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Effect of repeated Ribavirin treatment on grapevine viruses

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Summary. – The effect of Ribavirin treatment for the chemotherapy of several grapevine viruses was evaluated. Four grapevine cultivars were repeatedly treated with Ribavirin in two different concentrations and with three different lengths of treatment. Repeating the Ribavirin treatment always had a significant effect on the number of healthy grapevine plants obtained. Ribavirin concentration and length of exposure showed a significant difference in sanitation of the *Grapevine rupestris stem pitting-associated virus*. During sanitation of the *Grapevine Pinot gris virus* and *Grapevine fleck virus*, those two factors did not show significant differences in the elimination of grapevine viruses.

Keywords: chemotherapy; Ribavirin; grapevine; Grapevine rupestris stem pitting-associated virus; Grapevine Pinot gris virus; Grapevine fleck virus

Introduction

During our previous work (Komínek and Jandurová, 2011), two grapevine cultivars were sanitized from viruses using thermotherapy. Because the procedure had a very low success rate, chemotherapy was selected for the sanitation of grapevine cultivars in the Czech Republic. This work describes a study conducted to evaluate the possibility of the use of Ribavirin for this purpose, as well as the effects of different treatment conditions: Ribavirin concentration, length of treatment, repeating of the treatment, and the cultivar used.

Materials and Methods

Grapevine cultivars. Four grapevine cultivars, Kerner (aromatic cultivar for making white wine), Chardonnay (white wine), Blauer Portugieser (red wine), and Riesling (aromatic cultivar for making white wine) were selected for chemotherapy during the grapevine sanitation program at Crop Research Institute (CRI), Prague, Czech Republic. Currently, the area in the Czech Republic planted with the Chardonnay cultivar is ca. 830 ha, area planted with Blauer Portugieser is ca. 640 ha, and the area planted with Riesling is ca. 1,350 ha. Cultivar Kerner is only planted on 31 ha. Total area of grapevine cultivation in Czech Republic is ca. 18,500 ha.

Virus detection. From every grapevine cultivar taken into the sanitation program, several mother plants were selected and tested by RT-PCR for the presence of viruses. *Grapevine rupestris stem pitting-associated virus* (GRSPaV, the genus *Foveavirus*) *and Grapevine fleck virus* (GFkV, the genus *Maculavirus*) were detected as described earlier (Komínek *et al.*, 2009). *Grapevine Pinot gris virus* (GPGV, the genus *Trichovirus*) was detected with primers GPG-5637F and GPG-5939R according to Glasa *et al.* (2014). Mother plants, preferably infected with a single virus, were used for further experiments.

Chemotherapy procedure. Ribavirin (1-β-D ribofuranosyl-1H-1,2,4-triazole-3-carboxamide) was used for chemotherapy of the grapevine cultivars.

Explants based on meristematic tissues were taken from mother plants of four cultivars infected with individual viruses, propagated, and multiplied *in vitro* on Quoirin-Lepoivre medium (Quoirin and Lepoivre, 1977).

By several cycles of multiplication, at least 15 *in vitro* plants were prepared for each individual variant of the experiment.

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Abbreviations: GRSPaV = *Grapevine rupestris stem pitting-associated virus*; GFkV = *Grapevine fleck virus*; GPGV = *Grapevine Pinot gris virus*

Table 1. Results of RT-PCR detection of *Grapevine rupestris stem pitting-associated virus* in sanitized plants, percentage of negative plants

First treatment	Ribavirin concentration 10 mg/l			Ribavirin concentration 20 mg/l		
Variety	**4 weeks**	**6 weeks**	**8 weeks**	**4 weeks**	**6 weeks**	**8 weeks**
Kerner	0.0	6.7	6.7	16.7	13.3	50.0
Chardonnay	0.0	9.1	9.1	37.5	7.2	25.0
Blauer Portugieser	14.3	9.1	7.1	18.2	27.3	50.0
Riesling	10.0	0.0	9.1	11.1	57.1	60.0
Second treatment	**Ribavirin concentration 10 mg/l**			**Ribavirin concentration 20 mg/l**		
Variety	**4 weeks**	**6 weeks**	**8 weeks**	**4 weeks**	**6 weeks**	**8 weeks**
Kerner	14.3	30.8	27.3	25.0	42.9	66.7
Chardonnay	11.1	22.2	33.3	53.3	15.4	100.0
Blauer Portugieser	56.2	44.8	28.6	28.6	40.0	100.0
Riesling	25.0	28.6	62.5	50.0	66.7	82.4

Plants obtained *in vitro* were tested by RT-PCR before the experiment started to ensure that viruses were present in their tissues.

Two different Ribavirin concentrations were evaluated, as well as three different treatment lengths. All the plants were treated with Ribavirin by cultivation in the same media as above, having been supplemented with 10 or 20 mg/l Ribavirin. The treatment durations were 4, 6, or 8 weeks, respectively. After the treatment, the apical parts of the plants were transferred onto a fresh medium without Ribavirin for recovery (for 8 weeks), and then treated again with the same Ribavirin concentration and for the same period as in the first treatment. After each step, samples taken from the plants were analyzed by RT-PCR for the presence of viruses. Tests were repeated one year after the end of the Ribavirin treatment to verify the persistence of the sanitary status of the plants.

Mortality of grapevine plants *in vitro* was not evaluated during the experiments.

Data analysis. The effects of individual aspects of the experiments with Ribavirin treatments on the number of sanitized grapevine plants were compared. Statistical significance ($P < 0.05$) was evaluated using the analysis of variance calculated by Microsoft Excel (part of the Microsoft Office Standard 2010 package).

Results

Ribavirin effect on Grapevine rupestris stem pitting-associated virus

The sanitation of grapevine cultivars Kerner, Chardonnay, Blauer Portugieser, and Riesling from GRSPaV was evaluated after cultivation on Ribavirin-containing medium (for details see Table 1). Most of the factors studied, e.g., Ribavirin concentration, length of Ribavirin exposure, and repeating of the Ribavirin treatment had a statistically significant effect on grapevine sanitation. The effect of the cultivar used was not significant.

Concerning the length of treatment, differences between 4–8 and 6–8 weeks were significant; the 4–6 weeks difference was not.

Ribavirin effect on Grapevine Pinot gris virus

Sanitation of grapevines infected with GPGV was evaluated in cultivars Kerner, Blauer Portugieser, and Riesling after repeated cultivation on Ribavirin-containing medium for 8 weeks. The cultivar Chardonnay was not included in

Table 2. Results of RT-PCR detection of *Grapevine Pinot gris virus* in sanitized plants, percentage of negative plants

First treatment	Ribavirin concentration 10 mg/l	Ribavirin concentration 20 mg/l
Variety	**8 weeks**	**8 weeks**
Kerner	26.7	86.7
Blauer Portugieser	87.5	46.7
Riesling	66.7	25.0
Second treatment	**Ribavirin concentration 10 mg/l**	**Ribavirin concentration 20 mg/l**
Variety	**8 weeks**	**8 weeks**
Kerner	77.8	100.0
Blauer Portugieser	100.0	100.0
Riesling	100.0	75.0

Table 3. Results of RT-PCR detection of *Grapevine fleck virus* (GFkV) in sanitized plants, percentage of negative plants

First treatment	Ribavirin concentration 10 mg/l			Ribavirin concentration 20 mg/l		
Variety	**4 weeks**	**6 weeks**	**8 weeks**	**4 weeks**	**6 weeks**	**8 weeks**
Kerner	40.0	100.0	73.3	0.0	100.0	73.3
Chardonnay	73.3	86.7	100.0	0.0	93.3	33.0
Blauer Portugieser	46.7	93.3	40.0	53.3	100.0	100.0
Second treatment	**Ribavirin concentration 10 mg/l**			**Ribavirin concentration 20 mg/l**		
Variety	**4 weeks**	**6 weeks**	**8 weeks**	**4 weeks**	**6 weeks**	**8 weeks**
Kerner	100.0	100.0	75.0	100.0	100.0	100.0
Chardonnay	86.7	100.0	100.0	100.0	100.0	93.3
Blauer Portugieser	100.0	100.0	93.3	100.0	100.0	100.0

this experiment, because not a single mother Chardonnay plant was infected with the virus. Tests of Ribavirin exposure of four and six weeks were not established because low numbers of *in vitro* plants were obtained. For results, see Table 2.

Repeating the Ribavirin treatment had a statistically significant effect on the sanitation of grapevine cultivars, while Ribavirin concentration and cultivar taken into the experiment had no significant effect on the results of GPGV sanitation.

Ribavirin effect on Grapevine fleck virus

In the present experiment, *Grapevine fleck virus* was successfully removed from some plants of grapevine cultivars Kerner, Chardonnay, and Blauer Portugieser after repeated cultivation on Ribavirin-containing medium (Table 3). Cultivar Riesling was not included in the experiment, as not enough plants were available *in vitro*.

Repeating the treatment had a significant effect on elimination of the virus. The length of Ribavirin exposure and the concentration used did not result in statistically significant differences.

Discussion

Ribavirin was successfully used for chemotherapy sanitation of grapevine cultivars. Its utilization for grapevine sanitation has also been reported by other laboratories (Weiland *et al*., 2004; Panattoni *et al*., 2007, 2011; Skiada *et al*., 2013).

Ribavirin can be incorporated into RNA during replication as a base analogue, inducing mutations, which are lethal for RNA viruses (Crotty *et al*., 2002). This is also the principle of its effect in the case of grapevine viruses, because all of the viruses utilized in present experiment had a single-stranded positive RNA genome.

In the present work, the effect of repeated Ribavirin treatment was always statistically significant for sanitation from grapevine viruses. In evaluating the other factors of the Ribavirin treatment on individual viruses, different responses were observed. For *Grapevine rupestris stem pitting-associated virus*, the results showed that increasing both the length of the Ribavirin treatment and its concentration in the medium had a significantly positive effect on its elimination from grapevines cultivated *in vitro*.

Skiada *et al*. (2013) reported 26% of GRSPaV-free grapevines of cv. Agiorgitiko after 80 days treatment with Ribavirin at a concentration of 10 mg/l. In the present work, the comparable length of treatment was 12 weeks (6 weeks repeated), totaling 84 days. The results showed different rates of GRSPaV-negative grapevines depending on the cultivar, varying from 22.2% for Chardonnay to 44.8% for Blauer Portugieser. Those results are close to the data obtained by Skiada *et al*. (2013). Similarly, for higher Ribavirin concentrations, Skiada *et al*. (2013) reported 40% healthy plants using Ribavirin at a concentration of 20 mg/l. In the present work, it was from 15.4% (Chardonnay) to 66.7% (Riesling).

Results of repeated Ribavirin treatment were always statistically significant. Total sanitation from GRSPaV of all plants in a given experimental design was a rare occasion in the present work (Table 1). This is also in accordance with our previous work with thermotherapy (Komínek and Jandurová, 2011), where we obtained from 0% to 6% healthy plants, mainly due to GRSPaV persistence in sanitized plants.

Difficulties in GRSPaV sanitation were also reported by Gribaudo *et al*. (2006).

During sanitation from GPGV and GFkV, only the repeated Ribavirin treatment had a statistically significant effect on the numbers of sanitized grapevines *in vitro*. Both viruses were mostly totally removed from sanitized plants after the second Ribavirin treatment, see Tables 2 and 3. There are no data published on GPGV and GFkV sanitation using Ribavirin for comparison. GPGV is a newly emerged

virus, first identified in Italy (Giampetruzzi *et al*., 2012), later also found in other countries (Cho *et al*., 2013; Glasa *et al*., 2014; Mavrič Pleško *et al*., 2014).

The present work evaluated different conditions of Ribavirin-mediated sanitation from grapevine viruses. Repeating the Ribavirin treatment was always the key to obtain healthy plants.

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References

Cho IS, Jung SM, Cho JD, Choi GS, Lim HS (2013): First report of *Grapevine Pinot gris virus* infecting grapevine in Korea. New Dis. Rep. **27**, 10. https://doi.org/10.5197/j.2044-0588.2013.027.010

Crotty S, Cameron C, Andino R (2002): Ribavirin's antiviral mechanism of action: lethal mutagenesis? J. Mol. Med. 80, 86–95. https:/doi.org/10.1007/s00109-001-0308-0

Giampetruzzi A, Roumi V, Roberto R, Malossini U, Yoshikawa N, La Notte P, Saldarelli P (2012): A new grapevine virus discovered by deep sequencing of virus- and viroid-derived small RNAs in Cv Pinot gris. Virus Res. 163, 262–268. https:/doi.org/10.1016/j.virusres.2011.10.010

Glasa M, Predajňa L, Komínek P, Nagyová A, Candresse T, Olmos A (2014): Molecular characterization of divergent Grapevine Pinot gris virus isolates and their detection in Slovak and Czech grapevines. Arch. Virol. 159, 2103–2107. https:/doi.org/10.1007/s00705-014-2031-5

Gribaudo I, Gambino G, Cuozzo D, Mannini F (2006): Attempts to eliminate Grapevine rupestris stem pitting-associated virus from grapevine clones. J. Plant. Pathol. 88, 293-298.

Komínek P, Glasa M, Komínková M (2009): Analysis of multiple virus-infected grapevine plant reveals persistence but uneven virus distribution. Acta Virol. 53, 281–285. https:/doi.org/10.4149/av_2009_04_281

Komínek P, Jandurová OM (2011): Thermotherapy sanitation of two grapevine cultivars. Acta Virol. 55, 89–90. https:/doi.org/10.4149/av_2011_01_89

Mavrič Pleško I, Viršček Marn M, Seljak G, Žežlina I (2014): First Report of Grapevine Pinot gris virus infecting grapevine in Slovenia. Plant Dis. 98, 1014. https:/doi.org/10.1094/PDIS-11-13-1137-PDN

Panattoni A, D'Anna F, Cristani C, Triolo E (2007): Grapevine vitivirus A elimination in Vitis vinifera explants by antiviral drugs and thermotherapy. J. Virol. Methods 146, 129-135. https:/doi.org/10.1016/j.jviromet.2007.06.008

Panattoni A, Luvisi A, Triolo E (2011): Selective chemotherapy on Grapevine leafroll-associated virus-1 and -3. Phytoparasitica 39, 503–508. https:/doi.org/10.1007/s12600-011-0185-1

Quoirin M, Lepoivre P (1977): Improved media for in vitro culture of Prunus sp. Acta Hort. 78, 437–442. https:/doi.org/10.17660/ActaHortic.1977.78.54

Skiada FG, Maliogka VI, Katis NI, Eleftheriou EP (2013): Elimination of Grapevine rupestris stem pitting-associated virus (GRSPaV) from two Vitis vinifera cultivars by in vitro chemotherapy. Eur. J. Plant. Pathol. 135, 407–414. https:/doi.org/10.1007/s10658-012-0097-z

Weiland CM, Cantos M, Troncoso A, Perez-Camacho F (2004): Regeneration of virus-free plants by in vitro chemotherapy of GFLV (Grapevine fanleaf virus) infected explants of Vitis vinifera L. Cv 'Zalema'. Acta Hort. 652, 463–466. https:/doi.org/10.17660/ActaHortic.2004.652.61