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Diversity and distribution of viroids in German grapevines and possible future implications for product quality under global warming conditions

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Problem statement

Grapevine (*Vitis vinifera* L.) is a perennial crop, which is cultivated intensively over many decades. This provides optimal conditions for viroids, small single-stranded pathogenic RNA molecules, which need several months or years to fully infect their host plants. After the initial introduction of a viroid to a vineyard they can easily get transmitted through crop management practices. Since most viroids do not lead to strong regular symptoms in grapevine, they are considered to be latent, thus unimportant for viticulture. This might be the reason why viroids can be found in most vineyards worldwide. Currently, there are six viroids reported to infect grapevines, of which the Australian grapevine viroid (AGVd) is thought to have evolved as a result of the recombination of the genomes of coinfecting viroids [1]. The grapevine yellow speckle viroid 1 (GYSVd-1) is one of the potential sources of genomic fragments that AGVd consists of. This viroid has been detected all over the world. GYSVd-1 is named after the yellow spots, which it induces especially under warm weather conditions [2]. In contrast to GYSVd-1 the hop stunt viroid (HSVd) was described as generally symptomless for grapevine when it was discovered in Germany in the 1980s [3]. However, since HSVd is the causal agent of a stunting disease of hops (*Humulus lupulus* L.) this makes grapevine a reservoir whenever it is cultivated close to hop gardens. Consequently, it is almost surprising that HSVd has not yet been found in commercial hop gardens in Germany.

Here we present first results of a small viroid survey to get an up-to-date overview of the infection status of German grapevines.

Materials and methods

- German grapevine sampling (n=27) over three consecutive years (2018-2020)
- RNA extraction from healthy young leaves
- cDNA synthesis with reverse transcriptase
- High-fidelity PCR and gel electrophoresis
- PCR validation of exemplary amplicons with 2nd complementary PCR
- PCR amplicon clean-up and Sanger-sequencing of exemplaric amplicons at service provider
- Viroid genome assembly & BLAST analysis

Results

The PCR analysis showed that both viroids are widely distributed in Germany (Table 1). HSVd was present in 23 of 27 samples, while GYSVd-1 was detected in 17 of 27 samples. Only 2 samples did not contain any of those viroids, while GYSVd-1 was not found as single infection.

Table 1. PCR detection of HSVd & GYSVd-1 in different German grapevines.

Sample	Region	Variety	GYSVd-1-PCR	HSVd-PCR
1	Ahr	Müller Thurgau		seq
2	Ahr	Pinot Noir		
3	Baden	Pinot Noir		
4	Baden	Pinot Gris		
5	Baden	Pinot Noir	seq	
6	Franken	Riesling		
7	Franken	Müller Thurgau		
8	Hess. Bergstr.	Pinot Noir		seq
9	Hess. Bergstr.	Riesling		
10	Mosel	Müller Thurgau		
11	Mosel	Riesling		
12	Mittelrhein	Dornfelder		
13	Mittelrhein	Müller Thurgau		
14	Nahe	Müller Thurgau		seq
15	Pfalz	Riesling		
16	Pfalz	Dornfelder	seq	
17	Pfalz	Dornfelder		
18	Pfalz	Müller Thurgau		
19	Pfalz	Dornfelder		
20	Rheinhausen	Pinot Gris		
21	Rheinhausen	Riesling	seq	
22	Sachsen	Müller Thurgau		
23	Sachsen	Riesling		
24	Sachsen	Pinot Noir		
25	Saale-Unstrut	NA		seq
26	Württemberg	Lemberger		
27	Württemberg	Lemberger		
pos	-	-		
neg	-	-		
neg	-	-		

have been used for Sanger sequencing Eurofins seq

Sequencing	NCBI acc. ex.	%ID.
GYSVd-1	KU880715	99
HSVd	X06873, OK138974	95-99

Discussion

The high incidence of GYSVd-1 and HSVd shows that it is **almost impossible to find viroid-free grapevines**, which makes it difficult to estimate a potential yield gap or reduced overall vigor of infected plants compared to viroid-free plants. However, the evaluation of the hop latent viroid (HLVd) in hop clearly showed that the presence of this „latent“ viroid significantly reduced the concentration of valuable bitter acids up to 34% in the cultivar *Saazer* [4] or reduced yield by 8% and 27% in cultivars *Wye Challenger* and *Omega*, respectively [5]. Furthermore, it is important to consider that particularly for the GYSVd-1 there is evidence for temperature dependence of symptom induction in grapevine [2]. This hypothesis is supported by two assumptions; First, viroid-derived small RNAs (vd-sRNAs) result from the host RNA silencing defense systems. Those vd-sRNA are known to account for at least some of the viroid-associated symptoms [6].

Second, the plant RNA silencing is more active at higher temperatures [7]. Therefore, we assume that viroid-associated symptoms are enhanced through high temperatures as result of increased RNA silencing, thus **global warming could increase symptom severity of viroid infections**.

The role of grapevine as evolutionary boiling pot for viroids and as reservoir for hop viroids are further topics for future research.

More than 900 HSVd accessions have been uploaded to sequence databases and some have shown to be host specific. Three out of four HSVd-variants sequenced in this study did show to be highly similar to the accession X06873. This variant is very different from variants typically found in hops, citrus or even table grapes. In the next steps we will test if this so called *Riesling*-variant of HSVd is able to infect hops. At least other variants of HSVd did show to be able to adapt, infect and induce symptoms in hops [9].

Outlook

The risk of the **HSVd-Riesling-variant as pathogen for hop must be addressed urgently to prevent another concerning viroid epidemic** as currently observed for the citrus bark cracking viroids in hops. Further, the effect of the „latent“ viroid infections in times of global warming should be analyzed at least for frequent viroids as HSVd and GYSVd-1 in German grapevines.

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