

The influence of Hop latent viroid (HLVd) infection on secondary metabolites contents and genes expression in hop (*Humulus lupulus L.*) cone.

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Hop latent viroid (HLVd) (**Fig. 1**) is worldwide spread in all of hop growing regions without any visible symptoms on infected hop plants. It was found that HLVd infection have changed the content and the composition of secondary metabolites in maturated hop cones (Patzak et al., 2001). In our study, we evaluated the influence of HLVd infection on the content and the composition of secondary metabolites in maturated hop cones, together with gene expression analyses of involved biosynthesis and regulation genes for Saaz, Sládek, Premiant and Agnus cultivars. We confirmed that the contents of alpha bitter acids were significantly reduced by 30.6% and 34.2% by viroid infection for two years in cultivar Saaz (**Table 1, Fig. 2A**). There were found same trends for cultivars Sládek, Premiant and Agnus and the content of alpha bitter acids was significantly reduced by 28.2%, 14.8% and 8.8%, respectively (**Table 2, Fig. 2B**). New, we found that viroid infection significantly reduced the contents of xanthohumol in the range from 3.9% to 23.5% (**Table 1 and 2**). Secondary metabolites changes were supported by gene expression analyses as last step biosynthesis enzyme genes, humulone synthase 1 (HS1) and 2 (HS2) for alpha bitter acids (**Fig. 3A and B**) and O-methytransferase 1 (OMT1) for xanthohumol (**Fig. 3C**), were down-regulated by viroid infection. We found that the expression of ribosomal protein L5 (RPL5) (**Fig. 4**) and the splicing of transcription factor IIIA-7ZF (**Fig 5**) were affected by viroid infection and a disbalance in proteosynthesis can influence transcriptions of biosynthesis and regulatory genes involved in of secondary metabolites biosynthesis. We suppose that RPL5/TFIIIA-7ZF regulatory cascade can be involved in HLVd replication as for other viroids of the family *Pospiviroidae*.

# Table 1.

Hop bitter acids and xanthohumol contents (Average±SD) in dry cones of HLVd free and infected plants of Saaz cultivar.

HLVd infection Harvest year Number of samples	Negative 2019 28	Positive 2019 41	Negative 2020 14	Positive 2020 56			
Alpha acids (% of DW)	5.54±0.63	3.84±0.72***	5.46±0.99	3.59±0.60***			
Beta acids (% of DW)	3.78±0.32	3.96±0.47*	3.57±0.34	3.63±0.31			
Cohumulone (% of AA)	21.34±2.11	21.86±2.50	20.07±1.13	21.47±2.13**			
Colupulone (% of BA)	43.07±1.92	41.45±3.07**	38.79±0.90	39.22±2.28			
Xanthohumol (% of DW)	0.309±0.035	0.242±0.040***	0.289±0.032	0.221±0.034***			
Probability level: * - $P < 0.1$ , ** - $P < 0.05$ , *** - $P < 0.01$ , DW – dry weight, AA – alpha acids, BA – beta acids,							

# Table 2.

Hop bitter acids and xanthohumol contents (Average±SD) in dry cones of HLVd free and infected plants of Sládek, Premiant and Agnus cultivars.

Sládek		Premiant		Agnus	
Negative 9	Positive 7	Negative 3	Positive 3	Negative 3	Positive 4
9.31±0.98	6.68±0.17***	8.86±0.30	7.55±0.34***	11.53±0.45	10.52±0.27**
3.83±0.46	4.44±0.24***	3.32±0.41	3.23±0.33	3.90±0.28	3.52±0.20*
25.31±0.55	23.63±0.39***	17.13±1.62	16.33±0.51	29.97±1.08	30.55±0.61
49.21±0.82	49.29±0.47	37.30±3.32	38.23±2.12	52.80±1.31	53.50±0.57
	Slá Negative 9 9.31±0.98 3.83±0.46 25.31±0.55 49.21±0.82	Sládek   Negative 9 Positive 7   9.31±0.98 6.68±0.17***   3.83±0.46 4.44±0.24***   25.31±0.55 23.63±0.39***   49.21±0.82 49.29±0.47	Sládek Pren   Negative Positive Negative Negative   9.31±0.98 6.68±0.17*** 8.86±0.30 3.32±0.41   3.83±0.46 4.44±0.24*** 3.32±0.41 3.32±0.41   25.31±0.55 23.63±0.39*** 17.13±1.62   49.21±0.82 49.29±0.47 37.30±3.32	Sládek   Premiant     Negative 9   Positive 7   Negative 3   Positive 3     9.31±0.98   6.68±0.17***   8.86±0.30   7.55±0.34***     3.83±0.46   4.44±0.24***   3.32±0.41   3.23±0.33     25.31±0.55   23.63±0.39***   17.13±1.62   16.33±0.51     49.21±0.82   49.29±0.47   37.30±3.32   38.23±2.12	Sládek   Premiant   Ag     Negative 9   Positive 7   Negative 3   Positive 3   Negative 3     9.31±0.98   6.68±0.17***   8.86±0.30   7.55±0.34***   11.53±0.45     3.83±0.46   4.44±0.24***   3.32±0.41   3.23±0.33   3.90±0.28     25.31±0.55   23.63±0.39***   17.13±1.62   16.33±0.51   29.97±1.08     49.21±0.82   49.29±0.47   37.30±3.32   38.23±2.12   52.80±1.31

### **Materials and Methods**

From two to eight of acclimatized and well rooted viroid free in vitro plants of fourteen mericlones of Saaz, two of Sládek and one of both Premiant and Agnus cultivars, sixteen HLVd infected mericlones of Saaz and one of each hybrid cultivars were put under field conditions in 2018 for Saaz and in 2019 for hybrid cultivars to experimental hopgardens on Steknik farm of Hop Research Institute in Zatec. Hop latent viroid (HLVd) infection was analysed by molecular dot blot hybridisation (Patzak et al., 2001) and by real-time quantitative RT-PCR (Patzak et al., 2017). Chemical analyses of hop resins, polyphenols and essential oils from dried cone samples were carried out according to Patzak et al. (2010). Gene expression analyses of secondary metabolites biosynthesis and regulatory genes were done by qRT-PCR analysis (Patzak et al., 2021). For qRT-PCR detection of splicing variants of transcription factor IIIA were used PCR primer combinations.

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### References

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**Figure 2.** Contents of hop bitter acids in dry cones of HLVd free and infected plants of A) Saaz and B) hybrid cultivars. Probability level: \* - P < 0,1, \*\* - P < 0,05, \*\*\* - P < 0,01. strait line - median, dashed line – average, box – 95 % percentile ± standard deviation. **Figure 3.** The relative expression to reference genes (RE=1) for biosynthesis genes A) HS1, B) HS2 and C) OMT1 in young cones of HLVd free and infected plants of hop cultivars. Probability level: \* - P < 0,1, \*\* - P < 0,05, \*\*\* - P < 0,01. strait line - median, dashed line – average, box – 95 % percentile ± standard deviation.



**Figure 4.** The relative expression to reference genes (RE=1) for RPL5 gene in young cones of HLVd free and infected plants of hop cultivars. Probability level: \* - P < 0,1, \*\* - P < 0,05, \*\*\* - P < 0,01. strait line - median, dashed line – average, box – 95 % percentile ± standard deviation.



**Figure 5.** The percentage of relative expression of alternatively spliced transcription factor IIIA A) to standard and alternative variants TFIIIA-7ZF together (100%) detected by qRT-PCR and B) to tandard variant TFIIIA-7ZF (100%) detected by semiquantitative RT-PCR in young cones of HLVd free and infected plants of hop cultivars. Probability level: \* - P < 0,1, \*\* - P < 0,05, \*\*\* - P < 0,01. strait line - median, dashed line – average, box – 95 % percentile ± standard deviation.

