., 2001; Flores et al., 1998). The genus *Apscaviroid* includes *Citrus bent leaf viroid* (CBLVd, including *Citrus viroid-I-LSS*), *Citrus viroid-III* (CVd-III), *Citrus viroid-V* (CVd-V) and *Citrus viroid-OS* (CVd-OS) (Barbosa et al., 2005). While some of these citrus viroids are apparently harmless to citrus, others may cause important diseases such as exocortis (Semancik and Weathers, 1972) and cachexia (Diener et al., 1988; Semancik et al., 1988).

Citrus strains of HSVd, in addition to causing cachexia, have been implicated in other diseases including yellow corky vein of Kagzi lime (*Citrus aurantifolia*), reported from India (Roy and Ramachandran, 2003). The disease was characterized by yellow spots on leaf lamina, which soon spread along the mid and lateral veins. The veins turned rough on underside and developed corky tissues. A yield loss of 51.3–60.4 % was reported from Assam (Azad, 1993). HSVd (HSVd-lycv) and CEVd were associated with the disease (Roy and Ramachandran, 2006).

In recent years, a disease with specific symptoms of yellow corky vein has emerged in navel oranges in the Fars province of Iran (Figure 1, top). These symptoms are often associated with declining of affected trees. It has become of concern to the growers as it appears to be spreading from tree to tree.

Split bark is a disorder first described in declining sweet lime (*Citrus limettoides*) trees from Iran (Izadpanah, 1983). The disorder is characterized by cracks in bark of the stem, which spreads along main branches. The affected tree shows retarded growth and without any symptoms in fruits and leaves. Split bark disorder is becoming important in sweet lime which is a commercially important variety in Iran (Figure 1, bottom).

**Fig. 1** Yellow corky vein symptoms on Washington navel sweet orange leaves (top) and split bark symptoms on the main trunk of sweet lime (bottom).

In the present study we report two novel variants of hop stunt viroid each associated with yellow corky vein disease of sweet orange or split bark disorder of sweet lime.

## Materials and methods

Plant samples and Extraction of nucleic acids: Leaves of affected trees showing yellow corky vein and split bark symptoms were collected in 2007 and 2008 from Jahrom and Darab in the Fars province of Iran. Extraction of nucleic acids from samples was carried out by standard viroid extraction method (Semancik et al., 1975) designed to yield high viroid titers. The total nucleic acids were partitioned in 2M LiCl and the soluble fraction was concentrated by ethanol precipitation and resuspended in TKM buffer (10 mM Tris–HCl; 10 mM KCl; 0.1 mM MgCl2; pH 7.4). A citrus sample known to be infected with HSVd was used as positive control. The negative control consisted of leaf samples of a non-affected tree.

RT-PCR, cloning and sequencing of the amplified fragments: Reverse transcription polymerase chain reaction (RT-PCR) to detect CEVd, CBLVd, HSVd, CVd-III, CVd-IV and CVd-V were carried out following the method described by Bernad and Duran-Vila (2006). It involved the use of primers designed to amplify the full length of each target viroid (Table 1). Electrophoretic analysis in 1 % agarose gels was used to confirm the synthesis of a DNA product of the expected size. The PCR-amplified products were ligated into the vector pTZ57R/T (Fermentas) and the recombinant plasmids were used to transform DH5$\alpha$ *E. coli* cells. Plasmids from transformed cells were purified using the High Pure Plasmid Extraction Kit (Roche). PCR analysis was used to demonstrate the presence of correct insert.

**Tab. 1** Primers used in RT-PCR to amplify citrus viroids.

| Viroid | Direction | Primer Sequence | Reference |
|---|---|---|---|
| CEVd[a] | Reverse | 5′-CCGGGGATCCCTGAAGGA-3′ | Gross et al., 1982 |
| | Forward | 5′-GGAAACCTGGAGGAAGTCG-3′ | |
| HSVd | Reverse | 5′-GGGGCTCCTTTCTCAGGTAAGTC-3′ | Sano et al., 1988 |
| | Forward | 5′-GGGGCAACTCTTCTCAGAATCC-3′ | |
| CBLVd | Reverse | 5′-TTCGTCGACGACGACCAGTC-3′ | Ashulin et al., 1991 |
| | Forward | 5′-GGCTCGTCAGCTGCGGAGGT-3′ | |
| CVd-III | Reverse | 5′-TTCGTCGACGACGACAGGTA-3′ | Bernad and Duran-Vila, 2006 |
| | Forward | 5′-GGCAGCTAAGTTGGTGACGC-3′ | |
| CVd-IV | Reverse | 5′-GGGGATCCCTCTTCAGGT-3′ | Bernad and Duran-Vila, 2006 |
| | Forward | 5′-GGGGAAATCTCTTCAGAC-3′ | |
| CVd-V | Reverse | 5′-GGAACCACAAGGTTGTTCAC-3′ | Serra et al., 2007 |
| | Forward | 5′-TGTGGGTCACCCCGCCCC-3′ | |

[a] CEVd = Citrus exocortis viroid, CBLVd = Citrus bent leaf viroid, HSVd = Hop stunt viroid, CVd-III = Citrus viroid III, CVd-IV = Citrus viroid IV, CVd-V = Citrus viroid V.

Detection of viroids by dot-blot hybridization: Full-length cDNAs of CEVd, CBLVd, HSVd, CVd-III, CVd-IV and CVd-V were cloned into plasmid vector pTZ57R/T (Fermentas). DIG-labeled cDNA probes were prepared from these plasmid DNAs according to the method described by Li et al. (1995). Dot-blot hybridization followed the method of Li et al. (1995) with slight modification. Hybridization was carried out at 50 $^0$C and the membranes were washed at 60$^0$C. The signals were detected by a chromogenic assay.

Sequence analysis and prediction of RNA secondary structure: Eight independent clones from each disease were sequenced in both directions (Macrogen Inc., Seoul, South Korea). Sequence data were compiled, analyzed and compared with those available in GenBank, using NCBI/BLAST, to search for related sequences. Alignment of sequences was performed using the Vector NTI 9 software package (InforMax, Bethesda, MD). Comparison of nucleotide composition was performed employing the BioEdit (version 5.0.9) Sequence Alignment Editor program (Hall, 1999). Phylogenetic analysis was carried out using DNAMAN software (version 4.0.1.1) and a tree was constructed using the neighbor-joining method (Saitou and Nei, 1987) based on 10 000 replicates. Genetic distances were calculated using the MegAlign program in the DNASTAR software package (Madison, WI). The most stable secondary structure analysis was obtained with the RNAstructure software (version 4.6). The nucleotide sequence data reported in this paper were submitted to the EMBL, GenBank and DDBJ Nucleotide Sequence Databases.

## Results and discussion

Detection of viroids by RT-PCR: The PCR amplification with HSVd specific primers produced an amplicon of ~300 bp, as expected (Fig. 2, left). Nonspecific bands were seldom observed, and no fragments were detected from samples used as healthy controls (Figure 2).

CBLVd, CVd-IV and CVd-V were not detected in symptomatic trees by RT-PCR and dot-blot hybridization. CEVd and CVd-III were detected only rarely in some symptomatic trees (data not shown). However, CEVd has been reported in Kagzi lime with yellow corky vein disease (Roy and Ramachandran, 2006).

The nucleotide sequence analyses of HSVd from yellow corky vein affected sweet orange trees (HSVd-sycv) and split bark affected sweet lime trees (HSVd-sb) showed that those were new variants, similar to HSVd-lycv. The differences between the HSVd-sycv and HSVd-lycv are shown in Figure 4, top.

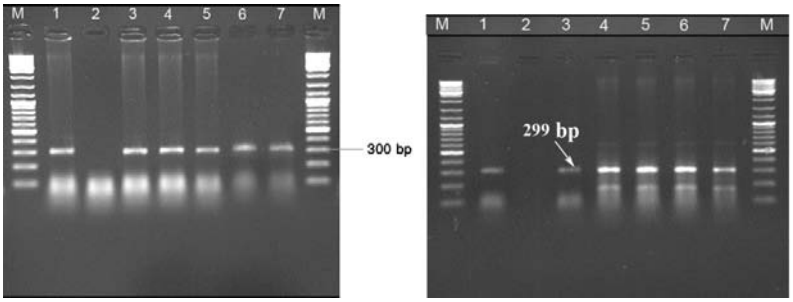


**Fig. 2** Electrophoresis pattern of RT-PCR products with sweet orange yellow corky vein (left) or sweet lime split bark affected (right) samples and specific hop stunt viroid primer pair: (1) Positive HSVd cDNA, (2) healthy citrus control, (3-7) sweet orange yellow corky vein or sweet lime split bark samples, (M) 100-bp DNA ladder.

Detection of viroids by dot-blot hybridization: All yellow corky vein and split bark samples showed a strong positive hybridization reaction against a DIG-labeled HSVd-cDNA probe (Figure 3). Dot-blot hybridization clearly detected the viroid in infected trees. The healthy control sample showed no reaction. No signal was obtained with CEVd, CBLVd, CVd-III, CVd-IV and CVd-V probes (data not shown). Neither RT-PCR nor dot-blot hybridization could detect HSVd-sycv and HSVd-sb in five samples of other citrus variants, which appeared healthy or had symptoms other than yellow corky vein or split bark, although other viroids could be detected occasionally (data not shown).

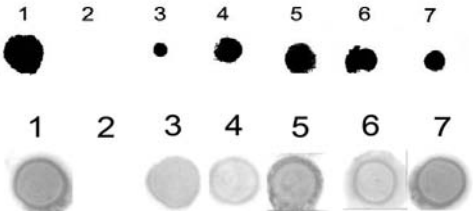


**Fig. 3** Dot-blot hybridization assay of sweet orange yellow corky vein (top) and sweet lime split bark affected (bottom) samples for HSVd cDNAs with specific Dig-labled probes. (1) Known HSVd cDNA, (2) Healthy control, (3-7) Samples from trees affected by yellow corky vein (top) or split bark (bottom) diseases.

Sequence analysis and prediction of RNA secondary structure: HSVd-sycv sequence [accession no. FJ465506] was 93.7 % homologous with HSVd-lycv while HSVd-sb sequence [accession no. FJ465507] was 94.8 % homologous with CVd-IIa-117 (Palacio-Bielsa et al., 2004), which according to the criteria of the International Committee for Taxonomy of Viruses (ICTV), are variants of HSVd rather than novel species (Flores et al., 2005).

Figure 4 illustrates the primary structures and alignments for maximum homology of HSVd-sycv, HSVd-sb and some other variants of HSVd. It shows that HSVd-sycv differs from HSVd-cit8 in a single nucleotide in the so-called “cachexia expression motif” in the variable domain. The HSVd-cit8 induces no symptoms in St. Michael orange-Wakayama trees in Japan (Ito et al., 2002). The “cachexia expression motif” plays a major role in inciting cachexia symptoms, and that changes within this motif affect symptom severity and may even suppress symptom expression. The lack of pathogenicity of HSVd-cit8, and association of symptoms with HSVd-sycv support the low flexibility of this motif (Serra et al., 2008).

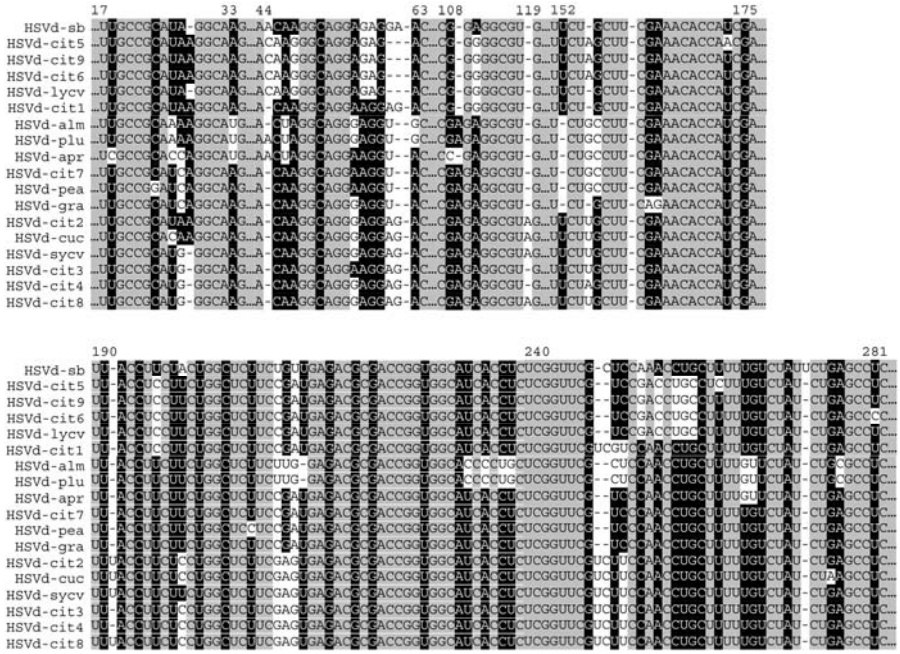


**Fig. 4** Primary structures of HSVd variants aligned for maximum homology using Vector NTI program (version 9.0.0). Portions of identical sequences are not shown. Nucleotides**:** Identical sequences, Nucleotides**:** Conserved sequences, -: lack of nucleotide

Figure 4 also shows that HSVd-sb differs from CVd-IIa-117 (HSVd-cit 2) in four nucleotides in "cachexia expression motif". The CVd-IIa-117 is a noncachexia variant of hop stunt viroid that induces mild bark-cracking symptoms on Pomeroy trifoliate orange rootstocks in Japan (Verniere et al., 2006). This difference between HSVd-sb and CVd-IIa-117 further suggests a low flexibility of this motif (Serra et al., 2008). It is yet to be determined whether these diseases are caused solely by HSVd or other factors, including those of host and environment, are involved in symptom expression. However, a single nucleotide change in the genome has been shown to affect the symptom expression (Serra et al., 2008).

Likewise, although viroid interactions are reported to alter plant symptoms (Verniere et al., 2006), none of the common citrus viroids other than HSVd-sycv or HSVd-sb were detected constantly in yellow corky vein or split bark affected plants, respectively. Point mutation experiments and testing the variants on the same host must be carried out to verify the role of nucleotide change in production of specific symptoms.

HSVd-sb is 4 nt shorter than CVd-IIa-117. Percent G+C in CVd-IIa-117 is somewhat higher than that of HSVd-sb. On the other hand, the secondary structure free energy of CVd-IIa-117 is higher than that of HSVd-sb. HSVd-sycv is 7 nt longer than HSVd-lycv. Percent G+C in HSVd-lycv is somewhat higher than that of HSVd-sycv. However, the secondary structure free energy of HSVd-sycv is higher than that of HSVd-lycv (Figure 5).

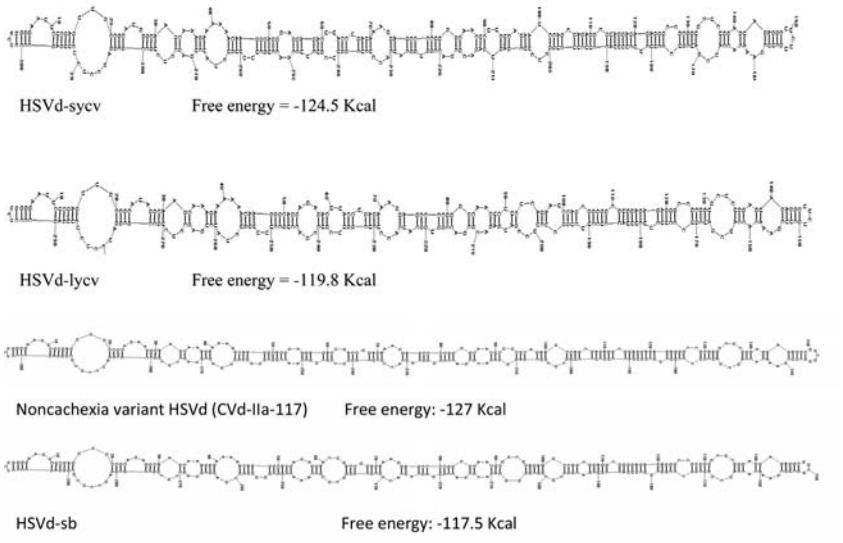


**Fig. 5** Comparison of secondary structures and minimum free energy of HSVd-sycv, HSVd-lycv, HSVd-sb and CVd-IIa-117 using RNAstructure software (version 4.6). The sequences are arranged so to obtain a maximum number of base pairs and taking into consideration the sensitivity of specific sites to controlled enzymatic digestions.

Sequence analysis revealed that the HSVd-sb consists of 299 nucleotides [accession no. FJ465507], composed of 63 A, 85 C, 80 G and 71 U residues, thus resulting in a G + C/A + U ratio of 1.23, suggestive of a highly base paired, heat stable molecule, characteristic of viroid-like low molecular weight RNAs. The most stable secondary structures of HSVd-lycv, HSVd-sycv, HSVd-sb and CVd-IIa-117 were a classical rod-like structure and all variants adopted a cruciform structure including various additional small hairpins. Comparison of the secondary structures of these variants indicates their close similarities in the rod-like structures, number of loops and the free energies.

Phylogenetic relationships: Sequence alignment between HSVd-sycv, HSVd-sb and other variants of the HSVd revealed identities of more than 90 %. A consensus phylogenetic tree based on the multiple sequence alignment illustrates the relationship between HSVd-sycv, HSVd-sb and other variants of the HSVd (Figure 6). HSVd-sycv formed a distinct cluster with HSVd-cit8 and HSVd-cuc. In BLAST analysis HSVd-sycv showed nearly 99% sequence identity with cucumber isolate of HSVd (HSVd-cuc) [accession no. X00524] and 99.7 % identity with citrus variant of HSVd from Japan (HSVd-cit8) [accession no. AB054615], but it has comparatively low homology with lime yellow corky vein and other isolates from citrus, grapevine, prune, plum and almond. The HSVd-sycv belongs to the cluster where cucumber and citrus variants of HSVd from Japan (HSVd-cit8) are present; HSVd-cuc is reported to cause cucumber pale fruit disease in Japan (Sano et al., 1984). HSVd-cit8 was reported as a noncachexia variant of HSVd from Japan (Ito et al., 2002).

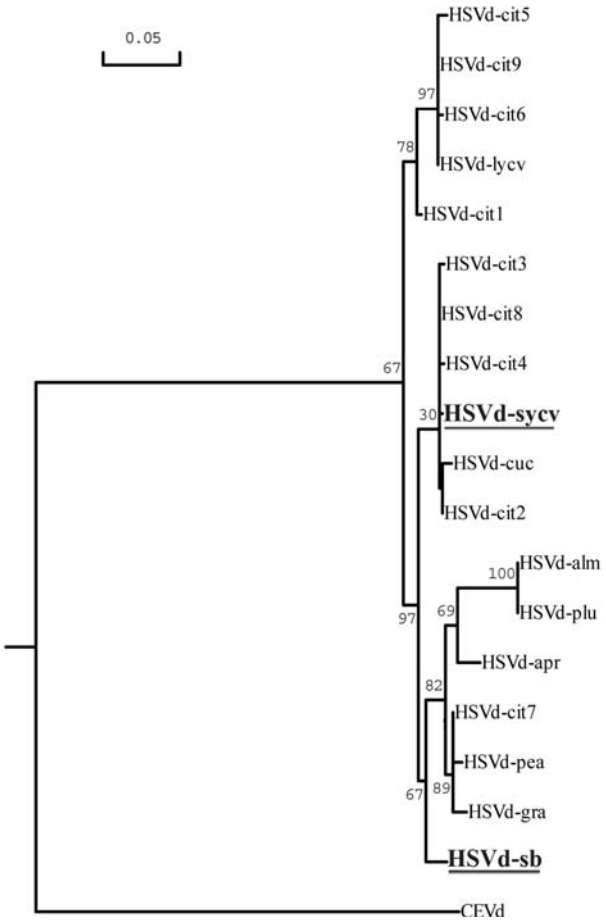


**Fig. 6** Phylogram, drawn by neighbor-joining Bootstrap Method in CLUSTAL X (1.81b) software, illustrating phylogenetic relationships based on multiple alignments of the complete sequences of hop stunt viroid variants and the sweet orange yellow corky vein variant and split bark variant of hop stunt viroid (HSVd-sycv and HSVd-sb). See Table 2 for viroid accession numbers. CEVd is used as an out group.

HSVd-sb formed a distinct cluster and most of citrus variant isolates are present in other clusters. In BLAST analysis HSVd-sb showed comparatively low homology with CVd-IIa-117 (inducing mild split bark symptoms) and other isolates from citrus, grapevine, prune, plum and almond. It was also seen that HSVd-sycv and HSVd-sb have distant relationship with variants belonging to other HSVd groups mentioned earlier. Following the nomenclature used to name citrus viroid variants the new viroid variants have been tentatively designated as sweet orange yellow corky vein variant of hop stunt viroid (HSVd-sycv) and sweet lime split bark variant of hop stunt viroid (HSVd-sb).

**Tab. 2** Characteristics of HSVd isolates used in this study.

| Variant | GenBank accession no. | Host | Reported from | Number of nucleotides |
|---|---|---|---|---|
| HSVd-sb | FJ465507 | Sweet lime | Iran (Fars) | 299 |
| HSVd- cit 2 (CVd-IIa-117) | AF213503 | Pomeroy trifoliate orange | Japan | 303 |
| HSVd-lycv | AJ490824 | Kagzi lime | India | 295 |
| HSVd-sycv | FJ465506 | Sweet orange | Iran (Fars) | 302 |
| HSVd- cit 8 | AB054615 | St. Michael orange-Wakayama | Japan | 302 |
| HSVd-cit 3 | EF126046 | Satsuma | Iran (Mazandaran) | 300 |
| HSVd-cit 4 | EF186992 | Satsuma | Iran (Mazandaran) | 300 |
| HSVd-cit 5 | AF213494 | Citrus | Spain | 297 |
| HSVd-cit 6 | AF213495 | Citrus | Spain | 297 |
| HSVd-cit 1 | AF131249 | Citrus | California | 299 |
| HSVd-cit 7 | X00009 | Citrus | Japan | 297 |
| HSVd-gra | M35717 | Grapevine | United States and Japan | 296 |
| HSVd-cuc | X00524 | Cucumber | Japan | 303 |
| HSVd-pea | D13765 | Peach | Japan | 297 |
| HSVd-plu | D13764 | Plum and Peach | Japan | 297 |
| HSVd-cit 9 | AF213491 | Citrus | Spain | 297 |
| HSVd-apr | AJ297840 | Prunus | Spain | 297 |
| HSVd-alm | AJ011813 | Almond | Spain | 296 |
| CEVd (out group) | FJ626865 | Citrus | Iran (Fars) | 370 |

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## Literature

Ashulin, L., Lachman, O., Hadas, R., and Bar-Joseph, M. 1991. Nucleotide sequence of a new viroid species, citrus bent leaf viroid (CBLVd) isolated from grapefruit in Israel. *Nucleic Acids Res.* 19: 4767.

Azad, P. 1993. Reduction in yield of acid limes infected with citrus corky vein virus in Assam. In Proceedings of the 12th Conf. Int. Org. Citrus Virol. IOCV, Riverside, p. 458.

Barbosa, C. J., Pina, J. A., Pérez-Panadés, J., Bernad, L., Serra, P., Navarro, L., and Duran-Vila,. N. 2005. Mechanical transmission of citrus viroids. *Plant Dis.* 89: 749-754.

Bernad, L. and Duran-Vila, N. 2006. A novel RT-PCR approach for detection and characterization of citrus viroids. *Mol. Cell. Probe* 20: 105–113.

Diener, T., Smith, D., Hammond, R., Albanese, G., La Rosa, R. and Davino, M. 1988. Citrus B viroid identified as a strain of hop stunt viroid. *Plant Dis.* 72: 691-693.

Flores, R., Randles, J.W., Bar-Joseph, M. and Diener, T.O. 1998. A proposed scheme for viroid classification and nomenclature. *Arch. Virol.* 143: 623-629.

Flores, R., Randles, J.W., Owens, R.A., Bar-Joseph, M. and Diener, T.O. 2005. Viroids. In Virus Taxonomy. VIII Report of the International Committee on Taxonomy of Viruses (C.M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger and A. L. Ball, Eds.), pp. 1145-1159. Elsevier/Academic Press, London.

Gross, H.J., Krupp, G., Domdey, H., Raba, M., Jank, P., Lossow, C., Alberty, H., Ramm, K. and Sanger, H.L. 1982. Nucleotide sequence and secondary structure of citrus exocortis and chrysanthemum stunt viroids. *Eur. J. Biochem*. 121: 249-257.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* 41: 95-98.

Ito, T., Ieki, H. and Ozaki, K. 2000. A population of variants of a viroid closely related to citrus viroid-I in citrus plants. *Arch. Virol.* 145: 2105-2114.

Ito, T., Ieki, H., Ozaki, K. and Ito, T. 2001. Characterization of a new citrus viroid species tentatively termed citrus viroid OS. *Arch. Virol.* 146: 975-982.

Ito, T., Ieki, H., Ozaki, K., Iwanami, T., Nakahara, K., Hataya, T., Ito, T., Isaka, M. and Kano, T. 2002. Multiple citrus viroids in citrus from Japan and their ability to produce exocortis-like symptoms in citron. *Phytopathology* 92: 542-547.

Izadpanah, K. 1983. An annotated list of virus and virus-like diseases of plants in Fars. College of Agriculture, Shiraz University, 187 p.

Li, S., Onodera, S., Sano, T., Yoshida, K., Wang, G. and Shikata, E. 1995. Gene diagnosis of viroids: comparisons of return-PAGE and hybridization using DIG-labeled DNA and RNA probes for practical diagnosis of hop stunt, citrus exocortis and apple scar skin viroids in their natural host plants. *Ann. Phytopathol. Soc. Jpn.* 61: 381–390.

Palacio-Bielsa, A., Romero-Durban, J. and Duran-Vila, N. 2004. Characterization of citrus HSVd isolates. *Arch. Virol.* 149: 537-552.

Roy, A. and Ramachandran, P. 2003. Occurrence of a hop stunt viroid (HSVd) variant in yellow corky vein disease of citrus in India. *Curr. Sci. India.* 85: 1608–1612.

Roy, A. and Ramachandran, P. 2006. Characterization of a Citrus exocortis viroid variant in yellow corky vein disease of citrus in India. *Curr. Sci. India*. 91: 798–803.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4: 406–425.

Sano, T., Hataya, T. and Shikata, E. 1988. Complete nucleotide sequence of a viroid isolated from Etrog citron, a new member of hop stunt viroid group. *Nucleic Acids Res.* 16: 347.

Sano, T., Uyeda, I., Shikata, E., Ohno, T. and Okada, Y. 1984. Nucleotide sequence of cucumber pale fruit viroid: Homology to hop stunt viroid. *Nucleic Acids Res*. 12 : 3427-3434.

Semancik, J., Roistacher, C., Rivera-Bustamante, R. and Durán-Vila, N. 1988. Citrus cachexia viroid, a new viroid of citrus: Relationship to viroids of the exocortis disease complex. *J. Gen. Virol.* 69: 3059-3068.

Semancik, J.S., Morris, T.J., Weathers, L.G., Rordorf, G.F. and Kearns, D.R. 1975. Physical properties of a minimal infectious RNA (viroid) associated with the exocortis disease. *Virology* 63:160–167.

Semancik, J.S. and Weathers, L.G. 1972. Exocortis virus: An infectious free-nucleic acid plant virus with unusual properties. *Virology* 46: 456–66.

Serra, P., Barbosa, C.J., Daròs, J.A., Flores, R. and Duran-Vila, N. 2007. Citrus viroid V: Molecular characterization and synergistic interactions with other members of the genus *Apscaviroid*. *Virology* 370: 102-112.

Serra, P., Gago, S. and Duran-Vila, N. 2008. A single nucleotide change in hop stunt viroid modulates citrus cachexia symptoms. *Virus Res*. 138: 130–134.

Verniere, C., Perrier, X., Dubois, C., Dubois, A., Botella, L., Chabrier, C., Bové, J.M. and Duran-Vila, N. 2006. Interactions between citrus viroids affect symptom expression and field performance of Clementine trees grafted on trifoliate orange. *Phytopathology* 96: 356–368.