

High Incidence of Hop Stunt Viroid in Hungarian Grapevines

ERZSÉBET FARKAS¹, L. PALKOVICS², J. MIKULÁS¹ and E. BALÁZS²

¹Research Institute for Viticulture and Enology, Kecskemét, Hungary

²Agricultural Biotechnology Center, H-2100 Gödöllő, P.O. Box 411, Hungary

The 297 nucleotide sequence of HSVd gvkr, a hop stunt viroid (HSVd gvkr) variant present in the Hungarian grapevines was determined from cDNAs amplified by the polymerase chain reaction. HSVd gvkr is very similar in its nucleotide sequences to several HSVd isolates previously detected in cucumber, *Citrus* and grapevine. It exhibits 97.6% sequence homology with a HSVd grapevine isolate from Germany. HSVd gvkr isolate sequence was included in the phylogenetic tree of different hop stunt viroid sequences. Using dot blot detection technique an intensive survey was performed in different Hungarian vineyards and a high incidence of this viroid was observed. More than 90% of the samples taken from three independent vineyards were positive for the HSVd gvkr probe. The wide distribution of this viroid could be attributed to the fact that grapevine nurseries were not screened before for HSVd.

Key words: Grapevine, hop stunt viroid sequence, presence in Hungarian grapevines.

At least five different viroids have been isolated and characterized from grapevines (Martelli, 1991). While there are no any indication that viroid infections affect the grapes yield, viroids presumably shorten the grape lifespan by interacting with other pathogens and disturbing the plant fundamental biosynthetic pathways. The decline of some of the grapevines in Hungary initiated an extensive study also on the grape pathogens including the viroids. By designing two 21 mer oligonucleotide primers based on known hop stunt viroid sequence data (5' - CTG GGG AAT TCT CGA GTT GCC - 3' and 5' - GGC TGA AGA GAG GAT CCG - 3') a viroid specific cDNA was amplified using polymerase chain reaction. The amplified fragments were cloned into Sma I linearised pUC 19 vector and the cloned DNA was sequenced by the dideoxy method (Sanger et al., 1977): Figure 1 presents the 297 nucleotide sequence of HSVd gvkr (EMBL accession number Y 14050).

The hop stunt viroid isolated from cv. Korona showed 97.6% sequence homology with a hop stunt viroid isolated from cv. Riesling in Germany in 1988 (Puchta et al., 1988). Sequence analysis was performed using the GCG package. The differences between the two viroid variants restricted to eight nucleotides. Phylogenetic tree was calculated and drawn by using the Phylogeny Interface Package (PHILIP) version 3. Sc. (Fig. 2). From the growtree phylogram one can conclude that hop stunt viroid variants isolated from grapevine separated from hop stunt viroid isolates originated from *Citrus* or stone fruit trees. To determine the distribution and presence of the hop stunt viroid in grapevines an extensive sample collection was performed.

Grapevine leaf samples were collected from three grapevine-growing regions in Hungary namely Kecskemét Kiszfái, Mátra Markaz and Szekszárd. Frozen leaf disks (0.133 g) were ground in pre-cooled mortar in the presence of chilled extraction buffer (400 µl 50 mM Tris, 0.1 M NaCl, 1 mM EDTA containing 0.5% SDS and 3% 2 -

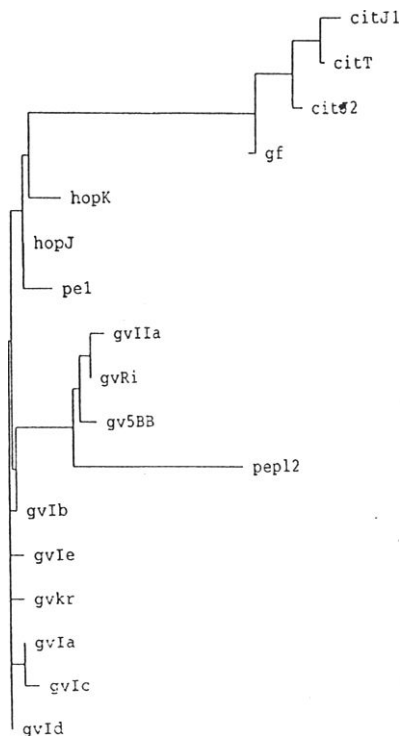


Fig. 2. Phylogenetic tree of HSVD isolates. List of viroid codes including the host of origin and the sequence accession numbers citJ1 - Citrus (Japan) X06718, citT - Citrus (Taiwan) U02527, citJ2 Citrus X06719, gf - Grapefruit X1 3838, hopK (Korea) - Hop, hopJ (Japan) - X00005, pe1 - peach D13765, gvIIa - grapevine X87928, gvRi - grapevine (Riesling) - X06873, gv5BB - grapevine (5BB) - X15330, pep12 - peach, plum D13764, gvIb - grapevine X87923, gvIe - grapevine X87927, gvkr - grapevine - Y14050, gvIa - grapevine X87924, gvIc - grapevine X87925, gvId - grapevine X87926

(data not shown) in our vineyards. This suggests the importance of screening of our nurseries and promote the use the developed shoot tip meristem culture for elimination of the viroids from mother stock plants (Duran-Vila et al., 1988).

Literature

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Table 1

List of hop stunt viroid infected grapevine varieties

Grapevine locations	Grapevine varieties
Kecskemét - Kisfűi nursery	Zöld veltelini Kr 100 Muscat Ottonel D-900 Rajnai rizling B7 Kövidinka 8/59 Leányka Hárslevelű K-9 Rizlingszilváni Sauvignon blanc Hárslevelű P41 Szürkeharát B 10/5 Cabernet Sauvignon E153 Teltfürtű kékfrankos Olaszrizling B20 Kerner Cabernet franc E11 Blauburger Chas. x Berl. 41B Pinot noir Mf. 2 Bianca Chasselas rouge Chasselas blanc Chardonnay R-75 Merlot Furmint T.85 Bürner B. x R. T5C

Table 1 (cont.)

Grapevine locations	• Grapevine varieties
	Fercal B. x R. TK 5 BB Cr.2 B. x R. TK 5 BB B. x R. K 125 AA
Mátravölgye Agricultural Cooperative Markaz, nursery	Rajnai rizling Zöldvelteleni Kr 100 Kerner Turán Karát Zengő K7 Badacsony 15 Zweigelt Szürkebarát Cabernet Sauvignon Zengő Leányka Teltfürtű kék oportó Pinot Blanc Királyleányka Medina Olaszrizling B20/7
Szekszárd Agricultural Cooperative Nursery	125AA Cr2 Fercal Teltfürtű kékfrankos Cabernet Sauvignon Rajnai rizling
Zomba nursery	5C Alicante Bouchet Zweigelt