VIROLOGICAL EXAMINATION OF A HUNGARIAN VINEYARD

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ABSTRACT

Winegrowing and winemaking have been known to humankind for thousands of years. Many abiotic, biological and anthropogenic factors have had remarkable effects on the development of viticulture. Numerous viruses may cause significant diseases to the grapes. Plant viruses belong to pathogens that draw attention to their presence in everyday cultivation only when the infected plants show apparent symptoms of a disease. According to our current knowledge, the control of viruses is challenging since infected plants cannot be cured. Furthermore, there are difficulties in the diagnostics of viruses that lead to the need to focus on the use of pathogen-free plant propagating material and the prevention of the infection. The degree of disease can be determined most reliably by molecular biological methods. The research aimed to assess the infection of Grapevine leafroll-associated viruses (GlRaV) and Grapevine fleck virus (GFkV) in grapevine from the Northern Transdanubia region of Hungary, using an enzyme-linked immunosorbent assay (ELISA) test. In 25 of the 60 samples, GlRaV and GFkV were detected. GlRaV 1, GLRaV 2 variants and GFkV were the most common viruses with serologically positive results, confirming previous studies showing GLRaV1-2 and GFkV as the dominant pathogens among grape viruses in Hungary and the Northern Transdanubia.

Keywords: Grapevine, Grapevine virus, ELISA, Northern Transdanubia, Hungary

1 INTRODUCTION

The cultivation of grapes and the making of wine have been known to humanity for thousands of years. From antiquity to the present day, it has evolved along with civilisation, social customs and traditions (Tamás and Tamás, 2013). However, human interventions, environmental changes, and the emergence of pathogens have impacted the development of grape production and, thus, wine production (Kozma 1993, Horváth-Gáborjányi 2000, Hluchy et al. 2007, Cseh et al. 2008, Pocsai 2017).

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In addition to selective breeding in the cultivation of grapes, plant protection also faces new challenges. Due to changing climatic factors and trade, pathogens that are not yet known may also appear in domestic vineyards. In addition, many viruses can cause significant symptoms and damage (Hull, 2002).
The control of viruses is challenging since the infected plants cannot be cured. The difficulties of diagnostics and the lack of therapeutic solutions emphasise the use of pathogen-free propagating material and the prevention of the development of infection in the control of viruses (Turcsán et al., 2020 Szabó, 2019 Balássy, 2016). Furthermore, the degree of contamination of plants can be determined most securely by molecular biological methods (Szabó 2019, Szegedi et al. 2012).
The research aimed to assess the infection of grape plantations in the Sopron wine region by grape viruses causing leafroll and latent spot symptoms (Grapevine fleck virus, GFkV) using the Enzyme-linked Immunosorbent Assay (ELISA). In addition, the study also extended to the evaluation of the relative dominance of leafroll disease variants (Grapevine leafroll-associated virus 1 (GLRaV 1), Grapevine leafroll-associated virus 2 (GLRaV 2), Grapevine leafroll-associated virus 3 (GLRaV 3), Grapevine leafroll-associated virus 6 (GLRaV 6), Grapevine leafroll-associated virus 7 (GLRaV 7).

2 MATERIALS AND METHODS

When choosing the sample collection dates, it was necessary to consider that the concentration of viruses in the plant is not constant. Instead, the virus concentration is influenced by the phenological stage of the vine and external environmental factors. Therefore, two-time intervals are suitable for determining grape viruses within a vegetation period. For detecting viruses belonging to the genus *Nepovirus*, *Maculavirus*, and *Alfamovirus*, the optimal time for collecting the samples starts from the grape flowering and lasts until the onset of summer heat.
The other sample collection period ranges from the fruit formation till the end of summer or the beginning of autumn. Viruses of the genus *Closterovirus*, *Ampelovirus*, and *Vitivirus* can be detected effortlessly (Apró et al., 2012). Samples of grape leaves were collected in August. The leaves were derived from the vineyard region of Kőszeg, located in the Sopron wine region, Hungary. Grape leaf samples from the lower two-thirds of the foliage, which showed symptoms typical of viral diseases, were collected during both collection periods.
Enzyme-linked Immunosorbent Assay (ELISA), an enzyme-linked antibody test, was first used by Avrameas (1969) to detect plant viruses (Clark-Adams, 1977). ELISA test investigates colour reactions where the reagents are tied to a plastic surface, and the response is tracked using an enzyme-linked antibody. In the plant virological practice, several types of procedures for the test are used. The so-called double antibody sandwich (DAS ELISA) method is generally used for diagnostic purposes. The samples have been considered negative when the extinction values did not exceed three times the extension value measured in the negative control plants.

3 RESULTS

From the 60 samples, 25 ones showed the symptoms of viral infection. The most common viral agent was the GLRaV 1 in 41,6 % of 25 samples. These studies confirm the previous results (Apró et al., 2012) that GLRaV 1 infection is one of the most common in Hungarian vineyards. The presence of GLRaV2 was successfully detected in 12 samples (Table 1).

Table 1: The viral infections in the collected samples.

Minta	GLRaV1	GLRaV2	GLRaV3	GLRaV6	GLRaV7	GFKV	Minta	GLRaV1	GLRaV2	GLRaV3	GLRaV6	GLRaV7	GFKV8
Kontroll	–	–	–	–	–	–	–	–	–	–	–	–	–
1	–	–	–	–	–	–	31	–	–	–	–	–	–
2	–	–	–	–	–	–	32	–	–	–	–	–	–
3	–	–	–	–	–	–	33	–	–	–	–	–	–
4	–	–	–	–	–	–	34	–	–	–	–	–	–
5	–	–	–	–	–	–	35	–	–	–	–	–	–
6	–	–	–	–	–	–	36	+	–	–	–	–	–
7	–	–	–	–	–	–	37	–	–	–	–	–	–
8	–	–	–	–	–	–	38	–	–	–	–	–	–
9	–	–	–	–	–	–	39	+	–	–	–	–	–
10	–	–	–	–	–	–	40	–	–	–	–	–	–
11	–	–	–	–	–	–	41	+	–	–	–	–	–
12	+	–	–	–	–	–	42	+	+	–	–	–	+
13	–	–	–	–	–	–	43	+	–	–	–	–	+
14	+	–	–	–	–	–	44	+	–	–	–	–	–
15	–	–	–	–	–	–	45	+	+	–	–	+	+
16	–	–	–	–	–	–	46	+	+	+	+	+	+
17	+	–	–	–	–	–	47	+	+	–	+	–	+
18	–	–	–	–	–	–	48	+	+	–	–	–	+
19	–	–	–	–	–	–	49	+	+	–	–	+	+
20	–	–	–	–	–	–	50	+	+	–	–	+	+
21	+	+	–	–	–	–	51	+	+	–	–	+	+
22	+	–	–	–	–	–	52	–	–	–	–	–	–
23	–	–	–	–	–	–	53	+	–	–	–	–	–
24	–	–	–	–	–	–	54	+	+	–	–	–	+
25	–	–	–	–	–	–	55	+	+	–	–	–	+
26	–	–	–	–	–	–	56	–	–	–	–	–	–
27	–	–	–	–	–	–	57	+	–	–	–	–	–
28	–	–	–	–	–	–	58	+	–	–	–	–	–
29	–	–	–	–	–	–	59	+	–	–	–	–	–
30	–	–	–	–	–	–	60	+	+	–	–	–	–

In addition to the agents mentioned so far, in 11 cases, the virus responsible for the latent spot of grapes (GFkV) could also be detected (Table 1). Based on previous studies, GFkV has proven to be one of the most common viruses (Martelli, 1993; Kovacs et al., 2001; Komar et al., 2007; Cretazzo et al., 2010).
Five leaf samples proved to be infected with the GLRaV 7, and two were infected with the GLRaV 6. In addition, there were a small number of cases of the GLRaV 3, which caused leafroll symptoms, too (Table 1).

According to the current Hungarian regulation (FVM No 87/2006) on placing propagating vine materials on the market, regular screening is mandatory for this significant grape pathogen virus and, in the case of infection, virus eradication (Lázár, 2016).
From the 25 samples, 13 ones showed the symptoms of simultaneous infections (Figure 1). The most common viruses that caused complex infections were the GLRaV 1 and the GLRaV 2, belonging to the leafroll symptom group. In 11 cases, the GFkV and the GLRaV 1 and/or GLRaV 2 could also be detected. In four samples, in addition to the pathogens mentioned above, the presence of the GLRaV 7 was also detected. Serological tests detected the GLRaV 2 two times. There was only one sample in which the GLRaV 1 and the GLRaV 2 showed complex infection with the GFKV, and every tested virus could be detected on another occasion.

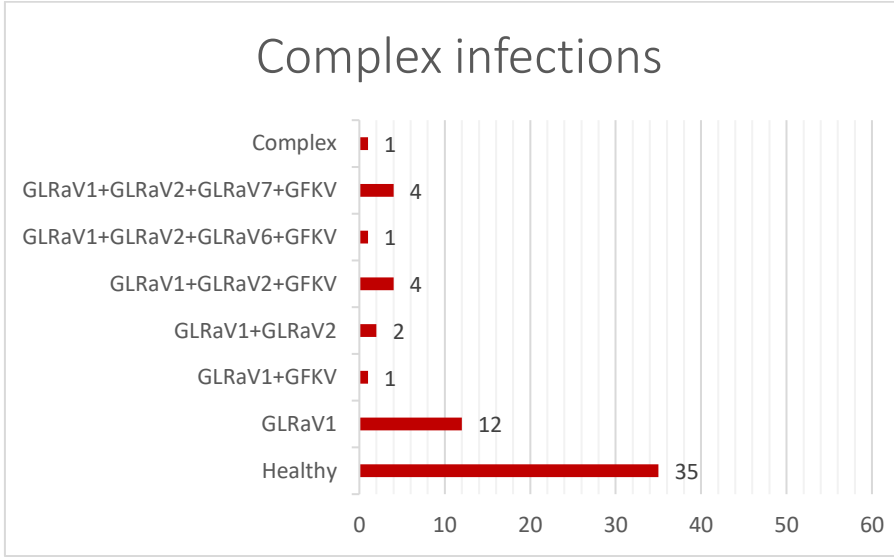

Figure 1: The complex infections.

4 DISCUSSION

Control of viruses in vineyards is a complex task. On the one hand, these pathogens are spread by contaminated propagating material and grafting at the time of planting, and later mechanically and by vectors (nematode and shield lice species). On the other hand, since we cannot chemically affect the diseases of the virus that have developed, we must strive for prevention to protect against them.
High-quality, pathogen-free propagating material plays a key role. Healthy, virus-free propagating material is essential to achieve a good quality and quantity of vine for the long term.

For the production of grape grafts, both the subject and the noble variety must be pathogen-free. Therefore, it is of paramount importance to regulate the production and sale of propagating material and monitor the health of imported propagating material.
Based on the symptoms, the exact identification of viruses is uncertain. The high number of symptoms found in the study and the virus-free plant individuals also raise the possibility of other diseases with similar symptoms. Because different variants of the same species have different disease characteristics, there is additional protection against them – it is essential to have a racial diagnosis.
In many cases, only diagnostic procedures performed in the laboratory can give an inevitable result of the ongoing infection. Therefore, in the future, it is necessary to develop and optimise methods that are more sensitive, faster and cheaper than the procedures used in practice. Furthermore, proper virus detection also allows monitoring of antivirus processes, with the help of which discharge protocols can be continuously optimised. Thus, we can contribute to the production of virus-free propagating material to preserve the health of vineyards.

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