

Grapevine viruses – a global challenge and biotechnological solutions to obtain healthy material

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ABSTRACT

Grapevine (*Vitis* spp.) is one of the most important horticultural species, but also one of the most vulnerable to viral infections. To date, 102 viruses are known to infect grapevines. Of these, some viruses cause significant economic damage worldwide, affecting the quantity and quality production, plant growth and development, and the vineyards long-lasting. Other viruses have a broad spectrum, affecting several crop plants and represent scientific curiosities for the grapevine, both because they are rarely encountered and the damages induced are negligible. This paper summarizes the main knowledge regarding the diversity of viral agents affecting grapevine and the associated symptomatology characteristics. The economic effects of viral diseases are analyzed, highlighting their influence on propagation material and grape production. Once virus-infected, grapevine plants remain infected for their entire life. Prevention of infection includes the production of certified virus-free planting material and virus testing. Thus, the propagation of healthy material, originating from authorized sources, tested and certified as virus-free, is essential for sustainable viticulture. Also, virus-elimination methods are presented (meristem/shoot tip cultures, thermotherapy, *in vitro* chemotherapy, electrotherapy, cryotherapy, and combined methods), which allow the regeneration of healthy, virus-free plants. The integration of these technologies into virus-free germplasm selection and conservation programs represents a priority direction for a modern viticulture, ensuring the maintenance of grapevine health, the protection of genetic diversity and the sustainability of grapevine production.

Keywords: *Vitis*, GFLV, GLRaVs, GFkV, economic effects, virus-elimination

INTRODUCTION

Grapevine (*Vitis* spp.) is one of the most important horticultural plants in the world due to its economic and social impact (www.oiv.int).

The grapevine market is considered to be one of the most dynamic and fastest-growing agricultural markets in recent years.

Like most vegetatively propagated plants, grapevines are exposed to attacks by many pests and pathogens, among which intracellular infectious agents (viruses, viroids, phytoplasmas) play a major role, causing important losses, shortening the productive life of vineyards and endangering the survival of affected plants (Martelli and Boudon-Padieu, 2006; Moutinho-Pereira *et al.*, 2012; Cuozzo *et al.*, 2018).

Therefore, timely monitoring of factors that may have a negative effect on plant growth and product quality, primarily the control of the causal agents of grapevine diseases, is of critical importance for grape production (Martelli and Boudon-Padieu, 2006; Zherdev *et al.*, 2018).

Due to their latent mode of manifestation and transmission through vectors or vegetative propagation material, grapevine viruses constitute a major challenge for viticulture, with long-lasting economic and genetic effects.

In recent decades, the research has focused on understanding the mechanisms of infection, assessing the physiological impact of viral diseases, and developing efficient technologies to obtain virus-free plants. Thus, the present synthesis aims to analyze the main viral infections of grapevine, their consequences on the biological and economic performance of the crop, as well as the advances made in the field of virus-elimination methods and healthy germplasm conservation.

Viral infections in grapevine

To date, 102 viruses are known to infect grapevine, making it the plant with the highest number of known viral entities. These viruses have single-stranded or double-stranded RNA or DNA genomes and are classified into 44 genera and 21 families or taxonomically unclassified, identified in various *Vitis* germplasm collections worldwide (Fuchs, 2023; 2025). Of these, 31 viruses that cause significant economic damage worldwide are associated with four major disease complexes: leafroll (5 viruses), rugose wood complex (6 viruses), infectious degeneration and decline (12 and 4 viruses, respectively) and grapevine fleck (4 viruses) (Martelli, 2017; 2018; Fuchs, 2020). The viruses associated with these diseases are transmitted by mealybugs, soft scale insects or nematodes (Maliogka *et al.*, 2015); for the fleck virus (GFkV) that causes fleck disease (marbrure), the vector is unknown (Bovey and Martelli, 1992; Fuchs, 2020). The application of biological and chemical phytosanitary treatments is recommended for the control of virus vectors (Maliogka *et al.*, 2015).

New viral species, such as *Grapevine red blotch virus* (GRBV) and *Grapevine Pinot gris virus* (GPGV), as well as new strains of known viruses, have been identified and detected using high-throughput sequencing (HTS) technologies. These viruses have a substantial negative impact on production by reducing grapevine growth, thereby affecting the quality and quantity of production (Fuchs, 2020). High-Throughput Sequencing (HTS), known as Next Generation Sequencing (NGS), facilitates the detection of known viruses, the discovery of new viruses, and viruses associated with a disease of unknown etiology.

The diseases caused by the presence of *Grapevine fanleaf virus* (GFLV), *Arabidopsis mosaic virus* (ArMV), *Grapevine leafroll-associated virus-1* and *-3* (GLRaV-1, GLRaV-3), *Grapevine fleck virus* (GFkV), *Grapevine virus A* (GVA) in single infections or in combinations are the most aggressive for grapevine. Other viruses have a broad spectrum, affecting several important crop plants (*Italian artichoke latent virus* – AILV, *Alfalfa mosaic virus* – AMV, *Bean wilt virus* – BBWV, *Potato virus X* – PVX); for grapevine, these represent scientific curiosities, both because they are rarely found and because the damages induced are negligible (Martelli, 1999).

All these viruses are spread through infected propagation material and lead to a considerable decrease in fruit quality and yield, causing significant economic losses to producers and nurseries. In addition, over the years, after planting in the field, even healthy grapevines can become infected with numerous pathogens in the absence of rigorous management involving early diagnosis of infections and vector control.

Diagnostic methods such as visual inspection (Bell *et al.*, 2017), biological indexing (Krake *et al.*, 1999; Engel *et al.*, 2010; Hao *et al.*, 2021), enzyme-linked immunosorbent assay (ELISA), molecular methods (Stewart and Nassuth, 2001; Pacifico *et al.*, 2011), electron microscopy (Corbetta *et al.*, 1984; Russo, 2005), and multispectral (Mahlein, 2016) or hyperspectral (Nguyen *et al.*, 2021) imaging methods are used to monitor and detect viruses. Of these,

ELISA is the most used virological diagnostic method (Clark and Adams, 1977) to check the grapevines' sanitary status.

According to the technical annex to Directive 68/93/EEC (Council Directive of 9 April 1968 on the marketing of material for the vegetative propagation of the grapevine), *Vitis* propagating material other than seeds must be free from ArMV, GFLV, GLRaV-1, GLRaV-3; rootstocks of *Vitis* spp. and their hybrids, except *V. vinifera* L. must be free from ArMV, GFLV, GLRaV-1, GLRaV-3, and GFkV (<https://eur-lex.europa.eu/legal-content/RO/TXT/PDF/?uri=CELEX:01968L0193-20050714&from=LV>).

The grapevine viruses tested in Romania comply with the legislation in force (Annex 5 to Order 1267/2005 for the approval of the Rules and technical norms regarding the production for commercialization, control, quality certification and commercialization of vegetative propagation material of grapevine) harmonized with EU legislation.

THE INFLUENCE OF VIRAL INFECTION ON GRAPEVINE

Grapevine, as a woody species, is a model plant for the study of plant-virus interactions both under controlled and field conditions (Perrone *et al.*, 2017), but evaluating the effects of a viral disease is not easy to do because a plant can be infected with multiple viruses at the same time. Viral infection produces physiological and structural changes, affecting: photosynthesis, gas exchange, chlorophyll content, carbon translocation, phloem transport, cellular metabolism (Bertamini *et al.*, 2005; Martínez-Lüscher *et al.*, 2019; Kappagantu *et al.*, 2020). Also, the losses caused by viral infection depend on the age of the plant, environmental and growing conditions, grapevine cultivars and rootstocks (Golino *et al.*, 2003; Mannini, 2003; Pesqueira *et al.*, 2012). In addition, it should not be forgotten that plants grown in the field are simultaneously subjected to different abiotic and biotic stress factors (Suzuki *et al.*, 2014).

The impact of viral diseases is amplified by the fact that grapevine is a vegetatively propagated woody species, and many grapevine cultivars have been distributed as vegetative cuttings throughout the world with reduced attention to the presence of possible intracellular pathogens (Naidu *et al.*, 2015). Of all the factors that could influence the success of grafting, none is more important than the sanitary condition of the biological material, since the virus can multiply and spread throughout the plant, both from the scion and the rootstock. Furthermore, it is possible for the scion to be infected with one virus and the rootstock with another, resulting in a grafted grapevine containing two viruses, causing more severe symptoms and synergistic effects on plant health, grape production, and quality (Rayapati, 2017).

Plants infected with GFLV (genus *Nepovirus*, family *Secoviridae*) often exhibit abnormal growth, such as vine debilitation and shortened or deformed internodes, in addition to yellowing or deformed leaves and clarification of veins. The presence of this virus decreases production profits by reducing plant longevity, scion-rootstock compatibility, and the ability of the vine to multiply (Samaali *et al.*, 2019; Poojari *et al.*, 2020). Infected vines may show reduced resistance to adverse climatic factors, such as drought or frost. Fanleaf degeneration or decline is one of the most severe grapevine viral disease complexes worldwide. Also, fanleaf is one of the oldest known viral diseases in *V. vinifera*, the symptoms reported in Europe since 1841. The disease caused by GFLV infection affects grapevines in all temperate regions where *V. vinifera* and hybrid rootstocks are present (Oliver and Fuchs, 2011).

GFLV infection can cause variable losses, with potential for total loss of production, depending on the virulence of the virus strain and varietal susceptibility. Bunches are reduced in size and number and have irregular ripening. Infected berries have uneven size, many are small and seedless, and some fail to ripen. Fruit quality is affected due to reduced sugar content and titratable acidity. Rooting ability of rootstocks and grafting are both

substantially reduced in GFLV-infected material. Also, the productive life of vineyards infected with GFLV is significantly reduced. Poor vine health predisposes the vines to winter damage and death in colder climates (<https://grapes.extension.org/grapevine-fanleaf-degeneration-disease/>).

Grapevine leafroll-associated viruses (GLRaVs) are among the most important pathogens affecting grapevines and have a considerable economic impact (Atallah *et al.*, 2012; Fuchs *et al.*, 2021; Ricketts *et al.*, 2015). These virus species are diverse and belong to the family *Closteroviridae*, with six species and numerous strains grouped into three genera: the genus *Closterovirus* – GLRaV-2, the genus *Ampelovirus* – GLRaV-1, GLRaV-3, GLRaV-4, GLRaV-13, and the genus *Velarvirus* – GLRaV-7 (Fuchs, 2020; Naidu *et al.*, 2015). Grapevines are often infected with several of these viruses simultaneously (Prosser *et al.*, 2007; Al Rwahnih *et al.*, 2009). Given the global impact and distribution of leafroll disease, efforts have been undertaken to manage the spread of GLRaVs, characterize their effects, and understand the interaction between grapevine and GLRaVs. Leafroll, one of the most important viral diseases affecting grapevines worldwide, causes yield losses of up to 14–40% and delays fruit ripening by 3 weeks to 1 month (Rayapati *et al.*, 2014). Some GLRaV-3 isolates (LR101, LR127 and PA94.148) affect grapevine yield performance and berry composition, but for most parameters, the effect is variable between isolates, and in the case of cut wood weight and berry composition, it also depends on the rootstock (Rowhani *et al.*, 2015). Leafroll negatively affects rooting ability, graft attachment, plant vigor, photosynthesis, as well as the modulation of host genes involved in a cultivar of biological functions (Vondras *et al.*, 2021). A strain of GLRaV-2, constantly associated with Kober 5BB incompatibility in Europe, appears to be involved in the decline of young vines in California and has been detected in affected vines in Chile and Argentina. Other molecular variants of GLRaV-2 have been reported in New Zealand, Chile and Australia in association with decline conditions in young vines. Based on the differential responses of a panel of 18 rootstocks, up to five different graft-transmitted incompatibility-inducing agents have been detected in California (Martelli and Gallitelli, 2008).

In grapevine cultivars infected with GVA (genus *Vitivirus*, family *Betaflexiviridae*), a reduction in shoot number and growth, reduced sugar accumulation in berries, and vine senescence have been observed. Grape production can be reduced by 20-30% (Martelli and Boudon-Padieu, 2006; Minafra *et al.*, 2017). The Shiraz cultivar is very sensitive to GVA infections; symptoms associated with infection include delayed shoot growth, decreased sugar accumulation in berries, and, in some cases, plant death (Habibi *et al.*, 2016).

The combination of GLRaV-1 and GVA infecting the scion caused incomplete healing of the grafting point and a decrease in the growth of the scions when Freedom and 101-14 rootstocks were used, while St. George (*V. rupestris*) and AXR#1 (*V. vinifera* × *V. rupestris*) rootstocks showed tolerance to viral infection under the same conditions (Golino *et al.*, 2015; Cui *et al.*, 2019). These observations suggested the existence of a virus-rootstock interaction that determines the destruction of the scions under the influence of viral infection. Furthermore, a synergistic effect between GLRaV and vitiviruses could increase the virulence on grapevine grafts, indicating an additional complexity underlying the virus-rootstock interaction. The genetic background determines the viral tolerance of the rootstock in the grafting system (Rowhani *et al.*, 2017).

Numerous observations and experiments highlighted the effects of the GFkV (genus *Maculavirus*, family *Tymoviridae*) presence, alone or in combination with other viruses and viral diseases. Many grapevine cultivars and rootstocks infected only with GFkV are asymptomatic, although the infection may be associated with graft incompatibility (<https://www.wineaustralia.com/getmedia/b1e5038d-cc30-48d8-b3c2-25844af8e795/201107-Grapevine-fleck-and-associated-viruses?ext=.pdf>). The presence of

GFkV reduces rhizogenesis and graft attachment; the effects depend on the virulence of the

viral isolate. GFkV alone or in the presence of leafroll induces a reduction in graft percentage and affects plant vigor in nurseries. Infection with GFkV associated with vein necrosis and vein mosaic reduced the amount of cutting wood on 420A and Kober 5BB rootstocks by 52% and 37%, respectively, but did not affect growth on Teleki 5A. Simultaneous infection with GFkV and leafroll produces ajinashika disease in the Japanese grape cultivar 'Koshu', characterized by a reduction in the amount of sugar in the fruit (Walter and Martelli, 1996). Following field experiments, it is considered that, in general, self-rooted grapevines are more tolerant to viral infections (Garau *et al.*, 1997).

GPGV, a member of the genus *Trichovirus* (family *Betaflexiviridae*), was first identified in the 'Pinot gris' cultivar in 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.

Although GPGV-infected grapevines are often asymptomatic, they may sometimes exhibit leaf deformation, chlorotic spots, dwarfing, and shorter internodes that typically develop early in the growing season, similar to symptoms caused by mite bites, but also to those produced by nepoviruses (Hily *et al.*, 2020). Symptomatic plants infected with GPGV showed reduced vigor and significant yield losses (up to 96%), depending on the severity of symptoms (Malossini *et al.*, 2014; Messmer *et al.*, 2024).

The vector of GPGV is the eriophyid mite *Colomerus vitis* (Pagenstecher) (Malagnini *et al.*, 2016). The virus has been reported in the last few years in major grapevine-growing regions of the world (Saldarelli *et al.*, 2017). Since GPGV is transmitted by grafting, it is mainly spread through propagation material (Saldarelli *et al.*, 2015). GPGV is currently considered a major grapevine pathogen in Europe (Cieniewicz *et al.*, 2020). Although this virus is not regulated, it is recommended that all grapevine propagation material (potted plants, cuttings, graft buds) be tested for the presence/absence of GPGV (<https://www.dpi.nsw.gov.au/agriculture/horticulture/grapes/publications/grapevine-pinot-gris-virus>).

Healthy mother plants are important because they are used by nurseries to produce propagating material and distribute it worldwide, and strict regulatory measures are necessary to protect agricultural systems from the viruses spread. In general, germplasm selection (thanks to diagnostic tools and procedures developed in plant virology) leads to the identification of healthy cultivars that can be included in the vegetative production systems. However, in some cases, the identification of healthy plants among selected local cultivars with limited distribution may be difficult or impossible, making the virus-elimination procedures necessary to recover the useful healthy plants (Panattoni *et al.*, 2013).

The influence of viral infection on the grapevine is presented in Table 1.

Table 1. The impact of virus diseases in grapevine

Disease/ Virus	Symptoms	Economic losses	Observations	References
Fanleaf/GFLV	Leaf deformation; fan-shaped leaves, short internodes, zig-zag growth, bushy appearance, reduced plant growth, reduced number of clusters, berries are small-sized and ripen irregularly, and compromised propagation material.	Production reduction of up to 80% in severely affected areas.	One of the most damaging viral diseases; seriously affects the longevity of the plantation and the wine quality.	Walter and Martelli, 1996. Malossini <i>et al.</i> , 2003. Martelli and Boudon-Padieu, 2006.

Disease/ Virus	Symptoms	Economic losses	Observations	References
Leafroll/ GLRaV-1, -2, -3	Downward of leaf edges, reddening or yellowing (in red and white cultivars, respectively), green veins, uneven ripening, delayed ripening of grapes, and decreased production.	Production reduction by 20–60%.	Globally widespread; major effects on grape ripening and sugar content, lower quality wine.	Martelli and Boudon-Padieu, 2006. Martelli, 2014. Naidu <i>et al.</i> , 2014.
Rugose wood/ (viral complex)	Bark abnormalities, cracks, swellings, zones of necrosis on the trunk, weakening of the plant, and gradual reduction of production.	Information on production losses specific to this viral complex is limited.	Affects sap circulation; cumulative effects over time.	Martelli and Boudon-Padieu, 2006. Cuozzo <i>et al.</i> , 2018.
Grapevine mosaic/ArMV	Yellow spots, mosaic, reduced growth.	It ranges from slight decrease to severe losses (30–70%).	Plantation compromise; difficult to control.	Martelli and Boudon-Padieu, 2006.
Marbrure/ GFkV	Clearing of upper order veins, deformed, thin, and wrinkled leaves, light mosaic (on the rootstock <i>V. ruperstris</i>)	0% in <i>vinifera</i> cultivars; over 20% in the sensitive rootstocks.	Without symptoms in most commercial cultivars (<i>V. vinifera</i>), but dangerous in nurseries.	Walter, 1988. Angelini <i>et al.</i> , 2001. Martelli and Boudon-Padieu, 2006.
Grapevine leaf mottling and deformation/ GPGV	Chlorosis, mosaic, leaf deformation, growth reduction, plant decline; symptoms are present and easier to identify in spring, when new shoots and leaves begin to grow.	Yield losses of up to 80% have been reported in some cases, especially in susceptible cultivars.	Production losses vary depending on the severity of symptoms.	Giampetruzzi <i>et al.</i> , 2012. Maliogka <i>et al.</i> , 2015. Saldarelli <i>et al.</i> , 2015. Bertazzon <i>et al.</i> , 2017. Messmer <i>et al.</i> , 2024.

VIRUS-ELIMINATION METHODS

Strategies to promote virus-free propagation material in culture, essential for maintaining the health, productivity and sustainability of vineyards, include the application of viral elimination methods, starting with meristem culture and thermotherapy, to alternative methods such as *in vitro* chemotherapy, electrotherapy, cryotherapy and their combinations.

Meristem /shoot tip culture

Meristem culture is the oldest method used for the eradication of plant viruses (Faccioli and Marani, 1998). Due to the uneven distribution of viruses in the plant, it is assumed that the meristem is free of viruses (Quak, 1987). The mechanism of virus eradication by meristematic culture has not been fully elucidated, but various hypotheses are accepted, such as: the absence of plasmodesmata at the meristematic cell level, competition between the synthesis of nucleoproteins necessary for cell division and viral replication or the intervention of inhibitory substances.

For Mellor and Stace-Smith (1977), viral replication requires the presence of enzymes normally available in cells close to the meristematic dome. The excision of the meristem causes a trauma that renders these enzymes temporarily unavailable and therefore viral replication is interrupted, the viral RNA is degraded and it is integrated into the cell. Thus, the smaller the explant is, the more the chance of getting healthy plants increases.

Despite the successes achieved by this method, there are some problems that prevent the use and recommendation of this technique (Altmayer, 1989; Hatjinikolakis and Roubelakis – Angelakis, 1993).

One of the major problems is the difficulty of isolating the meristem of the smallest possible size (0.1 - 0.3 mm) followed by the multiple requirements necessary to ensure the viability of the explant and the triggering of regeneration processes: the appearance of adventive buds at the base of the pseudo-petiole of the phylloid formation, the formation of shoot primordia from the adventive buds, the multiplication itself.

Most of the time, apical meristems are free of viruses, but some viruses are known to invade the meristematic region of the growth tips, in which case healthy plants cannot be regenerated, requiring the combination of several virus-elimination methods (Manu and Manju, 2018).

Increasing the size of the explant cultured on aseptic medium determined the maintenance of viability, with satisfactory results regarding viral elimination (Saygıç and Önder, 2021).

Thermotherapy

The thermotherapy is based on the reduction of the virus multiplication rate under the influence of high temperatures, 35-45°C. Heat-treated plants can produce virus-free tissues and the lack of vascular elements in the meristem makes it difficult for viral particles to be transported to the meristematic dome. These tissues, if taken and cultured *in vitro*, can generate new plants identical to the mother plant.

The combination of tissue culture with thermotherapy led to the elimination of viruses from various grapevine species (Stellmach, 1980; Barlass *et al.*, 1982; Goussard *et al.*, 1991; Savino *et al.*, 1990, Buciumeanu and Vişoiu, 1996).

The results of research conducted by Hatzinikolakis and Roubelakis – Angelakis (1993) demonstrated that high percentages of healthy plants can also be obtained through *in vitro* thermotherapy of explants formed from a fragment of a node.

In vitro thermotherapy of small explants (apices of 5 mm) led to their necrosis after 8 weeks (Leonhardt *et al.*, 1998). Thus, for the elimination of viruses by *in vitro* thermotherapy, the authors recommended rooted microshoots; 2 months after thermotherapy, almost all clones induced in the experiment were virus-free.

Rooted grapevine plantlets grown on hormone-free medium were subjected at temperatures of 34°C, from 34 to 115 days in growth chambers. At the end of the thermotherapy, approximately 2 mm apices and nearby 2-3 axillary buds were excised and cultured on multiplication medium. *Grapevine rupestris stem pitting-associated virus* (GRSPaV) elimination in a proportion of 9.1% was achieved by this method. The percentage is low compared to other recovery techniques for the same virus: 28.9% by meristem culture, 23.6% by *in vivo* thermotherapy, 100% by somatic embryogenesis (Gribaudo *et al.*, 2006).

In vitro thermotherapy applied to some rootstock genotypes infected with ArMV, GLRaV-3 and GFLV provided satisfactory results (Křižan, 2008).

Elimination of GFLV in grapevine rootstocks was achieved by *in vivo* and *in vitro* thermotherapy in parallel, at 37°C for 45 days. *In vivo* thermotherapy gave better results although it is more laborious (Křižan, 2009).

In vivo thermotherapy is a method that requires large, energy-consuming equipment, the time required to regenerate new plants is long (only thermotherapy is applied up to 120 days) and the tolerance of genotypes to high temperatures is different. Some of the shortcomings of this method have been overcome by applying the thermic treatment to *in vitro* grown plantlets.

***In vitro* chemotherapy**

Initially used against human viruses, the action of viricides has also been transferred to the plant world. In order to block viral multiplication in cultivated plants, viricides have been either inoculated/injected (de Fazio *et al.*, 1978), sprayed on plants (Cheplick and Agrios, 1983), or introduced into the culture medium in the micropropagation process (Bittner *et al.*, 1989).

Antivirals have been used in the *in vitro* culture of plants as therapeutic supplements in integrated control programs targeting plant viral diseases. The most frequently studied compounds are antimetabolites, substances able to block the viral nucleic acid synthesis. These compounds, natural or synthetic, have antiviral effects, but none of them has a satisfactory selective action that would allow their use in specific prophylaxis and large-scale therapy of viral plant diseases (Nascimento *et al.*, 2003).

Among the antivirals used to eliminate various types of viruses in horticultural plants, ribavirin was the most commonly used, followed by 5-azacytidine (AZA), acycloguanosine, azidothymidine, 2,4-dioxohexahydro-1,2,5-triazine (DHT), dihydroxypropyladenine (DHAP), oseltamivir, mycophenolic acid (6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methyl-hex-enoic acid) (MPA), selenazole (2-β-D-ribofuranosylselenazole-4-carboxamide) (SE), 3-deazauridine (DZD), cyanoguanidine, etc.

The streamline of the virus-elimination method by *in vitro* chemotherapy determined the correlation of treatment parameters (viricide concentration, treatment time) with the phytotoxic effect induced by the viricide on *in vitro* cultivated explants and the regeneration rate of new virus-free plants. Increasing the concentration of ribavirin in the culture medium determined a dwarfing effect on the plantlets and a decrease of the multiplication rate compared to oseltamivir (Guță *et al.*, 2009a,b).

The concentration of 30 mg/L ribavirin did not lead to the regeneration of any GLRaV-1+3-free plants in the Rânai Magaraci genotype, while the same concentration of oseltamivir determined a viral elimination rate of 71.42% after 30 days of treatment (Guță *et al.*, 2010). Testing of various concentrations of ribavirin and oseltamivir highlighted the lack of a correlation between the viricide concentration, the treatment time, and the viral elimination rate (Guță and Buciumeanu, 2011).

The need to improve the efficiency of virus-elimination methods led to the concomitant use of the viricides ribavirin and oseltamivir. GFKV was eliminated from both simple infections and viral complexes, the diagnosis being confirmed both by ELISA and RT-PCR (Guță *et al.*, 2014; 2017a). Similar results were not obtained in the case of GLRaV-1 and GFLV elimination, the rates being quite low, 13.3 and 6.8%, respectively (Guță *et al.*, 2017b). Increasing the viricides concentration to 60 mg/L ribavirin and 120 mg/L oseltamivir, used both individually and in combination, demonstrated once again the different behaviour of viral infections in grapevines to the application of virus-elimination methods. Thus, GFKV was completely removed with the viricide mixture only, while the best elimination rate for GLRaV-1 was recorded with oseltamivir (Guță *et al.*, 2017a).

In vitro chemotherapy, as a viral elimination method in grapevines, was also studied to maintain varietal identity in virus-free regenerated plants. RAPD analysis did not identify differences between plants subjected to chemotherapy treatments and healthy regenerated ones (Guță *et al.*, 2010).

The advantage of this method consists in the use of relatively large inocula (apices and nodal fragments), the success being overcoming the phytotoxic effect induced by the chemotherapeutic substance correlated with the regeneration of virus-free plants.

Electrotherapy

The use of electric current in the elimination of viral diseases in plants is known, although in-depth knowledge of these phenomena is not available. The description of the real causes of nucleoprotein inactivation will contribute to the optimization of future experiments aimed at streamlining the methods for the sanitation of different plant species. The energy dissipated in the form of heat depends on the specific treatment applied and on the properties of the plant tissues and the viral nucleoprotein.

Electrotherapy acts as a thermotherapy at the cellular level with controlled temperature at which the inactivation of the viral nucleoprotein occurs (Gonzales *et al.*, 2006).

Electrotherapy was effective in eliminating *Dasheen mosaic virus* (DMV) that affects 95% of *Xanthosoma sagittifolium* crops in Cuba (Igarza Castro *et al.*, 2001). Electrical treatment led to the regeneration of banana plants free of *Cucumber Mosaic Virus* (Helliot *et al.*, 2001).

The first studies on viral elimination in grapevines by electrotherapy reported a decrease in ELISA readings upon treatment of GFLV-infected plants with 1V/cm, with increasing treatment time (Burger, 1989).

The electric field applied to grapevine plants infected with GLRaV-3 led to high elimination rates (57.1-100%), without direct correlations between the exposure period and the efficiency of the treatment (Guță *et al.*, 2008 b,c).

The use of alternating electric current followed by *in vitro* culture was investigated as an alternative virus-elimination technique in grapevines with single or mixed infections. Electric current of 1000 and 10,000 kHz was applied to the ends of herbaceous grapevine cuttings; the treated axillary buds went through the micropropagation stages. ELISA results on regenerated and acclimatized plants showed encouraging results regarding the elimination of GLRaV-1 and -3 (12.5% elimination at 100 kHz and 4.5% at 10,000 kHz, respectively, after 10 min of treatment). The results were not reproducible in the case of GLRaV-1 for elimination from plants with mixed infections. Satisfactory results were not obtained regarding the elimination of GFLV and GFKV (Guță *et al.*, 2011; Guță and Buciumeanu, 2012). Electrotherapy in the horizontal electrophoresis tank led to satisfactory results for all viruses studied, GFKV being eliminated in simple infection and 50% in combination with GVA, GFLV and GLRaV-1, recording elimination rates of 50 and 30%, respectively (Guță *et al.*, 2019).

Cryotherapy

Cryotherapy is a relatively new method used to eliminate pathogens from infected plant tissue based on cryopreservation techniques.

The main methods employed in cryotherapy are encapsulation-dehydration, vitrification, encapsulation-vitrification and droplet vitrification, which are based on the immersion of preconditioned shoot tips in liquid nitrogen, followed by their recovery *in vitro* onto culture media for regeneration of healthy plantlets.

High frequency of virus-free plants among regenerants within a short time frame is the most desirable aspect of cryotherapy. Therefore, these advantages make the technique a promising tool for institutions mandated to the development of high-health planting materials with high genetic and agronomic potential for viticulture (Bettoni *et al.*, 2016).

The reported results show the dependence of the virus-elimination rate on the genotype, viral infection, and treatment method (Table 2).

Table 2. Efficiency of grapevine virus elimination by different methods

Virus/Viral complex	Species/cultivar	Efficiency (%)	References
Meristem culture			
GFLV	<i>V. vinifera</i> 'Merlot', 'Trebiana Toscana', and an unknown cultivar- JI	54	Barba <i>et al.</i> , 1992
	<i>V. vinifera</i> , 3 clones of the 'Refošk' cultivar	60	Koruza and Jelaska, 1993
	<i>V. vinifera</i> 'Thompson seedless'	85	Youssef <i>et al.</i> , 2009
	<i>V. vinifera</i> 'Flame seedless'	75	Fayek <i>et al.</i> , 2009
	<i>V. vinifera</i>	100	Miljanić <i>et al.</i> , 2022
GLRaV-1	<i>V. vinifera</i> 'Merlot', 'Trebiana Toscana', and an unknown cultivar - JI	73-93	Barba <i>et al.</i> , 1992
	<i>V. vinifera</i> , 3 clones of 'Refošk' cultivar	100	Koruza and Jelaska 1993
	<i>V. vinifera</i> 'Thompson seedless'	87.5	Youssef <i>et al.</i> , 2009
	<i>V. vinifera</i> 'Flame seedless'	82.5	Fayek <i>et al.</i> , 2009
GLRaV-3	<i>V. vinifera</i> 'Merlot', 'Trebiana Toscana' and an unknown cultivar- JI	73-93	Barba <i>et al.</i> , 1992
	<i>V. vinifera</i> 3 clones of 'Refošk' variety	100	Koruza and Jelaska, 1993
	<i>V. vinifera</i>	100	Miljanić <i>et al.</i> , 2022
GRSPaV	<i>V. vinifera</i> "Albarola"	14.2	Gribaudo <i>et al.</i> , 2006
	<i>V. vinifera</i> 'Bosco'	72.7	
	<i>V. vinifera</i>	100	Miljanić <i>et al.</i> , 2022
GFkV	<i>V. vinifera</i>	100	Miljanić <i>et al.</i> , 2022
GFLV+GFkV	<i>V. vinifera</i>	60 – 84.2	Vișoiu and Teodorescu, 2000
GLRaV-1+3	<i>V. vinifera</i>	83 – 9	
GPGV+GFkV	<i>V. vinifera</i> 'Negru mare'	90.9	Guță <i>et al.</i> , 2025
	<i>V. vinifera</i> 'Sangiovese'	98.4	
Shoot tip culture			
GLRaV-3	<i>V. vinifera</i>	100	Bi <i>et al.</i> , 2018
	<i>V. vinifera</i> × <i>V. labrusca</i>	100	
	<i>V. pseudoreticulata</i>	100	
GPGV	<i>V. vinifera</i>	100	Rieger, 2016
GPGV+GFkV	<i>V. vinifera</i> 'Negru mare'	62.5	Guță <i>et al.</i> , 2025.
		100	
	<i>V. vinifera</i> 'Sangiovese'	70	
Thermotherapy			
GLRaV-1	<i>V. vinifera</i> 'Rauschling'	100	Staudt and Kassemeyer, 1994
GVA	<i>V. vinifera</i>	60	Panattoni <i>et al.</i> , 2007
	<i>V. berlandieri</i> × <i>V. riparia</i> , 'Kober 5BB'	70.2	Panattoni and Triolo, 2010
GPGV + GFkV	<i>V. vinifera</i> 'Negru mare'	56.5	Guță <i>et al.</i> , 2025.
	<i>V. vinifera</i> 'Sangiovese'	100	
GFkV	<i>V. champini</i>	57.1	Hu <i>et al.</i> , 2021
	<i>V. berlandieri</i> × <i>V. riparia</i> 'Kober 5BB'	0	Panattoni and Triolo, 2010
	<i>V. vinifera</i>	100	Miljanić <i>et al.</i> , 2022
GLRaV-3	<i>V. champini</i>	42.9	Hu <i>et al.</i> , 2020
	<i>V. berlandieri</i> × <i>V. riparia</i> 'Kober 5BB'	24.7	Panattoni and Triolo, 2010
	<i>V. vinifera</i>	100	Miljanić <i>et al.</i> , 2022
GRSPaV	<i>V. vinifera</i> 'Mantilaria'	39.62	Maliogka <i>et al.</i> , 2009
	<i>V. vinifera</i> 'Prevezaniko'	92.85	
	<i>V. vinifera</i>	100	Miljanić <i>et al.</i> , 2022
GFLV	<i>V. vinifera</i>	100	Miljanić <i>et al.</i> , 2022

Virus/Viral complex	Species/cultivar	Efficiency (%)	References
In vitro chemotherapy			
GFkV	V. champini	79,5	Hu et al, 2021
	V. vinifera	100	Guță et al., 2008a
	V. vinifera	100	Komínek et al., 2016
	V. vinifera, 'Regina viilor'	100	Guță and Buciumeanu, 2021
GRSPaV	V. champini	13.6	Hu et al.,2021
GLRaV-1 + 3	V. vinifera	78.6	Guță et al., 2008a
GVA	V. vinifera	0	Guță et al., 2008a
GLRaV-1+GVA	V. vinifera 'Servant'	11	Guță and Buciumeanu, 2010; 2011
GPGV	V. vinifera	66.7	Komínek et al., 2016
		86.7	
		100	
GPGV+GFkV	V. vinifera, 'Negru mare'	100	Guță et al., 2025
	V. vinifera, 'Sangiovese'	100	
GPGV+GFkV	V. vinifera 'Pinot Gris 14 Șt.'	86.9	Guță and Buciumeanu, 2021
	V. vinifera 'Merlot 146 Mn.'	75	
	V. vinifera 'Balaban alb'	100	
Electrotherapy			
GVA	V. vinifera	40	Bayati et al., 2011
GLRaV-3	V. vinifera	57.1-100	Guță et al., 2008b,c
GPGV+GFkV	V. vinifera, 'Sangiovese'	100	Guță et al., 2025.
Cryotherapy			
GVA	V. vinifera	42	Bayati et al., 2011
	V. vinifera 'Bruti'	97-100	Wang et al., 2003
GLRaV-3	V. vinifera 'Cabernet Sauvignon'	100	Marković et al., 2015
GFLV	V. vinifera 'Chardonnay'	77.8	Marković et al., 2015
Combined methods: in vitro chemotherapy with thermotherapy			
GFkV	V. champini	100	Hu et al., 2021
GLRaV-3	V. champini	42.9	Hu et al., 2020
GRSPaV	V. champini	100	Hu et al., 2021
Combined methods: cryotherapy with shoot tip culture			
GLRaV-3	V. vinifera	100	Bi et al., 2018
	V. vinifera × V. labrusca	100	
	V. pseudoreticulata	100	

A study on the analytical hierarchy (Fig. 1) of the virus-elimination methods (meristem culture, intensely regenerative apex culture, *in vitro* thermotherapy, electrotherapy in uniform electric field, electrotherapy in horizontal electrophoresis tank), applied for elimination of GPGV+GFkV - led to the establishment of *in vitro* chemotherapy as the most efficient method in terms of the viral elimination rate, corroborated with the consumption of materials, energy and time required, until the regeneration of healthy plants (Guță *et al.*, 2025).

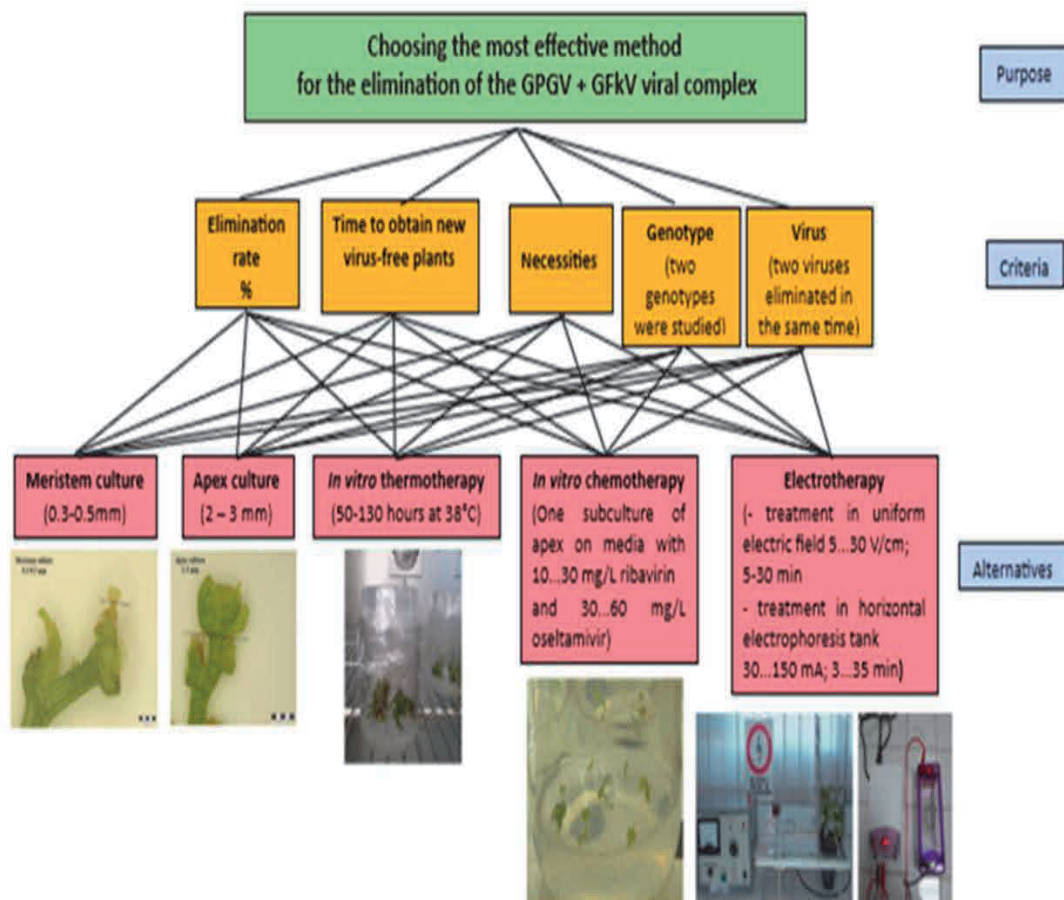


Figure 1. Analytic Hierarchy Process for GPGV + GFKVviruses elimination

CONCLUSIONS

Overall, grapevine viral infections constitute a complex problem, with major implications for the productivity, quality and sustainability of modern viticulture. The effects of these diseases, often cumulative and difficult to control, highlight the need for an integrated management that includes early diagnosis, the use of certified planting material, and the application of biotechnological methods of virus elimination. Progress registered in *in vitro* cultures, thermotherapy and chemotherapy techniques made it possible to obtain and preserve virus-free clones, essential for maintaining the genetic identity and economic value of cultivars. Therefore, the development and expansion of virus-free germplasm collections is not only a scientific necessity but also a fundamental condition for a sustainable viticulture capable of responding to current phytosanitary and economic challenges

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