The first detection of hop stunt viroid (HSVd) on hop in Czech Republic

Patzak J., Svoboda P., Henychová A. & Malířová I.
Hop Research Institute, Co., Ltd., Kadanska 2525, 438 01 Zatec, Czech Republic.
e-mail: patzak@chizatec.cz

The hop plant, Humulus lupulus L., is a dioecious perennial species, and only female cones are used for beer brewing. Hop as a perennial and vegetative-propagated crop is endangered by viruses and viroids.
Viroids are smallest known pathogen that consist of non-capsidated, single-stranded non-coding RNA replicons and they exploits host factors for their replication and propagation.
Viroids that can infect hop (Humulus lupulus L.) include Hop latent viroid (HLVd), Hop stunt viroid (HSVd), Citrus bark cracking viroid (CBCVd) and Apple fruit crinkle viroid (AFCVd).
HSVd infection of hop plants was previously found in Japan, Korea and USA and Slovenia. Symptoms are stunting, leaf curling, chlorosis, and poor plant vigor. Plants infected by HSVd produce less and smaller cones, yield is 50 % decreased, alpha and beta acids content is decreased up to 50 to 70 % compare to viroid-free plants (SANO 2013). The HSVd has been found in grapevine, citrus trees, plum, peach and several other fruit plants (HATAYA et al. 2017).
The qRT-PCR method was successfully used for detection of viruses and viroids in hop plants (PATZAK et al. 2017, GUČEK et al. 2017, GUČEK et al. 2019).

Material and Methods

For detection HSVd we used methods RT –PCR (PATZAK et al. 2017), we developed real-time quantitative RT-PCR (RT qRT-PCR) specific primers for detection of HSVd see table 1.

Results

We have used this method to control the health of hop plants in the World Collection of hop varieties from around the world in 2018 see table 2. We checked 18 varieties of hops from the Czech Republic, USA, England, Ukraine, Lithuania and Slovenia results are in table 3 a 4. HSVd was found in 4 plants of the Horizon variety see table 5. Samples were taken from the infected plants and deposited in the Hop Pathogen Collection.

Acknowledgement

This work was supported by Czech Ministry of Agriculture within Research Project MZE–RO1319.

References

GUČEK, T., TRDAN, S., JAKŠE, J., JAVORNIK, B., MATOUŠEK, J., & RADIŠEK, S. (2017). Diagnostic techniques for viroids. Plant Pathology, 66, 339–358.
GUČEK, T., JAKŠE, J., MATOUŠEK, J. et al. 2019. One-step multiplex RT-PCR for simultaneous detection of four viroids from hop (Humulus lupulus L.). European Journal of Plant Pathology, pp 1-14
HATAYA, T., TSUSHIMA, T., & SANO, T. (2017). Hop Stunt Viroid. In A. Hadidi, R. Flores, J. W. Randles, & P. Palukaitis (Eds.), Viroids and satellites (pp. 199–210). Academic Press.
PATZAK, J., SVOBODA, P., HENYCHOVÁ, A., MALÍŘOVÁ, I.2017: Detection of hop viruses and viroids by qRT-PCR in the Czech Republic. In: F. WEIHRAUCH, ed. Proceedings of the Scientific-Technical Commission 25–29 June 2017, St. Stefan am Walde, Austria. Wolnzach: Scientific-Technical Commission of the International Hop Growers´ Convention, 2017, p. 101. ISSN 2512-3785.
SANO, T. (2013). History, origin, and diversity of hop stunt disease and hop stunt viroid. Acta Horticulturae, 1010, ISHS, 87 96.

Table 1: Primer sequences for HSVd identification. Ta - PCR anneling temperature, TM melting temperature of PCR product

Primer	Primer sequence (5´- 3´)	Ta (°C)	Product size (bp)	Tm (°C)
HSVd	F: GCCGCATMAGGCAAGCAAAG	59	97	83,5
	R: CACGCCTCTYGCTGGATTCT			

Table 2: Plan World collection of hop varieties

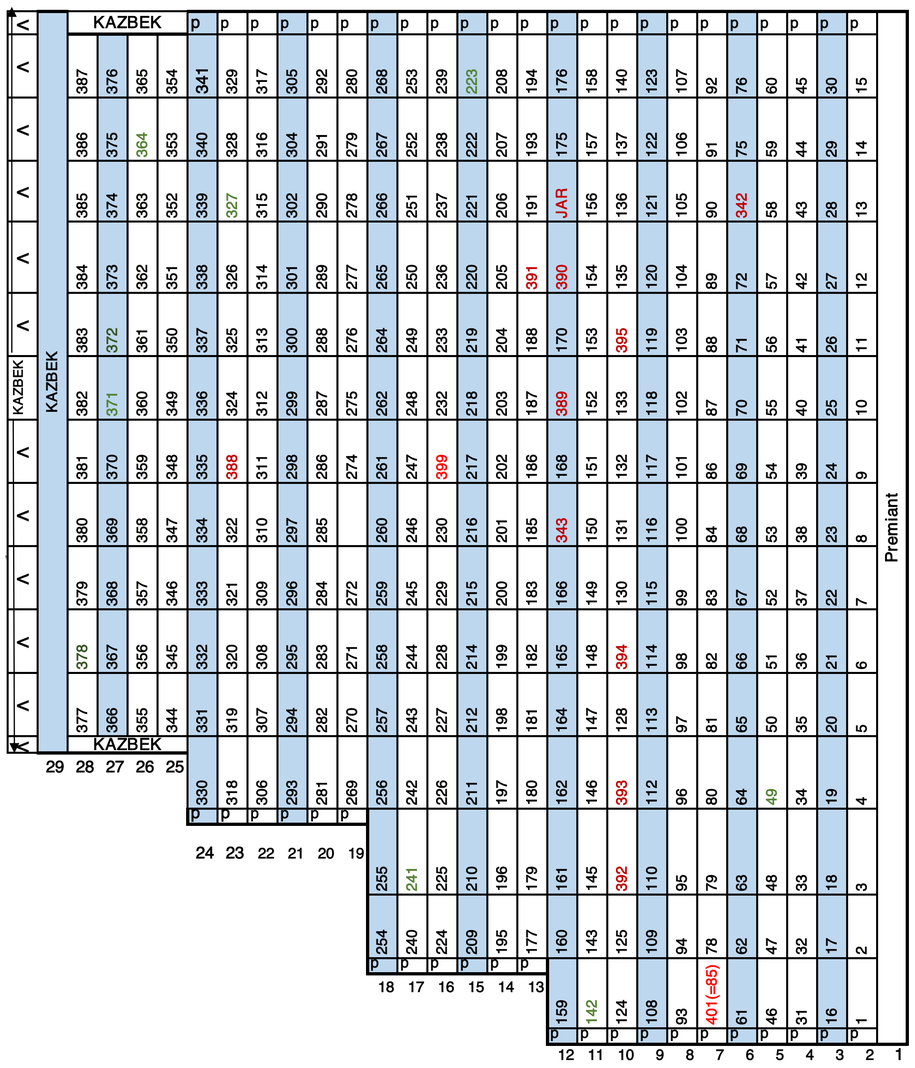

Table 3: Plan of evaluated hop varieties on Hop stunt viroid - HSVd

338 *N35 / Anglie*				
337 *Fredos Derlinglieji / Lithuania*	***325*** *Herold / England*		***300*** *Podvjaznyj / Ukraine*	***288*** *25 / CZ*
336 *Francuzy Ankstyvieji / Lithuania*	***324*** *Admiral / England*	***312*** *Sterling / USA*	***299*** *Agnus / CZ*	***287*** *45c / CZ*
335 *Bohemie / CZ*		***311*** *Santiam / USA*	***298*** *4528 / CZ*	***286*** *M2/27 / CZ*
		310 Horizon / USA		
		309 *Vanguard / USA*		
		308 *Glacier / USA*		
Row 24	Row 23	Row 22	Row 21	Row 20

Table 4: Results of varieties evaluation for HSVd infection

Row	No. Variety	Variety	Origine	HSVd
9	115	Atlas	Slovenia	–
20	286	M 2/27	CZ	–
20	287	45 c	CZ	–
20	288	25	CZ	–
21	298	4528	CZ	–
21	299	Agnus	CZ	–
21	300	Podvjaznyj	Ukraine	–
22	308	Glacier	USA	–
22	309	Vanguard	USA	–
22	310	Horizon	USA	+
22	311	Santiam	USA	–
22	312	Sterling	USA	–
23	388	N 35	England	–
23	324	Admiral	England	–
23	325	Herold	England	–
24	335	Bohemie	CZ	–
24	336	Francuzy Ankstyvieji	Lithuania	–
24	337	Fredos Derlinglieji	Lithuania	–

Table 5: Horizon infections with HSVd

310 *Horizon* / USA	HSVd infection
Plant 1	++
Plant 5	-
Plant 6	+++
Plant 7	+++
Plant 8	+/-