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INCIDENCE OF GRAPEVINE FANLEAF VIRUS (GFLV) AND GRAPEVINE LEAFROLL-ASSOCIATED VIRUSES (GLRaV 1−3) IN VOJVODINA PROVINCE

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SUMMARY

During May and June of 2021, a total of 123 grapevine leaf samples were collected and analyzed for infection by grapevine fanleaf virus (GFLV) and three viruses from the grapevine leafroll-associated virus complex -1, -2 and -3 (GRLaV-1, GRLaV-2 and GRLaV-3, respectively). The samples were collected from commercial vineyards, small backyard vineyards and grapevine nurseries located across the entire Vojvodina Province (Bačka, Banat and Srem). OEPP/EPPO sampling protocols were followed during the sampling of leaf tissues showing possible virus infection symptoms. Among the 123 samples, 47 were collected from Bačka region (Bačko Gradište − 11, Bečej − 10, Temerin − 15, Vrbas − 1, Hajdukovo − 10), 50 from Banat region (Srpska Crnja − 10, Vojvode Stepe − 10, Čoka − 10, Uljma −20) and 26 from Srem region (Šid − 6, Banoštor − 10, Sremski Karlovci − 10) and serological ELISA tests were performed for virus detection. GFLV was detected in five samples (4.06%), GLRaV-1 was detected in six samples (4.87%), while GLRaV-2 was not detected in any of the analyzed grapevine samples. GLRaV-3 was present in five samples (4.06%). When infection rates were examined in relation to cultivars, GFLV was detected in Cardinal, Zalagyöngye, Black Muscat, Italian Riesling and Dornfelder. GRLaV-1 was detected in cultivars Cardinal, Black Muscat, Italian Riesling and Merlot, and GRLaV-3 in cultivars Othello and Italian Riesling. Based on these results, it can be concluded that GFLV and GRLaV-1 and GRLaV-3 are present in vineyards across Vojvodina Province and affect different grapevine cultivars. To effectively control virus infections and their spreading, continuous monitoring of these viruses and their vectors is required along with the planting of healthy propagation material.

Key words: *grapevine, viruses, detection*

Abbreviations: ELISA - enzyme linked immunosorbent test; GFLV - grapevine fanleaf virus, GLRaV - grapevine leafroll-associated virus; SMEs - small and medium-sized enterprises

INTRODUCTION

There is a very long tradition of grapevine production in Vojvodina Province, dating back to vineyards cultivated by Celts in the 3rd century (Boros, 2006). During the ruling of King Mathias in the 15th century, the production, reputation and importance of wines originating from Srem region had especially increased (Vécsey, 2013). According to the Chamber of Commerce and Industry of Vojvodina (2022), vineyards cover about 4600 ha of the province territory, and secure regular income for a number of families and small and medium-sized enterprises (SMEs). Besides its economic importance, grapevine production in this region has important traditional and even cultural aspects, since many rural and urban manifestations, festivals and gatherings are closely connected to grape

harvest or wine competitions. Such manifestations are regularly held in villages like Temerin, Palić, Feketić, Horgoš, Gudurica, Rivica and Bajmok, but also in cities like Novi Sad, and have beneficial sociological and economic effects on local communities.

Successful grapevine production requires a number of agro-climatic factors, effective plant health control in particular, which includes adequate protection against plant pathogens. Besides fungal, bacterial, chromista and phytoplasma diseases, diseases caused by viruses can also be very harmful, as they are often not well-known to small producers. According to Martelli (2012), among 60 described grapevine viruses, grapevine fanleaf virus (GFLV) and virus species included in the grapevine leafroll-associated complex (GLRaV) are economically the most important.

Grapevine fanleaf virus belongs to family *Secoviridae* and genus *Nepovirus* (Bagi et al., 2016). It has spherical particles, and its only natural host is grapevine. Due to the exchange of grapevine propagation material, it is present in all parts of the world (Martelli et al., 2001). During vegetation, the virus is transmitted by nematodes, mostly by *Xiphinema index*, but *X. italiae* is nonetheless also an important vector species (Cohn et al., 1970; Van Zyl et al., 2012). As these vectors are characterized by low mobility, disease spread in vineyards is slow, and it is often confined to clearly demarcated patches.

GFLV disease symptoms can be divided into two groups: morphological deformations and chromatic changes (Maliogka et al., 2015). As a result of viral infection and loss of characteristic morphology for a specific cultivar, grapevine leaves change shape, while grapevine sprouts and branches are dwarfed (with shortened growth between nodes) as well as deformed, often forming irregular shapes. Yield from infected plants is significantly lower, with smaller numbers of grapes and uneven ripening. The sugar content of grapes from infected plants is also much lower. Chromatic changes are mostly manifested through leaf yellowing, especially during early stages of vegetation. Production losses can be significant (up to 90%), but are highly dependent on the interaction among a cultivar, virus strain and ecological factors (Bagi et al., 2016).

Species from GLRaV-1 to GLRaV-9 belong to three different genuses, namely *Ampelovirus*, *Closterovirus* and *Velarivirus*. The differentiation of species within the virus complex is usually performed on the basis of vector transmission and molecular characterization (Liu et al., 2013). Several authors have underlined the importance and distribution of GLRaV-3 as the most important member of the complex (Hanna et al., 2008; Bertolini et al., 2010; Jooste et al., 2010). However, according to Fuchs et al. (2009), GLRaV-1 and GLRaV-2 are as economically significant as GLRaV-3. In Serbia, Starović et al. (2008) reported the presence of all three species, but their investigation did not include vineyards from Vojvodina Province. GLRaV-1, 2 and 3 have elongated particles, which are transmitted during vegetation by insect vectors. However, due to well-developed trade, infected plant material can be highly geographically dispersed. In addition to typical symptoms such as leafroll, infected cultivars often also exhibit chromatic changes, which can be manifested as leaf yellowing or reddening depending on the cultivar type. In line with GFLV effects, GLRaV infection can also cause lower sugar content in berries and late or uneven ripening (Apró et al., 2014). Based on proposed OEPP/EPPO certification scheme (2008) GFLV, GLRaV-1 − GLRaV-3 are among the viruses mandatory for testing in grapevine varieties and rootstocks production.

The goal of this research was to determine the incidence of GFLV and GLRaV-1 − GLRaV-3 in vineyards across Vojvodina Province, and thus identify the most prevalent virus species among them. Based on the susceptibility of grapevine cultivars and age of infected plants, our further aim was to propose control measures to vineyard owners to prevent further yield losses.

MATERIAL AND METHODS

Sampling of the plant material

The plant material for this study was collected during May and June (2021) from commercial vineyards, small backyard vineyards and grapevine nurseries. Sampling protocols given by OEPP/EPPO (European and Mediterranean Plant Protection Organization) were followed when sampling leaf tissues showing possible virus infection symptoms (OEPP/EPPO, 2008). Every grapevine plant from which a sample was taken was marked and the grapevine age and cultivar type were recorded. The marking of the plants was important for two reasons: to inform the vineyard owner about the necessity of applying control measures, and to follow the symptom development if the owner did not immediately destroy the infected grapevine plants. The collected leaf samples were stored at +4 °C for one day, after which the serological detection was performed. The data pertaining to the collected samples are shown in Table 1.

Table 1. Grapevine tissue sampling data

Region	No. of collected samples by region	Locality	Cultivar	No. of collected samples by locality	Vineyard age (years)
Bačka	47	Bačko Gradište	Othello	11	30
		Bečej	Italian Riesling	10	25
		Temerin	Zalagyönygye	4	46
			Dornfelder	1	
			Orlovatski muskat	1	
			Peleškei muskat	2	
			Cserszegi fűszeres	2	
			Medina	5	
		Vrbas	Italian Riesling	1	0.5
		Hajdukovo	Cabernet Sauvignon	3	14
			Ezerjó	4	
			Bakator	3	
Banat	50	Srpska Crnja	Cardinal	10	16
		Vojvode Stepe	Cabernet Franc	10	15
		Čoka	Cabernet Sauvignon	3	17
			Merlot	3	
			Cabernet Franc	2	
			Black Muscat	2	
		Uljma	Cabernet Franc	4	8
			Black Burgundy	1	
			Black Muscat	2	
			Muscat Ottonel	2	
			Italian Riesling	11	
Srem	26	Šid	Italian Riesling	6	31
		Banoštor	Italian Riesling	10	20
		Sremski Karlovci	Italian Riesling	10	23

Serological analysis

The grapevine samples were subjected to the DAS ELISA (enzyme-linked immunosorbent assay) serological test using chemicals supplied by Loewe Biochemica GmbH for GFLV, GLRaV-1, GLRaV-2 and GLRaV-3 (Clark & Adams, 1977; Aydin, 2015; Sakamoto et al., 2018). The ELISA procedure and detection of positive samples was carried out as described by Bagi et al. (2021) and shown in Figures 1-4.

Figure 1. Measuring of leaf samples (Photo: G. Barać)

Figure 2. Sample homogenization in the extraction buffer (Photo: G. Barać)

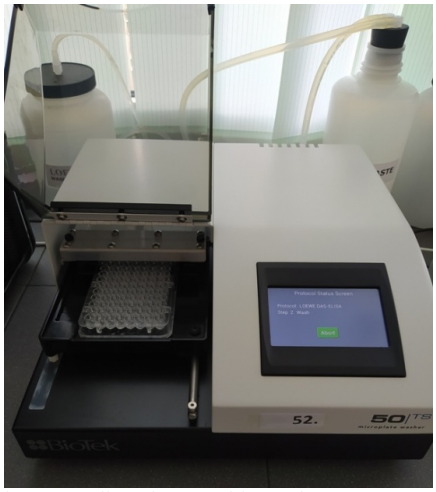

Figure 3. Elisa plate washing (Photo: G. Barać)

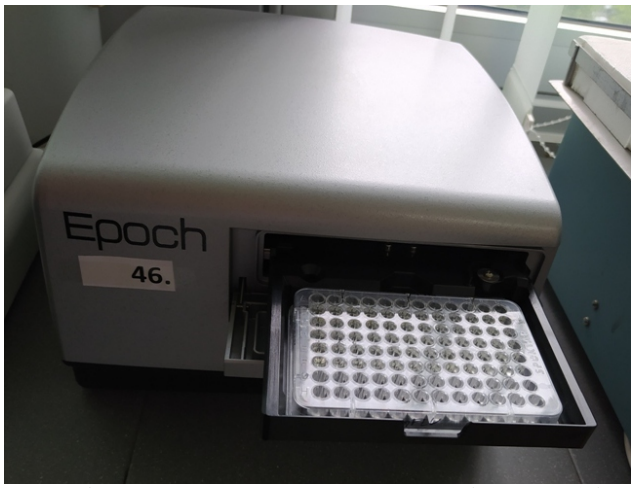

Figure 4. Reading of results by BioTek Epoch type spectrophotometer (Photo: G. Barać)

RESULTS AND DISCUSSION

Analysis of 123 grapevine samples revealed the presence of a virus in 16 samples (13.01%), none of which contained more than one virus. The distribution of locations from which infected samples originated is shown in Figure 5.

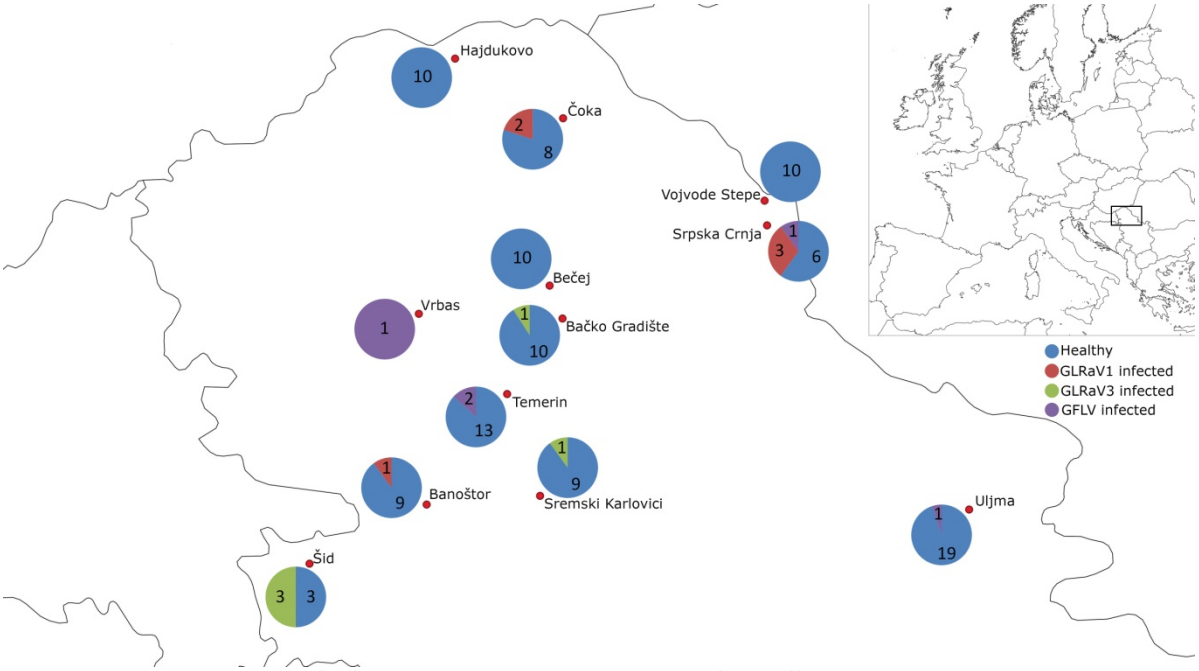

Figure 5. Number of infected and healthy grapevine samples from different geographic locations across Vojvodina

GFLV was detected in five grapevine samples (4.06%), two of which were from Temerin, while the remaining ones were from Srpska Crnja, Vrbas and Uljma (Fig. 6, 7). GLRaV-1 was detected in six samples (4.87%), three of which were from Srpska Crnja, two from Čoka and one from Banoštor (Figure 8). GLRaV-2 was not detected in any of the analyzed grapevine samples, while GLRaV-3 was present in five samples (4.06%), three of which were from Šid and the remaining two from Bačko Gradište and Sremski Karlovci.

Figure 6. GFLV symptoms at the beginning of vegetation season at Temerin locality (Photo: F. Bagi)

Figure 7. GFLV symptoms during the second part of vegetation season at Uljma locality (Photo: Z. Savić)

Figure 8. GLRaV-1 symptoms at Čoka locality (Photo: Đ. Konstantin)

Among the 47 samples originating from Bačka region, four (8.5%) were positive for one of the four analyzed viruses: three were infected by GFLV and one by GLRaV-3. In Banat region, the disease incidence was higher, since seven (14%) of the 50 analyzed samples proved to be infected, two of which were infected by GFLV and the remaining five by GLRaV-1. Even higher disease incidence was detected in Srem region, as five (19.2%) of the 26 analyzed samples were infected, four with GLRaV-3 and one with GLRaV-1. Although a greater number of samples would assure more reliable conclusions about the virus incidence in different geographical parts of Vojvodina, it is

clear that the regions with higher grapevine production are at a greater risk of virus infection. Srem is the most important grapevine production area in Vojvodina, due to which propagation material is more frequently exchanged, resulting in a greater likelihood of vector transmission and virus infection.

These results confirm the importance of GFLV, GLRaV-1 and GLRaV-3 (Starović et al., 2008; Apró et al., 2014; Hančević et al., 2021). Apró et al. (2014) analyzed 543 samples from Hungarian vineyards during a 7-year period and detected GFLV in only five samples, which is much lower disease incidence compared to our research. With respect to grapevine cultivar infection and age of the infected plants, GFLV was detected in Cardinal (aged 16 years), Zalagyöngye (aged 46 years), Black Muscat, which is also known as Muscat Hamburg (aged 8 years), Italian Riesling (aged 0.5 years) and Dornfelder (aged 46 years). GRLaV-1 was detected in Cardinal (three plants, each aged 16 years), Black Muscat (aged 17 years), Italian Riesling (aged 20 years) and Merlot (aged 17 years). GRLaV-3 was identified on cultivars Othello (aged 30 years) and Italian Riesling (three plants aged 30 years, and one plant aged 31 years).

As the analyses revealed, cultivar Italian Riesling was infected by three of the four tested plant viruses, concurring with the results reported by Dida et al. (2018), who found high infection levels among Italian Riesling and Black Muscat cultivars. Hančević et al. (2021) tested 16 grapevine cultivars from Mediterranean Croatia for the presence of ten most economically important grapevine viruses. While these authors found GLRaV-3 to be the most widespread, they did not draw any conclusions regarding the level of susceptibility of different cultivars. On the basis of our results and scale of investigations, we posit that our findings stem from the current cultivar ratio in the investigated area and targeted cultivars, rather than being influenced by the susceptibility/resistance level of these cultivars. According to Fuchs (2003) and Sastry & Zitter (2014), as genes proffering resistance to GFLV do not exist inside genus *Vitis*, none of the grapevine cultivars are resistant to this virus. As the same applies to the GLRaV complex, transgenic resistance is used against these viruses in some countries (Ling et al., 1997).

As older vineyards have been exposed to virus vectors and infection for longer periods, the probability of infection in these vineyards is significantly higher than in the younger ones. That is especially true if the grapevine propagation material is certified according to OEPP/EPPO (2008) certification schemes. Our findings concur with these observations, as the examined viruses were detected predominantly in older vineyards, with just one case of infection in a 6-month-old specimen. In this young vineyard, the propagation material was the most likely source of infection.

Vineyard owners were informed about our results and were advised to destroy all infected grapevine plants. By adopting this measure, the potential for virus transmission was eliminated, thus preventing infection of neighboring healthy plants and further losses in grape quantity and quality. Threat of virus infection can be considered as an emerging plant protection problem, as climatic changes favor greater vector's geographical distribution and population. On the other hand, the less educated farmers cannot easily recognize the signs and symptoms of virus infections which in some cases can be latent, and can lead to prioritizing the protection measures against well-known fungal or chromista diseases.

Virus control is only possible with timely adoption of preventive plant protection measures. The most important among them is planting a tested, virus-free propagation material. Other measures include destroying the virus-infected plants, agro-technical measures (avoiding the transfer of soil clods via shoes or mechanization and thus preventing nematode dispersion), weed control, etc.

CONCLUSION

Among the four monitored viruses, GFLV, GLRaV-1 and GLRaV-3 are present in vineyards across Vojvodina Province on different grapevine cultivars. Older vineyards are more susceptible to infection, and require more stringent application of plant protection measures. For effective virus control, continuous virus and virus vector monitoring and planting of healthy propagation material are necessary.

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