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Originalarbeit

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# Detection and molecular analysis of *Hop latent virus* and *Hop latent viroid* in hop samples from Poland

Nachweis und molekulare Analyse des *Hop latent virus* und *Hop latent viroids* in polnischen Hopfenproben



## Abstract

Monitoring the occurrence of virus diseases in plants is important for the implementation of early control measures and prevention of further disease spread. In Poland, in 2004 a health programme for hop was started to eliminate viruses and viroids. In 2012/13, *in vitro* plants, samples from the IUNG-PIB experimental station and commercial hop gardens in Poland were tested for *Hop latent virus* (HpLV), and *Hop latent* and *Hop stunt viroids* (HpLVd and HpSVd). For virus testing, RT-PCR and ELISA methods were used. In order to detect hop viroids, RT-PCR was employed. The overall incidence of HpLV and hop viroids was lower than reported before the start of the programme. Cloning and sequencing revealed that the HpLV and the HpLVd from Polish sources are very similar to the type sequences and the Czech sources.

**Key words:** *Hop latent virus*, *hop latent viroid*, *hop stunt viroid*, RT-PCR, real time RT-PCR, ELISA

## Zusammenfassung

Die Überwachung von Viruskrankheiten bei Pflanzen ist wichtig für die Durchführung frühzeitiger Kontrollmaßnahmen und die Verhinderung der weiteren Ausbreitung der Erreger. In Polen wurde im Jahr 2004 ein Programm zur Eliminierung von Viren und Viroiden im Hopfen gestartet. In den Jahren 2012/13 wurden *in vitro* Pflanzen, Proben aus der IUNG-PIB Versuchsstation und aus kommerziellen polnischen Hopfengärten auf das *Hop latent virus*, *Hop latent viroid* und *Hop stunt viroid* getestet. Für die Virustestung wurden RT-PCR und ELISA eingesetzt. Die Viroide wurden mittels RT-PCR nachgewiesen. Insgesamt war die Nachweishäufigkeit für Viren und Viroide geringer als vor dem Start des Programms. Klonierung und Sequenzierung lassen den Schluss zu, dass das *Hop latent virus* und das *Hop latent viroid* aus den polnischen Proben den „type" Sequenzen und den tschechischen Viren/Viroiden sehr ähnlich sind.

**Stichwörter:** *Hop latent virus*, *hop latent viroid*, *hop stunt viroid*, RT-PCR, real time RT-PCR, ELISA

## Introduction

*Hop latent virus* (HpLV) is a carlavirus infecting hop. The virus was first described by Schmidt (1966). There are no apparent symptoms; however, some impairment of growth and reduction in yield has been noted. HpLV is transmitted by the aphid *Phorodon humuli* in a non-persistent manner (Adams and Barbara, 1982). Other aphids (eg *M. persicae*) and mechanical means (scissors, contact of bines) are also implicated in HpLV transmission (Crowle et al., 2006).

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Hop is the only known natural host. However, hemp can serve as an experimental host for HpLV (Ziegler et al., 2012).

*Hop latent viroid* (HpLVd, genus *Cocadviroid*; Puchta et al., 1988) is distributed worldwide and appears on hop usually symptomless. However, a decrease in contents of alpha acids, important for brewing, has been reported for infected plants (for example, Patzak et al., 2001).

*Hop stunt viroid* (HpSVd, genus *Hostuviroid*) has recently been detected in hops for the first time in Europe, in Slovenia (Radisek et al., 2012). The viroid severely stunts the plants, leads to smaller yields, loss in alpha acids (Sano, 2003) and changes in expression of important genes involved in regulation of the metabolome (Füssy et al., 2013). Typical symptoms also include leaf curling and small cones. Stunting appears 3 to 5 years after plants become infected (Eastwell and Nelson, 2007). Just like HpLVd, HpSVd can be transmitted by hands, scissors or through bine contact. HpSVd has a wide host range which includes woody plants (plum, peach: Sano et al., 1989; citrus: Diener et al., 1988; pistachio: Elleuch et al., 2013) and grapevines (e.g. Matoušek et al., 2003). These can serve as a natural reservoir of viroid. There are no genetic resources available for breeding for HpLV, HpLVd or HpSVd resistance, therefore growing healthy hop plants is important to prevent spread of these pathogens.

Since hop yards are kept for 20 years and longer, continuous monitoring is necessary to be able to remove any infected plants as early as possible and to prevent spread of the disease.

Poland is one of the largest producers of hop. After it became evident that Polish hop yards had a high incidence of hop viruses and viroids (Solarska and Grudzinska, 2001), a programme was started at the Institute of Soil Science and Plant Cultivation-State Research Institute (IUNG-PIB), Pulawy, to produce disease-free stocks. To estimate the impact of the programme, in 2012/2013 samples from commercial hop gardens established using disease-free stocks and from experimental field "Kepa IUNG-PIB", where genetic resources of hop from around the world are maintained and could be a source of these pathogens, were collected and analysed by RT-PCR. In addition, using RT-PCR, the presence of HpLV, HpLVd and HpSVd in the vegetatively propagated *in vitro stock* collection IUNG-PIB of most popular Polish hop cultivars and in hop seedlings obtained from seeds was studied to establish the health status of the plants. Additionally, at the Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Quedlinburg, a polyclonal antiserum was produced for the detection of HpLV and tested for application in ELISA and Western blot.

## Material and methods

*Plant material*

Hop samples were collected from IUNG-PIB experimental field "Kepa", where various hop cultivars from all over the world are maintained. The health of these hop accessions had not been tested for virus and viroid infections so far. Samples were also collected from 8 commercial hop gardens located in different regions of Poland. These commercial hop gardens were established between 2005 and 2008 using disease free stocks of cultivars Sybilla, Magnum, Iunga, and Lubelski produced at IUNG-PIB. Testing included 136 samples from the hop collection and 74 samples from commercial hop gardens. From each hop garden, 6–12 leaf samples had been collected, depending on the size of the plantation. Samples were frozen at −80°C immediately after collection. The *in vitro* plants were from IUNG-PIB stockcollection of the most popular Polish hop cultivars. 18 samples of *in vitro* plants obtained using the method of regeneration of apical tips were tested, and 14 samples of plants obtained from sterilized seeds germinated in LS medium.The source for the HpLV and HpLVd samples used for comparison was infected hop from the glasshouse. HpLV originated from the Czech hop virus collection (provided by J. Patzak).

*Virus purification, ELISA, Western blot*

Hemp (*Cannabis sativa*) is a useful propagation host for HpLV. Unfortunately it could not be used for virus purification as it contains a cryptic virus (Ziegler at al., 2012). Consequently, we had to use HpLV infected hop grown in the greenhouse. Before starting the purification, the material was tested by electron microscopy for the presence of virus particles. Plant material (200 g leaves and shoots) was harvested and homogenized in a Warren blender with 500 ml of ice cold 0.1 M potassium citrate/0.02 M EDTA buffer pH 7.0 containing 1% (w/v) Polyclar AT. Plant debris was removed by filtering through cheese cloth; the filtrate was centrifuged for 10 min at 20.000 g. The supernatant was clarified with 0.1 volume tetrachloromethane. Virus particles were pelleted by centrifugation for 1 h 30 min at 110.000 g in a vertical rotor. The pellet was redissolved overnight in 20 ml potassium citrate buffer containing 0.5% Triton X 100. Aggregates were removed by centrifugation for 5 min at 20.000 g. The supernatant was layered onto a sucrose cushion (40%) and centrifuged at 98.000 g in a vertical rotor. The pellet was redissolved in 3 ml sodium phosphate buffer (0.1 M, pH7), loaded onto a preformed sucrose gradient (10–40%) and centrifuged at 110.000 g for 1 h 30 min. Two light scattering bands were visible; the material from these 2 bands was removed from the tubes, diluted with an equal volume of sodium phosphate buffer and centrifuged for 1 h 20 min at 150.000 g. This resulted in pellets containing highly purified virus, which was used for immunization of a rabbit using a common intravenous immunization scheme. The rabbit was bled after 3.5 months and serum used for immunodetection.

For coating of ELISA plates (Nunc) leaf extracts were diluted 1:5 in PBS and kept overnight at 4°C. After 3 washes with PBSTw and blocking with 2% MPBSTw, the anti-HpLV antiserum was added (1:5.000 in MPBSTw) to the wells and incubated for 3 hrs at room temperature. An incubation with the secondary antibody (anti rabbit AP conjugate) for 1 hour followed. After 4 washes (PBSTw)

substrate pNPP was added (1 mg/ml in substrate buffer). Results were recorded after 30 min.

For Western Blotting, samples of leaf extracts were mixed with an equal volume of Laemmli buffer (Laemmli, 1970) and electrophoresed in a 12.5% SDS-PAGE gel. The proteins were then electroblotted onto Hybond ECL nitrocellulose. Subsequently, the membrane was incubated with the antibody preparation, and then with the AP-conjugated secondary antibody. Detection was done with BCIP/NBT.

*RNA, cDNA and PCR*

RNA was prepared from leaf tissue samples using RNA tri-liquid (Bio&Sell, Feucht, Germany) according to the manufacturer's instructions. cDNA synthesis and PCRs were performed according to standard procedures using primers (Tab. 1).

*Real-time RT-PCR*

Primers and the probe for real-time PCR were acquired from Biomers. cDNA was prepared as described for the conventional PCR. For the amplification a reaction mix from Bioline (Sensi FAST Probe No ROX) was used. The qPCR was carried out in a qTower 2.2 (Jena Analytik). A serial dilution of cloned HpLV DNA was used as standard (Tab. 2).

*Cloning and sequencing*

PCR products were recovered from agarose gel (Gene Jet Gel Extraktion Kit, Thermo Sientific) and ligated to pGEM-T vector (Promega). After transformation to competent *E. coli* cells (NEB Turbo), clones with inserts were selected by colony PCR using M13 forward and reverse primers. Plasmid DNA was prepared (Gene Jet Plasmid Miniprep Kit, Thermo Scientific) and sequenced (GATC, Cologne, Germany). Sequence analysis was performed using BLAST and CLUSTAL W (Larkin et al., 2007).

The full-length nucleotide sequence for the HpLV strain Zatec 2008 was determined earlier and submitted to the European Nucleotide Archive (accession HG793797). The sequence can be obtained from http://www.ebi.ac.uk/ena/data/view/HG793797.

## Results and discussion

RT-PCR for HpLVd was carried out using primers HpLVd5-100 and HpLVd3-120 (Tab. 1). About 40% of the tested plants from the vegetatively propagated *in vitro* stock collection contained HpLVd. The viroid was not detected in hop plants obtained from seeds (Tab. 3). RT-PCR products encompassing the whole viroid genome (256 bp) were cloned to vector pGEM-T (Promega) and subsequently sequenced. The sequences were compared with the HpLVd complete genome sequence (Puchta et al., 1988). Two of the clones (45FC59 and 45FC60) showed a 100% homology to the published type sequence (Puchta et al., 1988). Clone 45FC58 has an A to G change at nucleotide 187; clone 45FC61 has an A to G change at nucleotide 187, and another A to G change at nucleotide 79 (Fig. 1). Nucleotide 79 is just outside the upper part of the central conserved region. Nucleotide 187 is in the lower part of the central conserved region (Matoušek et al., 2001). However,

**Tab. 1. Primers for PCR and cloning viral and viroid sequences**

| *Primer* | *Sequence* |
|---|---|
| PST-HpLVd5-100 | 5' AGGGATCCCCGGGGAAACCT 3' |
| PST-HpLVd3-120 | 5' AGGTTTCCCCGGGGATCCCT 3' |
| HpSVd3-160 | 5' GACGATCGATGGTGTTTCGAAG 3' |
| HpSVd5-160 | 5' ATCGATCGTCCCTTCTTCTTTAC 3' |
| HpLV 5' Mlu | 5' CGCACGCGTGGATAAACAAACATACAA 3' |
| HpLV 3'-1100 | 5' GCTTAGCAATTGCGGATTGCAC |

**Tab. 2. Primers and probes for HpLV real time RT-PCR**

| *Primer* | *Sequence* |
|---|---|
| HpLV5-3908 | 5' GGTGCATCTCTTCCTCATA 3' |
| HpLV3-4038 | 5' GCATGTTGTACTTATAAGTCTCATC 3' |
| HpLV-Fam3993as | 5' ACTCCGCAAGGCAAGACAACA 3' |
| HpLV3-1654 | 5' TCCACAAAAGACTCCAGC 3' |
| HpLV5-1578 | 5' AAGCGTCTAGCAGTACAG 3' |
| HpLV-Fam1622as | 5' CCTCCTGCGATGCTCACCTC 3' |

**Tab. 3. The distribution of HpLV, HSVd and HpLVd in the *in vitro* stock collection IUNG-PIB**

| Number of sample | Flask | Genotype | HpLV | HSVd | HpLVd |
|---|---|---|---|---|---|
| Iv 1 | 1 | hop seedlings | – | – | – |
| Iv 2 | | | – | – | – |
| Iv 3 | 2 | hop seedlings | – | – | – |
| Iv 4 | | | – | – | – |
| Iv 5 | 3 | hop seedlings | – | – | – |
| Iv 6 | | | – | – | – |
| Iv 7 | 4 | hop seedlings | – | – | – |
| Iv 8 | | | – | – | – |
| Iv 9 | 5 | hop seedlings | – | – | – |
| Iv 10 | | | – | – | – |
| Iv 11 | 6 | hop seedlings | – | – | – |
| Iv 12 | | | – | – | – |
| Iv 13 | 7 | hop seedlings | – | – | – |
| Iv 14 | | | – | – | – |
| Iv 15 | 8 | Sybilla | – | – | + |
| Iv 16 | | | – | – | + |
| Iv 17 | 9 | Marynka | – | – | – |
| Iv 18 | | | – | – | – |
| Iv 19 | 10 | Iunga | – | – | + |
| Iv 20 | | | – | – | + |
| Iv 21 | 11 | Sybilla | – | – | + |
| Iv 22 | | | – | – | + |
| Iv 23 | 12 | Marynka | – | – | – |
| Iv 24 | | | – | – | – |
| Iv 25 | 13 | Iunga | – | – | + |
| Iv 26 | | | – | – | + |
| Iv 27 | 14 | Marynka | – | – | + |
| Iv 28 | | | – | – | + |
| Iv 29 | 15 | Magnat (new cultivar, Dr. U. Skomra) | ++ | – | + |
| Iv 30 | | | + | – | + |
| Iv 31 | 16 | Pulawski (new cultivar, Dr. U. Skomra) | – | – | – |
| Iv 32 | | | + | – | – |

– no viroid/virus detected; + low level of viroid/virus; ++ high level of viroid/virus

since position 187 is situated within a large loop, a destabilisation of the viroid secondary structure is not expected. Since we have not sequenced large numbers of clones, we don't know if there are any other HpLVd variants in the samples. However, the occurrence of variants is in line with the quasispecies theory (EIGEN, 1993).

In the IUNG-PIB hop collection maintained in the open field only one plant with HpLVd was found, and none in the samples from commercial hop gardens established using the disease-free stocks (Tab. 3). An absence of HpLVd infection in hop seedlings, although this viroid is well spread in analyzed cultivars (Tab. 3), is consistent with the fact that this viroid is not pollen and seed transmissible (e.g. MATOUŠEK et al., 2008). Previous studies had found a high incidence of HpLVd in Polish hop gardens established using strap-cuttings (root stocks) (SOLARSKA and GRUDZINSKA, 2001). These findings are in sharp contrast to those of LU et al. (2012), who detected extremely high incidences of HpLVd and HpSVd in China. However, the majority of the hop gardens included in our study is relatively young. New disease-free stocks have been planted between 2005 and 2008 in Poland, notably of the cultivars Sybilla, Magnum, Iunga and Lubelski.

The low viroid incidence found by this study could also be due to the time of sampling. HpLVd survives in the rootstocks (MORTON et al., 1993), and after the dormancy period only slowly spreads to the new shoots. During active plant growth, the concentration of HpLVd in shoot tips increases (MATOUŠEK et al., 1995). In young plants a strong tissue-specific gradient of viroid was observed, the highest level was found in roots and the lowest in the stem apex. The samples from the Polish commercial hop gardens were collected in September 2012. This is late in the growing season, and viroid levels may be too low again for detection at this point.

Results for the detection of HpLVd demonstrated that it is extremely important to use highly sensitive detection methods to guarantee that only healthy plant material is used for multiplication. This was the reason why we have developed a RT-PCR method for viroid detection.

Three of the vegetatively propagated *in vitro* plants were found to contain HpLV (Tab. 3). A PCR product for part of the replicase region (the 5'1100 base pairs) was amplified using primers HpLV5-Mlu and HLV3-1100 (Tab. 1), cloned and sequenced. There are two changes at the amino acid level compared to the genomic sequence (HATAYA et al., 2000) in the database, and only one difference to the Czech strain (HpLV Zatec 2008) (Fig. 2). However, this is a valine for isoleucine exchange, and these amino acids are structurally similar. Therefore, the virus sequences from the Polish and the Czech sources seem quite conserved.

In the 136 samples collected from the hop collection IUNG-PIB and the 74 samples from commercial hop gardens HpLV was not found.

HpLV is a virus that is difficult to detect. Detection depends on the time of year and on the actual sample taken. This has also been shown for Grapevine leafroll-associated virus 3 in grapevine cultivars (FIORE et al., 2009;

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45FC58        CTGGGGAATA CACTACGTGA CTTACCTGTA TGGTGGCAAG GGCTCGAAGA
45FC59        CTGGGGAATA CACTACGTGA CTTACCTGTA TGGTGGCAAG GGCTCGAAGA
45FC60        CTGGGGAATA CACTACGTGA CTTACCTGTA TGGTGGCAAG GGCTCGAAGA
45FC61        CTGGGGAATA CACTACGTGA CTTACCTGTA TGGTGGCAAG GGCTCGAAGA
gi|20153409   CTGGGGAATA CACTACGTGA CTTACCTGTA TGGTGGCAAG GGCTCGAAGA
Consensus     CTGGGGAATA CACTACGTGA CTTACCTGTA TGGTGGCAAG GGCTCGAAGA


45FC58        GGGATCCCCG GGGAAACCTA CTCGAGCGAG GCGGAGATCG AGCGCCAGTT
45FC59        GGGATCCCCG GGGAAACCTA CTCGAGCGAG GCGGAGATCG AGCGCCAGTT
45FC60        GGGATCCCCG GGGAAACCTA CTCGAGCGAG GCGGAGATCG AGCGCCAGTT
45FC61        GGGATCCCCG GGGAAACCTA CTCGAGCGGG GCGGAGATCG AGCGCCAGTT
gi|20153409   GGGATCCCCG GGGAAACCTA CTCGAGCGAG GCGGAGATCG AGCGCCAGTT
Consensus     GGGATCCCCG GGGAAACCTA CTCGAGCGaG GCGGAGATCG AGCGCCAGTT


45FC58        CGTGCGCGGC GACCTGAAGT TGCTTCGGCT TCTTCTTGTT CGCGTCCTGC
45FC59        CGTGCGCGGC GACCTGAAGT TGCTTCGGCT TCTTCTTGTT CGCGTCCTGC
45FC60        CGTGCGCGGC GACCTGAAGT TGCTTCGGCT TCTTCTTGTT CGCGTCCTGC
45FC59        CGTGCGCGGC GACCTGAAGT TGCTTCGGCT TCTTCTTGTT CGCGTCCTGC
gi|20153409   CGTGCGCGGC GACCTGAAGT TGCTTCGGCT TCTTCTTGTT CGCGTCCTGC
Consensus     CGTGCGCGGC GACCTGAAGT TGCTTCGGCT TCTTCTTGTT CGCGTCCTGC


45FC58        GTGGAACGGC TCCTTCTTCA CACCAGCCGG AGTTGGGAAC TACCCGGTGG
45FC59        GTGGAACGGC TCCTTCTTCA CACCAGCCGG AGTTGGAAAC TACCCGGTGG
45FC60        GTGGAACGGC TCCTTCTTCA CACCAGCCGG AGTTGGAAAC TACCCGGTGG
45FC61        GTGGAACGGC TCCTTCTTCA CACCAGCCGG AGTTGGGAAC TACCCGGTGG
gi|20153409   GTGGAACGGC TCCTTCTTCA CACCAGCCGG AGTTGGAAAC TACCCGGTGG
Consensus     GTGGAACGGC TCCTTCTTCA CACCAGCCGG AGTTGGaAAC TACCCGGTGG


45FC58        ATACAACTCT TGAGCGCCGA GCTTTACCTG CAGAAGTTCA CATAAAAAGT
45FC59        ATACAACTCT TGAGCGCCGA GCTTTACCTG CAGAAGTTCA CATAAAAAGT
45FC60        ATACAACTCT TGAGCGCCGA GCTTTACCTG CAGAAGTTCA CATAAAAAGT
45FC61        ATACAACTCT TGAGCGCCGA GCTTTACCTG CAGAAGTTCA CATAAAAAGT
gi|20153409   ATACAACTCT TGAGCGCCGA GCTTTACCTG CAGAAGTTCA CATAAAAAGT
Consensus     ATACAACTCT TGAGCGCCGA GCTTTACCTG CAGAAGTTCA CATAAAAAGT


45FC58        GCCCCT
45FC59        GCCCCT
45FC60        GCCCCT
45FC61        GCCCCT
gi|20153409   GCCCCT
Consensus     GCCCCT
```

**Fig. 1.** Alignment of full-length HpLVd sequences.

```
HpLV Zat      MALTYRTPMEDIVISFEPQVQSLIANSAAELYKNLENENCKYFNYYLPAVAKKKLSAAGI
NC_002552     MALTYRTPMEDIVISFEPQVQSLIANSAAELYKNLEKENCKYFNYYLPAVAKKKLSAAGI
29iv          MALTYRTPMEDIVISFEPQVQSLIANSAAELYKNLENENCKYFNYYLPAVAKKKLSAAGV
              ************************************:**********************:

HpLV Zat      YLSPYSAVVHSHPVCKTLENYMLYSVLPNYLDGKYFFVGIKNKKINLLKSRNKKLESVIC
NC_002552     YLSPYSAVVHSHPVCKTLENYMLYSVLPNYLDGKYFFVGIKNKKINLLKSRNKKLESVIC
29iv          YLSPYSAVVHSHPVCKTLENYMLYSVLPNYLDGKYFFVGIKNKKINLLKSRNKKLESVIC
              ************************************************************

HpLV Zat      INRLVTSADRLRYSNDFVTFESVSHEDLRRHGPGL------------------------
NC_002552     INRLVTSADRLRYSNDFVTFESVSHEDLRRHGPGLSEPALQGIIEPLKRRKATHLFLHDE
29iv          INRLVTSADRLRYSNDFVTFESVSHEDLRRHGPGLSEPALQGIIEPLKRRKATHLFLHDE
              ***********************************
```

**Fig. 2.** Alignment of deduced amino acid sequences for different HpLV sources.

TSAI et al., 2012), and an irregular distribution of virus in systemically infected plants is well known for other viruses (for example, *Plum pox virus*, MARTINEZ-GOMEZ and DICENTA, 2001). There is a seasonal and a tissue age effect, and, according to KOMINEK et al. (2009), several random samples from different parts of a plant should be analyzed.

In order to evaluate the most reliable sampling strategy, we have taken 10 samples from one greenhouse hop plant in early spring (April 2013). HpLV was detected using ELISA and RT-PCR. The virus was found only in mature leaves, nearer the bottom of the bine (see Fig. 3). The results for ELISA and RT-PCR show a good agreement (Tab. 4), and seem to support the notion that the virus (similarly to HpLVd) only moves slowly out of the root to the younger leaves after the dormancy phase. They support our sampling strategy of collecting samples late in the season and support our findings that plants have been free of HpLV.

We have also shown that our HpLV antiserum is efficient in detecting HpLV coat protein in leaf extracts in a Western blot (Fig. 5).

From our experiments (data not shown) we have no indication that HpLV encodes a silencing suppressor. In *Potato*

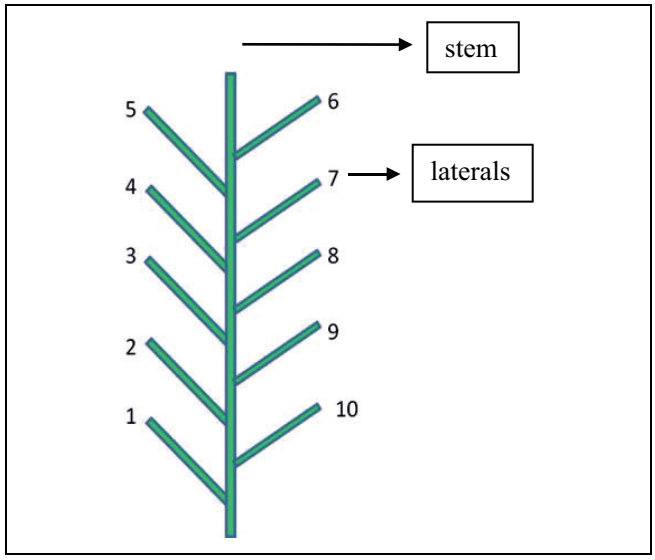


**Fig. 3.** Scheme of hop sampling for HpLV detection: 1–5, young leaves; 6–10, mature leaves.

**Tab. 4. Testing hop leaf tissue from different parts of the bine by ELISA and RT-PCR for the presence of HpLV**

| Hop 2013/1 | ELISA OD400 | RT-PCR |
|---|---|---|
| 1 | 0,04 | – |
| 2 | 0,06 | – |
| 3 | 0,04 | – |
| 4 | 0,04 | – |
| 5 | 0,04 | – |
| 6 | 0,04 | – |
| 7 | 0,10 | + |
| 8 | 0,07 | – |
| 9 | 0,19 | ++ |
| 10 | 0,10 | + |
| buffer | 0,036 | – |

*virus M* carlavirus the TGBp1 and the cystein-rich protein were found to be suppressors of RNA silencing (SENSHU et al., 2011). However, LUKHOVITSKAYA et al. (2005) have shown that the cystein-rich protein of another carlavius, *Chrysanthemum virus* B carlavirus, does not have silencing suppression activity. The lack of an active suppressor of gene silencing in HpLV may explain our observations that in some cases infected plants have recovered from infection after the winter dormancy period and that in some cases the virus rapidly disappeared from infected leaves.

For the detection of HpSVd primers HpSVd3-160 and HpSVd5-160 were used. None of the samples from the hop collection and commercial hop gardens contained HpSVd. Viroid was also not detected in vegetatively propagated *in vitro* plants and plants obtained from seeds. (Tab. 3). As a positive control for HpSVd we have used leaf material from tomato infected with PSTVd, and primers were designed such that they could detect both viroids. In this material as well as in PSTVd-spiked hop samples we were able to detect PSTVd, and we are therefore satisfied that our primers and the RT-PCR setup were working. Even though we did not find HpSVd-infected hop samples, continued monitoring is vital, because grapevine is a host for HpSVd and the viroid could spread to hop gardens.

The results confirm the efficiency of the Polish hop disease curation programme carried out in the last decade.

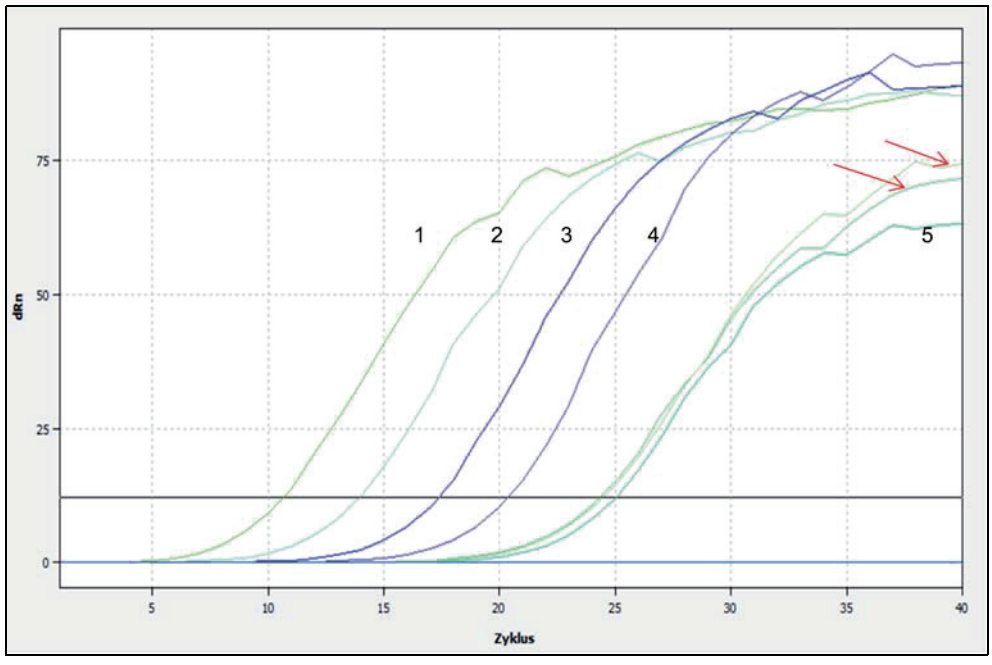


**Fig. 4.** Real time RT-PCR HpLV Standard curve: $y = -3{,}51x + 16{,}14$; $R^2 = 0{,}994$; efficiency = 0,93
Numbers 1–5 show the standard tenfold dilutions (42 ng to 4,2 pg per µl), the arrows point to the samples from HpLV infected hop.

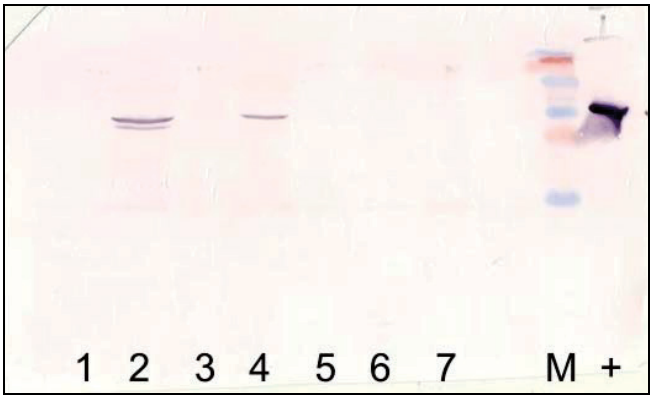


**Fig. 5. Detection of HpLV in leaf material using a polyclonal antibody**
**M: Pageruler Plus Prestained Protein Ladder, Fermentas**
**1–7, hop leaf extracts**
**+ purified HpLV particles, 34kD**
**Samples 2 and 4 clearly show an infection with HpLV.**



Though HpSVd has a wide host range, including grapevine and fruit trees which are grown in the Polish hop growing region, the hop plants remained viroid-free.

Since highly sensitive methods are needed for monitoring, we decided to establish a real-time PCR assay for the detection of HpLV.

Real-time PCR detection of HpLV in hop leaf samples was done using a FAM-labelled probe (Tab. 2).

This method proved very specific, the negative control samples did not result in any amplification. The assay enables sensitive quantification of viral RNA (Fig. 4).

Therefore, for further monitoring studies, this may be the method of choice.

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