Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

VALIDATION OF *GRAPEVINE PINOT GRIS VIRUS* ELIMINATION IN GRAPEVINE BY *IN VITRO* CHEMOTHERAPY

Ionela-Cătălina GUȚĂ, Elena-Cocuța BUCIUMEANU

National Research and Development Institute for Biotechnology in Horticulture Ştefăneşti - Argeş, 37 București-Pitești Road, Ștefănești, Argeș County, Romania

Corresponding author email: gutaionelacatalina@yahoo.com

Abstract

Distributed worldwide, Pinot Gris Grapevine Virus (GPGV) is considered a major pathogen of grapevine. Virus-free propagation material is the first step in the control of viral diseases, often requiring the application of methods to regenerate healthy plants. In vitro chemotherapy is the sanitation method used in this work, its validation being necessary to improve the efficiency of obtaining healthy biological material. To obtain GPGV-free grapevines, the treatment was applied to recover valuable clones from INCDBH Ștefănești, Fetească albă 97 Șt. and Cabernet Sauvignon 131 Șt. The apices and axillary buds were collected during the active growth period in two stages from plants grown in the protected space and in the field. A subculture of these explants on medium with ribavirin and oseltamivir, followed by three multiplication and one rooting on medium without antivirals, led to the regeneration of new plants with elimination rates of 50-100%, effective elimination being achievable with a precision of 21%, independent of genotype, explant type and sampling period.

***Key words**: Vitis, GPGV, sanitation, precision, ELISA.*

INTRODUCTION

Grapevine Pinot gris virus (GPGV), a member of the genus Trichovirus (family *Betaflexiviridae*), was first identified in Pinot gris in the Trentino vineyards of Italy by next-generation sequencing (NGS) techniques (Giampetruzzi et al., 2012), although symptoms of the new disease called Grapevine Leaf Mottling and Deformation (GLMD) (Martelli, 2014) have been reported since 2003.
The reports on GPGV have revealed the widespread of this virus in many European and non-European wine-producing countries, such as Korea, China, United States and Canada.
Molecular characterization and phylogeny of different isolates have indicated the existence of symptomatic and latent GPGV variants (Saldarelli et al., 2015; Bertazzon et al., 2017). In addition, a correlation between GPGV concentration and GLMD expression has also been proposed (Bertazzon et al, 2016). Furthermore, GPGV has been detected in several non-*Vitis* hosts (*Silene latifolia*, *Chenopodium* sp., *Asclepias syriaca*, *Rosa* sp., *Rubus* sp., *Fraxinus* sp.) (Gualandri et al., 2017; Demián et al., 2018). However, mechanical transmission to herbaceous plants has not been observed (Malagni et al., 2016).
Being transmitted by grafting (Saldarelli et al., 2015), GPGV is mainly spread through propagation material. Without regular testing of mother plants of fruiting varieties and the absence of symptoms on rootstocks, the virus spreads and cannot be safely controlled. As GPGV is widespread in Slovakia (Glasa et al., 2014); Moravia (Eichmeier et al., 2016) and Hungary, it is believed that there is a possibility that GPGV could spread from Eastern Europe to Italy and from Europe to other areas of the world (Bertazzon et al., 2016). On the other hand, GPGV is assumed to originate from Asia, with China being the most probable source of the virus (Hily et al., 2020).
GPGV has been detected in the body of the eriophyid mite *Colomerus vitis* (Pagenstecher) and it is transmitted to healthy grapevines by this infested mite (Malagnini et al., 2016).
Preliminary data on the presence of GPGV in Romania were obtained by analyzing grapevine material collected after 2010 year from germplasm collections in several European countries (Bertazzon et al., 2016). During 2019-2020 period, 199 grapevine samples were

analyzed by ELISA (Enzyme-linked immunosorbent assay) for the presence of GPGV, *Grapevine fanleaf virus* (GFLV), *Grapevine leafroll-associated virus 1+3* (GLRaV-1+3) and *Grapevine fleck virus* (GFkV). Of these, GPGV was detected in 107 samples (53.76%), in single or mixed infections with GFkV or GLRaV-1+3 (Guță & Buciumeanu, 2021).

Currently, in Europe, GPGV is considered a major pathogen of grapevines (Cieniewicz et al., 2020). GPGV control can be achieved by using healthy propagation material, using virus elimination methods (Gualandri et al., 2015; Guță & Buciumeanu, 2022), vector control (Gualandri et al., 2015) and removing alternative hosts (https://www.awri.com.au/wp-content/uploads/2015/09/GPGV-fact-sheet.pdf).

The paper aims to evaluate the influence of genotype, origin and type of explant on the application of *in vitro* chemotherapy for the elimination of *Grapevine Pinot Gris virus* in grapevine.

MATERIALS AND METHODS

The biological material was represented by grapevine plants (*Vitis vinifera* L.) belonging to the Fetească albă 97 Șt. and Cabernet Sauvignon 131 Șt. genotypes, from two locations: plants grown in vegetation pots in a protected space (germplasm collection G0) and plants selected in experimental fields.

The plants were virological tested ELISA (Clark & Adams, 1977) with commercial reagents Bioreba (Switzerland). They were ELISA - positive for GPGV and negative (control) for GPGV. All plants were free of GFLV, GLRaV-1+3 and GFkV.

The experiment consisted of using two types of explants (apices and nodal fragments) from a grapevine infected with GPGV and a control plant, one stored under controlled conditions in the G0 storage greenhouse and the other in the field. Six apexes and six nodal fragments were collected from each plant. The reason for starting from one plant was that all explants taken from it should have similar viral concentrations. Since plants stored in a protected space start the vegetation earlier as compared to those in the field, the initiation of the cultures was carried out in two stages: April and May (explants taken from the protected space), May and June (explants taken from the field).

For the initiation of aseptic cultures, growth tips were collected and after an insistent wash under jet of water for 1 h, they were sterilized with 6% sodium hypochlorite for 7 minutes. These plant fragments constituted the source of biological material for the excision of intensely regenerative apices and nodal fragments.

In vitro chemotherapy as a viral elimination method consisted of explants cultivating, apices and nodal fragments on grapevine specific culture medium (MS basic medium - Murashige and Skoog, 1962), containing 1 mg/L benzylaminopurine (BAP) + 0.5 mg/L indolylacetic acid (AIA) and 20 mg/L glucose, solidified with agar-agar (7g/L), supplemented with the mixture of viricides, ribavirin 10-30 mg/L and oseltamivir 30-60 mg/L, for one subculture (30-40 days), followed by the subcultivation on media (multiplication, rooting) without viricides, until the regeneration of new grapevine plants, 3 subcultures (SI, SII, SIII) (according to Patent no. 123133/2010 – Procedure for obtaining virus-free grapevine plants).

Ribavirin (1-β-D-ribofuranosyl-1, 2,4 triazone-3-carboxamide) pure substance (produced by Sigma-Aldrich, USA) (R) and oseltamivir ([(3E,4R,5S)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexane-1-carboxylic acid)] (O) as capsules containing oseltamivir phosphate (Tamiflu, Hofmann-La Roche, Germany) have been used.

Culture media were sterilized by autoclaving for 20 minutes at 120°C and 1 atm.

After inoculation, under aseptic conditions, in a laminar airflow hood, the tissue cultures were stored in the growth chamber where the conditions for triggering organogenesis processes were ensured (24°C, 16/8 photoperiod, day/night).

The multiplication rate (X) was expressed as the number of resulting explants reported to the number of initiated explants.

The evolution of the inocula during the three post-treatment subcultures was expressed by quantifying the multiplication rates [number of multiplication formations (adventitious bud glomeruli and shoot primordia)], which were statistically interpreted by regression analysis for the 95% confidence interval.

The phytotoxic effect of viricides was assessed by cultivation of explants on medium without antivirals, after the culture on medium containing ribavirin and oseltamivir.

The efficiency of the GPGV elimination method by *in vitro* chemotherapy was expressed as no. ELISA negative plants/no. tested plants x100.

The calculation of precision expressed as the coefficient of variation (standard deviation/mean of measurements) as a validation parameter reflects the repeatability of the results when applying the method.

RESULTS AND DISCUSSIONS

After 30 days on aseptic medium containing the mixture of viricides ribavirin and oseltamivir, the cultures belonging to the two genotypes studied behaved relatively similarly, considering the accumulation of disruptive factors: the phytotoxic effect of the chemotherapeutics, the genetic regenerative potential of the genotypes studied and last but not least the presence of viral infection.

In the case of Fetească albă 97 Șt.:

- Of the six apices grown on the medium with antivirals, only two developed organo-regenerative processes that led to the continuation of the culture, so that, after the first subculture, the multiplication rate was 1:1 (three explants resulted from each of the two viable explants);
- Under the influence of the chemotherapeutics, the nodal fragments behaved similarly: two explants out of the five initiated retained their viability and generated three explants each, that were subcultured on regular medium, the multiplication rate being 1:1.2;
- The phytotoxic effect of antivirals was assessed by the cultivating the explants on medium without antivirals, without omitting the presence of viral infection that can influence the regenerative potential of the genotype. The apices showed a viability rate of 1:0.5. The multiplication rate of the apex culture on basic medium was 1:1.66;
- The nodal fragments were treated similarly: the viability rate was 1:0.6; the multiplication rate was 1:2.2; 4 explants each from two initiated on control medium were subcultured on medium with chemotherapeutics.

In the case of Cabernet Sauvignon 131 Șt.:

- The treatment with ribavirin and oseltamivir for of GPGV elimination in the Cabernet Sauvignon 131 Șt. had a similar effect to the previous genotype (Table 1).

Table 1. *In vitro* chemotherapy for GPGV elimination to Cabernet Sauvignon 131 Șt. genotype. Values are averages of three replicates ± SD

Treatment	Explant type	Initiation No. of explants initiated	Subculture 1 Multiplication rate (X)*
R+O	Apex	5.0 ± 0.573	2.0± 0.897
	nodal fragment	5.0 ± 0.798	2.0 ± 0.768
Control	Apex	4.0 ± 0.836	2.5 ± 0.258
	nodal fragment	4.0 ± 0.798	3.0 ± 0.675

*multiplication rate = number of resulting explants reported to the number of initiated explants

For the construction of the regression curves of multiplication rate, the polynomial equation of degree 2 was chosen at which the coefficient of determination r^2 has the value 1 in all the analyzed cases, which shows a linear functional dependence between the analyzed variables, in our case, each explant in a subculture corresponds to a multiplication rate (Figures 1, 2, 3, 4).

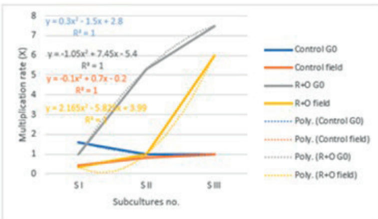

Figure 1. Multiplication rate of apices belonging to Fetească albă 97 Șt. genotype during 3 consecutive subcultures (SI, SII, SIII)

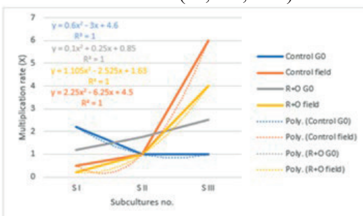

Figure 2. Multiplication rate of nodal fragments belonging to Fetească albă genotype 97 Șt. during 3 consecutive subcultures (SI, SII, SIII)

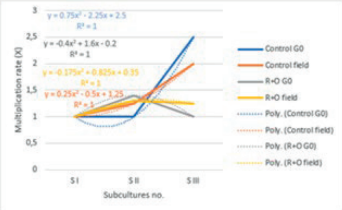

Figure 3. Multiplication rate of apices belonging to Cabernet Sauvignon 131 Șt. genotype during 3 consecutive subcultures (SI, SII, SIII)

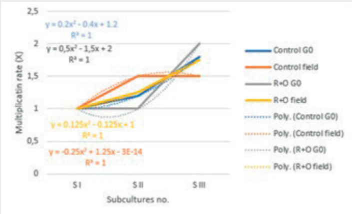

Figure 4. Multiplication rate of nodal fragments belonging to Cabernet Sauvignon 131 Șt. genotype during 3 consecutive subcultures (SI, SII, SIII)

Visually, the phytotoxic effect was manifested by chlorosis of explants cultivated on media with viricides after 30 days of treatment (Figure 5).
Released from the effect of viricides, explants subcultured on regular multiplication medium during three consecutive subcultures proliferated adventitious buds that differentiated into shoot primordia that rose, having normal appearance. These were excised and transferred to rooting medium.

Figure 5. Chlorosis of explants (apices and nodal fragments) of Cabernet Sauvignon 131Șt. (right) compared to the untreated control (left) after 30 days of culture on medium with ribavirin and oseltamivir

The regeneration rates reflect the regenerative potential of the genotype in the presence of viral infection and the effect of chemotherapeutics on the evolution of regenerative processes during consecutive subcultures, without omitting the fact that each explant initiated in culture is a on one hand, and on the other hand for obtaining virus-free plants distinct individual with specific behaviour (Table 2).

Table 2. Regeneration by *in vitro* chemotherapy of Fetească albă 97Șt. and Cabernet Sauvignon 131Șt. genotypes infected with GPGV

Genotype	Treat-ment	Explant type*	Period and place of explant sampling				Average	Average regeneration rate**
			April, protected space	May, protected space	May, field	June, field		
Fetească albă 97Șt.	Control	apex	3	2	2	3	2.50	0.41
		nodal fragment	6	4	0	1	2.75	0.45
	R+O	apex	5	4	4	3	4.00	0.66
		nodal fragment	3	5	0	1	2.25	0.38
Cabernet Sauvignon 131Șt.	Control	apex	10	11	8	9	9.50	1.58
		nodal fragment	8	6	5	4	5.75	0.95
	R+O	apex	6	5	4	3	4.50	0.75
		nodal fragment	5	3	4	2	3.50	0.58

*cultures were initiated with 6 inocula/type of explant
**regeneration rate = number of *in vitro* regenerated (rooted *in vitro*)/number of initiated inocula

Tissue culture is a very effective approach for obtaining a large number of plants in a short time However, some research has shown that viral elimination in grapevine was not possible by shoot tip culture (Wang et al., 2016; Sim et al., 2019). Therefore, tissue culture has been combined with thermotherapy, chemotherapy, electrotherapy, etc. (Panattoni et al., 2013).
The grapevine genotypes behave differently when applying the micropropagation method.

Studies undertaken to evaluate the regenerative potential of some grapevine clones from germplasm collection belonging to the National Research & Development Institute for Biotechonoly in Horticulture Ștefănești - Argeș led to their grouping into three classes, following the quantification of the multiplication rate, *in vitro* rhizogenesis, and the acclimatization of the *in vitro* regenerated plants. Thus, the Fetească albă 97 Șt. genotype was declared to have a low regenerative potential, at the opposite pole being the Cabernet Sauvignon 131Șt. genotype (Vișoiu et al., 2008). On the other hand, it is self-evident that the presence of a viral infection in the explant donor plants influences the behaviour of these genotypes in the *in vitro* culture. In Cabernet Sauvignon infected with the viral complex *Grapevine rupestris stem pitting-associated virus* (GRSPaV), *Grapevine leafroll-associated virus 2* (GLRaV-2) and GFkV, no plants could be regenerated by micropropagation (Hu et al., 2022).

The influence of viral infection on the behaviour of a grapevine genotype in the *in vitro* culture was studied in Fetească neagră genotype. *In vitro* studies conducted with explants collected from healthy grapevine and those infected with GFLV, GLRaV1+3 or GFkV highlighted the influence of the presence of the virus on the quantitative and qualitative characteristics of the culture. Significant differences in the multiplication rate were recorded between healthy and virus-infected material. On the contrary, shoot elongation and rooting capacity were not significantly influenced by virus infection. The type of virus did not influence the behaviour of the genotype during the cultures, highlighting the fact that the Fetească neagră genotype has a tolerant response to various viral infections under *in vitro* conditions (Guță et al., 2009b). The use of chemotherapeutics in aseptic culture media to block viral multiplication and to regenerate new virus-free plants caused problems regarding phytotoxicity on explants from the beginning. Different viricides, different concentrations of them and different treatment times have been studied so that the method generates viral elimination: ribavirin (Weiland et al., 2004; Hu et al., 2018); tiazofurin, 6-thioguanine, oseltamivir (Panattoni et al., 2011), dihydroxypropyladenine (Panattoni et al., 2007a); mycophenolic acid (Panattoni et al., 2007b), salicylic acid (Khassein et al., 2024).

Increasing the treatment time (1-3 subcultures) induced a significant decrease in the multiplication rate for each concentration of the viricides ribavirin and oseltamivir in GFkV - infected Canner genotype. The phytotoxic effect diminished with the transfer of the plants to the rooting medium without antivirals (Guță et al., 2009a). In the process of GLRaV-1 and *Grapevine virus A* (GVA) elimination in Servant variety, using 80 μmol/L ribavirin in the culture medium for 30-90 days, the phytotoxic effect on the multiplication formations (axillary buds, shoot primordia) was also highlighted, which diminished at the transfer to regular medium (Guță & Buciumeanu, 2011). Studies on the GFLV elimination in Fetească albă genotype, GLRaV-1+3 in Ranâi Magaraci, and GFkV in Canner mentioned the occurrence of explants vitrification and necrosis phenomena as an effect of the presence of viricides in the culture medium (Guță & Buciumeanu, 2012). Throughout time, *in vitro* chemotherapy with ribavirin and oseltamivir used single each one or in combination has proven to be effective in viral elimination, the phytotoxic effect being neutralized by subsequent cultivation on regular medium without antivirals, and by the regenerative potential specific to the grapevine genotype (Guță et al., 2014; 2017a; 2017b).

Successful application of the *in vitro* chemotherapy method must consider the cultivation of a sufficient number of explants that lead to the regeneration of new plants, some of which are virus-free. After acclimatization, as a result of the process of adapting the vitroplants to ex vitro conditions, the number of regenerated plants decreased, but all experimental variants were generally represented. These were individually tested by ELISA to establish the virological status, namely the presence of GPGV (Table 3).

Table 3. Virological status of grapevine plants regenerated by *in vitro* chemotherapy

Genotype	Treatment	Explant type *	No. of regenerated plants – Period and place of explant sampling: April, protected space – tested**	April, protected space – ELISA negative***	May, protected space – tested**	May, protected space – ELISA negative***	May, field – tested**	May, field – ELISA negative***	June, field – tested**	June, field – ELISA negative***
Fetească albă 97Șt.	Control	apex	3	1	1	0	2	0	1	0
		nodal frag.	3	0	2	1	-	-	1	0
	R+O	apex	1	1	4	4	3	2	1	1
		nodal frag.	2	1	3	2	0	-	1	1
Cabernet Sauvignon 131Șt.	Control	apex	8	0	3	0	6	0	4	0
		nodal frag.	7	0	6	0	2	0	2	0
	R+O	apex	6	5	2	1	3	3	1	1
		nodal frag.	3	3	1	0	2	2	1	1

*cultures were initiated with 6 inocula/type of explant
**plants regenerated from *in vitro* rooted plants after going through the acclimatization process
***regenerated plants were ELISA tested for GPGV

The efficiency of the GPGV elimination method by *in vitro* chemotherapy was calculated as no. ELISA negative plants/no. of tested plants x 100 (Table 4).

Table 4. Efficiency of the method of GPGV elimination, simple infection, by *in vitro* chemotherapy
(expressed as no. of ELISA negative plants/no. of plants tested x100)

Genotype	Treatment	Explant type	GPGV elimination rate (%) – Period and place of explant sampling: April, protected space	May, protected space	May, field	June, field
Fetească albă 97Șt.	Control	apex	33	0	0	0
		nodal frag.	0	50	-	0
	R+O	apex	100	100	66	100
		nodal frag.	50	66	-	100
Cabernet Sauvignon 131Șt.	Control	apex	0	0	0	0
		nodal frag.	0	0	0	0
	R+O	apex	83,3	50	100	100
		nodal frag.	100	0	100	100

The calculation of precision, as the variability of the results obtained when repeating the experiments of GPGV-free plants regeneration by *in vitro* chemotherapy, dependent on the genotype and the type of explant, independent of the period and place of sampling of the explants subjected to chemotherapy treatment, showed that the apexes give the best results in both genotypes studied. Cultivation of apices from donor plants grown in protected culture or in the field, regardless of the sampling period, led to the regeneration of GPGV-free plants with a variation of ±0.19 in Fetească albă 97Șt., respectively ± 0.28 in Cabernet Sauvignon 131Șt. as compared to the nodal fragments (Table 5).

Eliminating the influence of genotype led to a precision of 0.22 at an average of 87.41 and SD of 19.52 when cultivating apices on viricidal medium.

There are cases when a small amount of biological material requires the use of both apices and nodal fragments in the application of *in vitro* chemotherapy.

Table 5. Validation the parameters of the GPGV elimination method by *in vitro* chemotherapy in two grapevine genotypes infected with GPGV

Genotype	Explant type	Average*	Standard deviation** (SD)	Precision*** (Variation coefficient VC)
Fetească albă 97Șt.	apex	91.5	17.0	0.19
	nodal fragment	54.0	41.6	0.77
Cabernet Sauvignon 131Șt.	apex	83.3	23.6	0.28
	nodal fragment	75.0	50.0	0.66

*average of viral elimination rate (number of GPGV - ELISA negative plants/number of plants tested x100)
**SD = standard deviation of the average of the 4 values corresponding to the four initiation periods
***VC = SD/average

The precision calculated when standardizing all parameters that could influence the successful application of the viral elimination method (genotype, explant type, donor plant, sampling period) was 0.21 for an average of 75.95 and a standard deviation of 16.10. This indicates a minimal influence of the type of explant on the repeatability of the method.

CONCLUSIONS

In vitro chemotherapy is an effective method for regeneration of ELISA negative grapevine plants for GPGV.
The method can be applied by culturing apexes and nodal fragments on medium supplemented with antivirals taken from donor plants grown in protected areas or in the field during the grapevine vegetation period.
In the case of the genotypes Fetească albă 97 Șt. and Cabernet Sauvignon 131 Șt., GPGV was eliminated at rates ranging from 50 to 100% with a precision of ± 0.21.

ACKNOWLEDGEMENTS

The study is part of the Nucleus Program 23.41/2023-2026, Project 01 01 "Procedure of *Grapevine Pinot gris virus* elimination under conditions of economic efficiency, in order to increase the biological value of viticultural propagation material" which is supported jointly by Ministry of Research, Innovation and Digitalization.

REFERENCES

Bertazzon, N., Filippin, L., Forte, V., & Angelini, E. (2016). Grapevine Pinot gris virus seems to have recently been introduced to vineyards in Veneto, Italy. Archives of Virology, *161*(3), 711-714.

Bertazzon, N., Forte, V., Filippin, L., Causin, R., Maixner, M., & Angelini, E. (2017). Association between genetic variability and titre of Grapevine Pinot gris virus with disease symptoms. *Plant Pathology*, 66, 949-959.

Cieniewicz, E.J., Qiu, W., Saldarelli, P., & Fuchs, M. (2020). Believing is seeing: lessons from emerging viruses in grapevine. *Journal of Plant Pathology*, 102, 619-632.

Clark, M.F., & Adams, A.N. (1977). Characteristics of the microplate method of the enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology, 34, 475-483.

Demián, E., Czotter, N., &Várallyay, É. (2018). Detection of Grapevine pinot gris virus in different non-*Vitis* hosts in Hungary. Proceedings of the 19th ICVG, Santiago, Chile, April 9-12, 2018, 24-25.

Eichmeier, A., Peňázová, E., Pavelková, R., Mynarzová, Z., & Saldarelli, P. (2016). Detection of Grapevine Pinot gris virus in certified grapevine stocks in Moravia, Czech Republic. *Journal of Plant Pathology*, 98(1), 155-157.

Gualandri, V., Asquini, E., Bianchedi, P., Covelli, L., Brilli, M., Malossini, U., Bragagna, P., Saldarelli, P., & Si-Ammour, A. (2017). Identification of herbaceous hosts of the Grapevine Pinot gris virus (GPGV). *European Journal of Plant Pathology*, 147, 21-25.

Gualandri, V., Bianchedi, P., Morelli, M., Giampetruzzi, A., Valenzano, P., Bottalico, G., Campanale, A., & Saldarelli, P. (2015). Production of Grapevine pinot gris virus-free germplasm: techniques and tools. *Proceedings of the 18th ICVG*, 7-11 September 2015, Ankara, Turkey, 246-247.

Giampetruzzi, A., Roumi, V., Roberto, R., Malossini, U., Yoshikawa, N., La Notte, P., Terlizzi, F., Credi, R., & Saldarelli, P. (2012). A new grapevine virus discovered by deep sequencing of virus- and viroid-derived small RNAs in cv. Pinot gris. *Virus Research*, 163, 262-268.

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. *Archives of Virology*, *159*(8), 2103-2107.

Grapevine Pinot Gris virus. Fact Sheet, Viticulture. Retrieved January 21, 2025, from https://www.awri.com.au/wp-content/uploads/2015/09/GPGV-fact-sheet.pdf.

Guta, I.C., & Buciumeanu, E.C. (2011). Grapevine chemotherapy for elimination of multiple virus infection. *Romanian Biotechnological Letters*, *16*(5), 6535-6539.

Guţă, I.C., & Buciumeanu, E.C. (2012). Results of chemotherapy and electrotherapy on virus elimination in grapevine. *Proceedings of the 17th ICVG*, Davis, California, USA, October 7-14, 2012, 264-265.

Guță, I.C., & Buciumeanu, E.C. (2021). Grapevine Pinot gris virus infecting grapevine in Romania – Short Communication. *Horticultural Science* (Prague), *48*(1), 47-50.

Guță, I.C., & Buciumeanu, E.C. (2022). Procedeu de eliminare a virusului Grapevine Pinot gris virus la vița-de-vie prin cultură de meristem. In: Tabăra V. (coord.), *Oferta cercetării științifice pentru transfer tehnologic în agricultură, industria alimentară și silvicultură, XXV*, 173-175. Bucharest, RO: Ceres Publishing House.

Guţă, I.C., Buciumeanu, E.C., Gheorghe, R.N., & Teodorescu, Al. (2009a). Examination of phytotoxic effect of viricides on grapevine in controlled medium. *Scientific Papers U.S.A.M.V.B., Serie B, Horticulture*, LIII, 559-563.

Guţă, I.C., Buciumeanu, E.C., Tătaru, L.D., & Topală, C.M. (2017a). Regeneration of grapevine virus-free plants by *in vitro* chemotherapy. *Acta Horticulturae*, 1188, 319-322.

Guţă, I.-C., Buciumeanu, E.-C., Topală, C.M. & Tătaru, L.D. (2017b). The use of *in vitro* chemotherapy in grapevine virus elimination. *Acta Horticulturae*, 1155, 425-430.

Guţă, I.-C., Buciumeanu, E.-C., & Vişoiu, E. (2009b). Comparative study of *in vitro* behavior of grapevine (*V. vinifera* L., Fetească neagră cv.) under the influence of various infections. *Extended abstracts 16th ICVG*, Dijon, France, 31 Aug.- 4 Sept., 2009, 247.

Guţă, I.C., Buciumeanu, E-C., & Vişoiu, E. (2014). Elimination of Grapevine fleck virus by *in vitro* chemotherapy. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *42*(1), 115-118.

Hily, J.-M., Poulicard, N., Candresse, T., Vigne, E., Beuve, M., Renault, L., Velt, A., Spilmont, A.S., & Lemaire, O. (2020). Datamining, genetic diversity analyses, and phylogeographic reconstructions redefine the worldwide evolutionary history of Grapevine Pinot gris virus and Grapevine berry inner necrosis virus. *Phytobiomes Journal*, *4*(2), 165–177.

Hu, G., Dong, Y., Zhang, Z., Fan, X., Ren, F., Li, Z., & Zhang, S. (2018). Elimination of Grapevine rupestris stem pitting-associated virus from *Vitis vinifera* 'Kyoho' by an antiviral agent combined with shoot tip culture. *Scientia Horticulturae*, 229, 99-106.

Hu, G., Dong, Y. Zhang, Z., Fan, X., & Ren, F. (2022). Effect of *in vitro* culture of long shoot tip on variant structure and titer of grapevine viruses. *Plants*, 11, 1907.

Khassein, A., Suleimanova, G., Malakhova, N., & Kizildeniz, T. (2024). Grapes recovery from grapevine fan leaf virus by chemotherapy using salicylic acid. *Research on Crops*, *25*(4), 605-610.

Malagnini, V., de Lillo, E., Saldarelli, P., Beber, R., Duso, C., Raiola, A., Zanotelli, L., Valenzano, D., Giampetruzzi, A., Morelli, M., Ratti, C., Causin, R., & Gualandri, V. (2016). Transmission of grapevine Pinot gris virus by *Colomerus vitis* (Acari: Eriophyidae) to grapevine. *Archives of Virology*, 161, 2595-2599.

Martelli, G.P. (2014). Directory of Virus and Virus-like Diseases of the Grapevine and their Agents. *Journal of Plant Pathology*, 96(1S), 105-120.

Murashige, T., & Skoog, F. (1962). A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiologia Plantarum*, *15*(3), 473–497.

Panattoni, A, D'Anna, F, Cristani, C, Triolo, E. (2007a). Grapevine vitivirus A eradication in *Vitis vinifera* explants by antiviral drugs and thermotherapy. *Journal of Virological Methods*, *146*(1-2), 129-135.

Panattoni, A., D'Anna, F., & Triolo, E. (2007b). Antiviral activity of tiazofurin and mycophenolic acid against Grapevine Leafroll-associated Virus 3 in *Vitis vinifera* explants. *Antiviral Research*, *73*(3), 206-211.

Panattoni, A., Luvisi, A., & Triolo, E. (2011). Selective chemotherapy on Grapevine leafroll-associated virus-1 and -3. *Phytoparasitica*, 39, 503–508.

Panattoni, A., Luvisi, A., & Triolo, E. (2013). Review. Elimination of viruses in plants: twenty years of progress. *Spanish Journal of Agricultural Research*, *11*(1), 173-188.

Saldarelli, P., Giampetruzzi, A., Morelli, M., Malossini, U., Pirolo, C., Bianchedi, P., & Gualandri, V. (2015). Genetic variability of Grapevine Pinot gris virus and its association with grapevine leaf mottling and deformation. *Phytopathology*, *105*(4), 555–563.

Sim, S.T., Khuu, N., Shoulders, J.R., Pudlo,W., Hoang, N.H., & Golino, D.A. (2019). Elimination of rose viruses using microshoot tip tissue culture. *Acta Horticulturae*, 1232, 241–246.

Wang, M.R., Li, B.Q., Feng, C.H., & Wang, Q.C. (2016). Culture of shoot tips from adventitious shoots can eradicate apple stem pitting virus but fails in apple stem grooving virus. *Plant Cell, Tissue and Organ Culture*, 125, 283-291.

Weiland, C.M., 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 Horticulturae*, 652, 463-466.

Vișoiu, E., Zmărăndoiu, I.L., Buciumeanu, E.C., & Guță, I.C. (2008). Use of *in vitro* culture as a method for valorification of grapevine genetic resources. *Book of abstracts, XXXVIII*, ESNA Annual Meeting Krakow, Poland, 27-31 August, 2008, 307.