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# MANAGEMENT OF GRAPEVINE VIRAL DISEASES

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KEYWORDS

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ABSTRACT

Plant viruses represent the most damaging pathogens causing production losses and endangering the survival of infected plants. In grapevine, one of the most valuable horticultural crop in the world, one hundred and one viruses have been identified so far, being the plant where the most viral entities are known. Of these, grapevine is severely affected by viruses belonging to four major disease complexes: leafroll, rugose wood, infectious degeneration and decline, and fleck. The viruses associated with these diseases are transmitted by mealybugs, insects, or nematodes. In this review, several methods of grapevine viruses and virus diseases management have been presented, such as: virus identification, producing the virus-free propagating material, vector monitoring, rouging the diseased grapevines, using resistant and transgenic plants.

## INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the most valuable horticultural plant in the temperate climate on the strength to its economic and social impact. The areas covered with grapevine all over the world are more than 7 million hectares. The area cultivated with grapevine in Romania is of 191,000 hectares, occupying the tenth place in the world (https://www.onvpv.ro/ro/content/date-statistice-oiv), and it is organized into eight regions, as follows: Transylvanian plateau, Hills of Moldova, Hills of Muntenia and Oltenia, Hills of Banat, Hills of Crisana and Maramures, Hills of Dobrogea, Danube Terraces, and Sands and other favourable lands of the south of the country (Order no. 1205/2018).
As a vegetatively propagated plant, grapevine is exposed to the action of many pests and pathogens. Among them, intracellular infectious agents (viruses, viroids, phytoplasmas) have a considerable negative involvement, causing major production losses, shortening the productive life of vineyards, and endangering the survival of affected plants (Martelli et al., 2006; Cuozzo et al., 2018).
Until now, 101 viruses are known in grapevine, being the plant in which the most viral entities have been identified. These viruses have single- or double-stranded RNA or DNA genomes and are classified into 21 families or, some of them are not yet taxonomically classified (Fuchs, 2023). Of these, about a third cause significant economic damage worldwide are associated with four major disease complexes: leafroll (5 viruses), wood rugose (6 viruses), infectious degeneration and decline (16 viruses), and fleck or marbrure (4 viruses) (Martelli, 2017; 2018). Viruses associated with these diseases are transmitted by mealybugs, insects, or nematodes (Maliogka et al., 2015).
Due to the diseases produced by the presence of *Grapevine fanleaf virus* (GFLV), *Arabis mosaic virus* (ArMV), *Grapevine leafroll associated viruses type 1 and 3* (GLRaV-1, GLRaV-3), *Grapevine fleck virus* (GFkV), *Grapevine virus A* - GVA) in simple infections or in combination, are the most damaging grapevine viruses. Other viruses have a broad spectrum, infecting several important crop plants (*Italian artichoke latent virus*, *Alfalfa mosaic virus*, *Broad bean wilt virus*, *Potato virus X*). For the grapevine they are scientific curiosities, both because they are rarely found and the losses caused are negligible (Martelli, 1997).
As an effect of diseases produced in the presence of viruses, it must be taken into account that some viruses induce definite symptoms at the time when the selection is done, while others express the symptoms later or remain in a latent state, being influenced by multiple factors, suchs as: the agro-climatic conditions, scion-rootstock combination, virus-host combination, and not in the last place, the plant senescence (Pesqueira et al., 2012).

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According to the technical annex of Directive 68/93/EEC, grapevine propagating material must be free by ArMV, GFLV, GLRaV-1, GLRaV-3, and GFkV. The International Council for the Study of Viruses and Viral Diseases of Grapevine - ICVG in 2003 recommended the screening of propagating material for agents associated with grapevine infectious degeneration and decline (nepoviruses), leafroll disease (serotypes 1, 2, 3), rugose wood (grapevine viruses A, B, D), and phytoplasmas (mainly flavescence dorée, bois noir) (https://icvg.org/data/recomm.pdf). Two additional grapevine leafroll-associated viruses (GLRaV-4 and GLRaV-7) were added in the ICVG - 2012 recommendations. It has been specified that in the future rapidly advancing diagnostic technologies will make it possible to include other viruses that cause diseases in certified planting material (https://icvg.org/data/ICVG-2013-Recommendation-Final-after-Sterring-Commitee-Approval.pdf).

In 2003, in the Trentino region of Italy, a new disease was observed in grapevine plants, that caused leaf spotting and deformation, and a decrease of plant vigor. This was called Grapevine Leaf Mottling and Deformation - GLMD. Infected plants showed a reduced number of shoots and clusters, with a significant decrease of cluster weight (Malossini et al., 2015). Subsequently, in 2012, a new virus was discovered in this region, called *Grapevine Pinot gris virus* (GPGV), after the variety in which it was found (Giampetruzzi et al., 2012). In 2019, the causal role of GPGV in GLMD symptomatology was established (Tarquini et al., 2019). In a short time, it was proven that GPGV has a wide spread, on all continents (Saldarelli et al., 2015). GPGV is not listed in the frame of regulations of grapevine propagation materials production, whose testing is recommended (Saldarelli et al., 2017), but due to its widespread incidence and association in complexes with other aggressive viruses, it should be included in the grapevine certification schemes (Guta and Buciumeanu, 2021).

In 2014, a survey of viruses (GFLV+ArMV, GLRaV-1+3, GFkV) was carried out in the Hills of Muntenia and Oltenia region, targeting Romanian grapevine varieties, in vineyards of different ages. Virological analyses have been done by DAS-ELISA (Double antibody sandwich – Enzyme- linked immunosorbent assay)(Clark and Adams, 1977), with commercial reagent kits (Bioreba, Switzerland). All analyzed viruses were present, complex infections being recorded in all varieties and plantations, with an infection level between 11.9% and 55.8%. In the new plantations, the viral infection level was higher than in the old ones, as a consequence of the propagation material circulation without severe phytosanitary control (Buciumeanu et al., 2015). During 2019-2020, 199 samples were analysed by the same method for the presence of GPGV, GFLV, GLRaV-1+3, and GFkV. Among them, GPGV has been detected in 53.76%, in simple or mixed infections with GFkV or GLRaV-1+3 (Guta et al., 2021).

The spread of viruses occurs directly or indirectly to human activity. Long-distance transport of plant material and virus vectors, and vegetative propagation are the most efficient ways of virus dissemination.

## MATERIALS AND METHODS

Since diseases caused by viruses cannot be treated in the plantation, various management techniques must be adopted to prevent and control the spread of viruses.

In this review, strategies for the management of grapevine viruses are briefly presented: virus detection (Joo-Jin et al., 2014), using healthy propagating material (Order no. 1267/2005), vector monitoring (Fuchs, 2020), rouging the symptomatic grapevines (Oliver and Fuchs, 2011), and using resistant plants and transgenic plants (Laimer et al., 2009).

## RESULTS AND DISCUSSION

### Virus detection

The most important action in managing a virus disease is the accurate identification of the viral entity. Diagnostic methods should be fast, accurate and inexpensive (Joo-Jin et al., 2014). Current diagnostic techniques are divided into four groups, in function of the properties of the virus on which it is based: biological activity, physical properties of the viral particle, properties of the capsid protein, properties of the viral nucleic acid (Koenig et al., 2008).

The first diagnostic methods were based on biological properties related to the interaction of the virus with its host (visual observation), and physical properties determined by its size and shape (electron microscopy investigations). Unfortunately, all of them are time consuming and not feasible in a high number.

Using the viral protein properties, Clark and Adams (1977) demonstrated the efficiency of serological method DAS-ELISA to quantify the virus concentration in different plant tissues.

A multidisciplinary review on plant virus diagnostic methods mentioned that molecular analyzes provide the most accurate diagnosis, although these tests are expensive, time-consuming, require advanced specialization, and are labor-intensive to process a large number of samples (Wang et al., 2022).

Continuous scientifical and technological innovations have led to the development and application of new analysis methods in the plant virus diagnosis field. High sensitivity and specificity are requested for a reliable plant virus detection. Combinations of multiple techniques are preferred for effective detection, in particular *in situ* (Joo-Jin et al., 2014).

Early diagnosis is an important part of management and control strategy of virus diseases in different crop species, giving the chance to growers to take fast and efficient phytosanitary measures (such as removing infected plants, restricting the movement of agricultural machinery, the control of the vector population), to limit the spread of viruses/viral diseases and offer better certification procedures of propagating material before germplasm import (Giampetruzzi et al., 2012; Wallingford et al., 2015). Some common diagnostic tools have been used to follow and detect viruses, such as: serological (Borges et al., 2020), based on nucleic acid amplification (Rowhani et al., 2017), microarray (a grid of DNA segments of known sequence that is used to test and map DNA fragments, antibodies or proteins) (Engel et al., 2010), and multispectral (Mahlein, 2016) or hyperspectral imaging methods (Mishra et al., 2017; Nguyen et al., 2020; Wang et al., 2023). Spectral sensors allow the non-invasive assessment of plant characters, they are well suited to follow dynamic processes, such symptom development.
In recent years, techniques such as the lateral flow immunoassay, methods for the detection of several viruses in a single test (e.g., multiplex-PCR) and cutting-edge technologies suitable for the discovery of new viruses (e.g., next generation sequencing) were developed. Compared to the traditional methods (visual assessment, biological indexing), laboratory methods (serological, molecular) having the advantage of increased safety, offer an early detection of viral disease, but they are expensive, involving specific reagents and equipment, and cannot be used on a large scale. In recent years, spectral sensors have proven to be a promising tool for disease diagnosis, being independent from genetic and phenotypic information about pathogens (Mahlein, 2016).

**Obtaining the healthy propagating material**

Once virus infected, the grapevine remains infected for its entire life. Prevention of infection includes production of certified plant material and viral testing. Therefore, the propagation of healthy vegetative material (including cuttings, grafts, buds, rooted cuttings, and grafted plants), is decisive.
The initial material category is the vegetative propagation material of grapevine "consisting of virus diseases free clones recognized by the regulations in force, planted on sanitary families" (Decision no. 512/2016).
The grapevine mother plants belonging to the initial propagation material category, intended for Base propagation material production, are analyzed virologically at least once every 6 years starting from the third year after the establishment of the mother plantation, according to Order no. 1267/2005. Mother plants destined to Base propagation material production must be free of viruses as follows: GFLV and ArMV (infectious degeneration complex); GLRaV-1 and GLRaV-3 (leafroll disease); GFkV (fleck/marbrure, with the specification that GFkV testing must be done for rootstocks only). According to Annex no. 5 (List of specific harmful organisms affecting the propagation material quality) from Order no. 1267/2005, GVA testing (which causes stem grooving), is applied optionally, upon the maintainer or multiplier request.
Identification and production of negative tested grapevines is a preventive measure that can be combined, when it is necessary, with viral sanitation methods (Golino et al. 2017).
Methods of virus elimination in plant are grouped like this: thermotherapy (that includes cryotherapy and meristem culture associated techniques), chemotherapy and tissue culture (including somatic embryogenesis) (Panattoni et al., 2013), electrotherapy (Burger, 1987), and their combinations (Hu et al., 2020). The success of virus elimination method depends on the grapevine genotype, the virus species, the virus - plant interactions, and the treatment conditions (Maliogka et al., 2009; Guta et al., 2017; Miljanić et al., 2022). However, the main disadvantages of electro- and cryotherapy are their low percent of virus elimination, and the possible induction of genetic modification in the regenerated plant (Baranek et al., 2009).
A high efficiency of virus-free grapevine regeneration was achieved by thermo- or chemotherapy associated with meristem and shoot tip culture (Buciumeanu et al., 2000; Weilland et al., 2004; Basso et al., 2017).

***Vector monitoring***
Grapevine viruses are spread by various vectors (Table 1).
The production and use of certified virus tested propagative material reduce the virus spreading, mainly in zones where vectors are present (Martelli, 2014).
Due to their large distribution in soil, nematodes are known as the most difficult crop pests to manage. Soil fumigation (chemical method) used against *X. index* proved to be ineffective due to nematodes resistance, their widespread distribution in soil, and movement in depth (Lear et al., 1981). The use of intercropping, and the bacterial and fungal preparations represent a potential that can be taken into account for nematode control (Polanšek et al., 2023). Some of the control methods known against nematodes and mealybugs vectors have not yet been evaluated in grapevine plantations (Maliogka et al., 2015). Establishing new vineyards in nematodes-free areas reduces local and long-distance virus spreading viruses (Laimer et al., 2009; Villate et al., 2008).
Mite control strategies include spraying programs that can stop the spread of GPGV. How mite population density and management influence the efficiency of GPGV transmission are not yet well understood (Constable et al., 2019).

**Table 1. Vectors of the main grapevine viruses (adapted after Martelli et al., 2006; Fuchs, 2020; Malagnini et al., 2016)**

| Virus | Disease produced | Vector |
|---|---|---|
| GFLV | Fanleaf | Nematodes: *Xiphinema index* |
| ArMV | | Nematodes: *X. diversicaudatum* |
| GLRaV-1 | Leafroll | Mealybugs: *Heliococcus bohemicus*, *Phenacoccus aceris*<br>Soft scale insects: *Pulvinaria vitis, Parthenolecanium corni, Neopulvinaria innumerabilis* |
| GLRaV-3 | | Mealybugs: *Planococcus ficus, Pl. citri, Pseudococcus longispinus, Ps. calceolariae, Ps. maritimus, Ps. affinis, Ps. viburni, Ps. comstocki*<br>Soft scale insects: *Pulvinaria vitis, Neopulvinaria innumerabilis* |
| GFkV | Fleck | Unknown |
| GVA | Rugose wood | Mealybugs: *Planococcus ficus, Pl. Citri, Pseudococcus longispinus, Ps. affinis, Heliococcus bohemicus*<br>Soft scale insects: *Neopulvinaria innumerabilis* |
| GPGV | Mottling/deformation | Eriophyid mite: *Colomerus vitis* |

***Rouging the symptomatic grapevines***

Rouging the symptomatic grapevines, and removing any remaining roots and the plants in the neighborhood are possible strategies for viral disease management (Oliver and Fuchs, 2011).

*X. index* can survive in soils and retain GFLV for many years with or without host plants (Demangeat et al., 2005). In the case of grapevine plantations establishment on areas previously cultivated with grapevine without a rest of 3-5 years after clearing the crop, special treatments against nematodes must be applied (Raski et al., 1983). Atallah et al. (2011) reported that grapevine leafroll-associated viruses can cause damage of tens of thousands of $/ha, without being counted the costs of removing the plantation, leaving the soil out of crop for a few years.

***Resistant plants and transgenic plants***

In vineyard, alternatives to the use of chemical nematicides with high toxicity, the nematode-resistant rootstocks (Esmenjaud et al., 2011), and plants having an antagonistic effect on *X. index* (Villate et al., 2012), are promising leads for nematode and virus control.

Resistance to *X. index* has been identified in several *Vitis* species including *V. arizonica*, *V. candicans*, *V. rufotomentosa*, V. *smalliana*, and V. *solonis* (Kunde et al. 1968). *Muscadinia rotundifolia* have been infected with GFLV by grafting but was resistant to virus infection by *X. index* feeding (Bouquet, 1981). Grapevine resistance to the *X. index* was durable in Muscadine-derived plants obtained from woody cuttings but not from *in vitro*, where the increased nematode multiplication might be mainly due to the modification of root architecture in the micropropagation method (Nyugen et al., 2020). Resistance to GFLV infection can appear as a hypersensitive reaction to nematode feeding (Staudt et al., 1992).

Also, transgenic plants may represent a possible choice for nematode control (Laimer et al., 2009; Maliogka et al., 2015). Production of transgenic plants is the ability to regenerate plants from transformed tissues (Altpeter et al., 2016). It has been possible to obtain grapevine varieties resistant to fungal (Nirala et al., 2010), viral (Mauro et al., 1995), and bacterial diseases (Dandekar et al., 2019).

The insertion of virus capsid protein through *Agrobacterium*-mediated transformation has been used to increase the resistance to GFLV in grapevine, both vinifera and rootstocks (Gambino et al., 2005, Tsvetkov et al., 2000).

## CONCLUSION

Because the diseases caused by viruses cannot be treated in plantation, various management strategies must be followed to prevent and control their spread.

Diagnosis is an important part of the management of grapevine virus diseases, allowing viticulturists to take effective sanitary measures. Identification and production virus-free grapevines are preventive measures that can be combined with virus-elimination procedures, when it is needed. Rouging the symptomatic grapevines, removing any remaining roots and adjacent plants are methods that can be applied for viral diseases management. Against vectors, control strategies include chemical programs that can stop the viruses spread. The use of nematode-resistant rootstocks or transgenic plants are promising directions for nematode vectors and, also virus control in grapevine.

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