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Grapevine Pinot gris virus infecting grapevines in Romania - Short Communication

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Abstract: *Grapevine Pinot gris virus* (GPGV) has been identified in many grape growing countries of the world since 2012. The aim of this work was to investigate the presence of GPGV on some accessions collected from a germplasm collection, in addition to the propagation material and clonal selection samples. During 2019–2020, a total of 199 samples have been analysed by a double antibody sandwich – enzyme-linked immunosorbent assay (DAS-ELISA) for the presence of GPGV, Grapevine fanleaf virus (GFLV), Grapevine leafroll-associated virus-1+3 (GLRaV-1+3) and Grapevine fleck virus (GFkV). Among them, 107 samples (53.76%) showed a GPGV-infection, associated with or without symptoms on the leaves (deformations, chlorosis, mosaic, wrinkles) or stunting plants. The distribution of infected varieties showed a high rate of infection in old varieties (37.38%), followed by clones (32.71%), rootstocks (11.21%), clonal selections (9.35%) and new varieties (9.35%). The tests revealed the association of GPGV with GFkV (5 cases) and GLRaV-1+3 (2 cases). GPGV should be included in the rules of grapevine certification schemes for the production of virus-free mother plants.

Keywords: GPGV; incidence; symptoms; DAS-ELISA; grapevine

The first report on the existence of *Grapevine Pinot gris virus* (GPGV) was undertaken by professor Martelli who, in 2012, announced at the meeting of virologists in the framework of the 17th Congress of the International council for the study of viruses and virus-like diseases of the grapevine (ICVG), the discovery of four new grapevine viruses, including GPGV, although the characteristic symptoms had already been reported since 2003 in northern Italy (Martelli 2012). The circulation of propagating material in the grape growing countries of the world caused the rapid spread of this virus, so that it crossed the borders of Europe, being reported in South Korea since 2013, in China and Uruguay in 2015, in the United States, Canada and Georgia in 2016 (Saldarelli et al. 2017). At the last meeting of the ICVG, the GPGV detection and characterisation was reported in countries such as Pakistan (Rasool et al. 2018), Greece (Sassalou et al. 2018), Canada (Poojari et al. 2018), France (Spilmont et al. 2018), Chile (Medina et al. 2018), Hungary (Demián et al. 2018), Italy (Gentili et al. 2018), and Turkey (Ulubaş Serçe et al. 2018). The preliminary data on the presence of GPGV in Romania reported no details on the grapevine material collected after 2010, by analysing some genotypes from germplasm collections in several European countries (Bertazzon et al. 2016).

The aim of this work was to investigate the presence of GPGV on some accessions collected from a germplasm collection, in additions to the propagation material and clonal selection samples. The possible relationships between the virus con-

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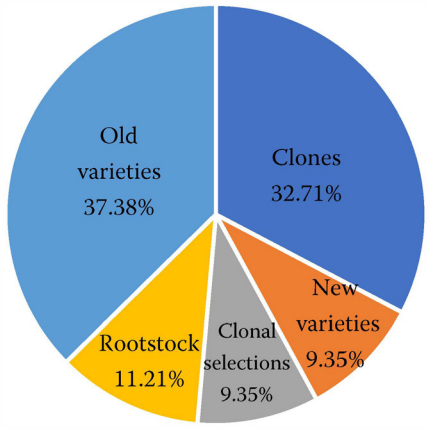

Figure 1. The distribution of GPGV-infected grapevine genotypes

centration, symptomatology and serological diagnosis were also investigated.

During 2019–2020, 199 samples belonging to some newly created varieties/clones (49/46), clonal selections (31), old varieties (39) from the grapevine collection in the country and rootstocks (4) were analysed to evaluate the incidence of GPGV in Romania. Twenty-nine samples were tested from woody material, the others being young leaves collected from plants either showing symptoms or without symptoms, at the beginning of the vegetation period, to the genotypes stocked in the germplasm collection. The symptoms were visible on the grapevine leaves (deformations, chlorosis, mosaic, wrinkles), the shoots (short internodes) or the whole plant (stunting). The samples were analysed by a DAS-ELISA (double antibody sandwich – enzyme-linked immunosorbent assay) (Clark, Adams 1977) with commercial reagents (Bioreba, Switzerland) for the diagnosis of GPGV, Grapevine fanleaf virus (GFLV), Grapevine leafroll-associated viruses-1+3 (GLRaV-1+3) and Grapevine fleck virus (GFkV). The ELISA readings (optical density – OD) were performed with a double filter at 405/492 nm.

The analysis from the present study showed an incidence of GPGV in 53.76% of the samples, 107 positive ELISA samples (21 from wood, 86 from leaves) out of 199 tested. Of these, the old varieties, from the germplasm collections, registered the highest percentage (37.37%). As expected, the clones closely followed (32.71%) the old varieties. The presence of GPGV in the clonal selections confirms, again, that it is necessary to use grapevine virus-free material in the breeding programmes. A small percentage (9.35%) of plants belonging to varieties created after 1994 have been infected with GPGV (Figure 1) due to the fact that the grapevine viruses are at low risk of seed transmission (Gasparro et al. 2016).

Within the study, twelve samples collected from four rootstock genotypes were analysed. All of these were ELISA positive for GPGV.

Our study, regarding the GPGV incidence, started with the collection of the samples for a laboratory diagnosis, based on symptoms described by other researchers as being related to the presence of the virus. Symptoms like deformations, chlorosis, mosaic and wrinkled leaves, the poor development of the whole plant have been identified. Also, in the GPGV infected plants, short internodes, double nodes, fan-shaped leaf symptoms were observed, without the GFLV presence. Other plants with wrinkled and upward twisted leaves (characteristic symptom for GFkV) were subsequently diagnosed with the GPGV infection.

The results of the analysis of the samples collected from the plants with and without symptoms showed that there is not a clear relationship between the laboratory diagnosis and the symptoms of the GPGV presence. Factors such as the varietal tolerance, resistance, sensitivity, susceptibility to viruses, environment, virus strain and titre in the grapevine clones or varieties can influence the expression of the symptoms. Thus, out of 95 samples from plants presenting specific symptoms to GPGV infection, 60 were confirmed as infected. On the other hand, out of 75 samples from asymptomatic grapevines, 22 were ELISA positive (Figure 2).

On the contrary, our study showed high viral concentrations in the positive ELISA plants with-

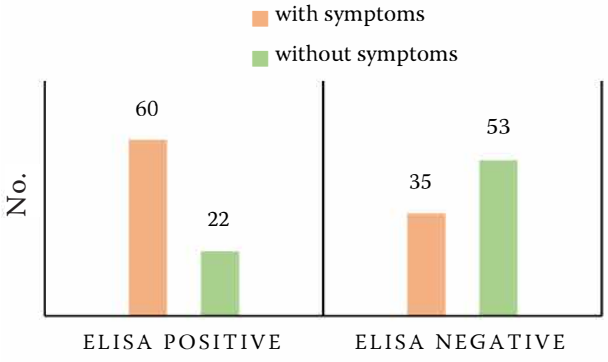

Figure 3. ELISA readings on the symptomatic (orange) or asymptomatic (green) tested grapevine

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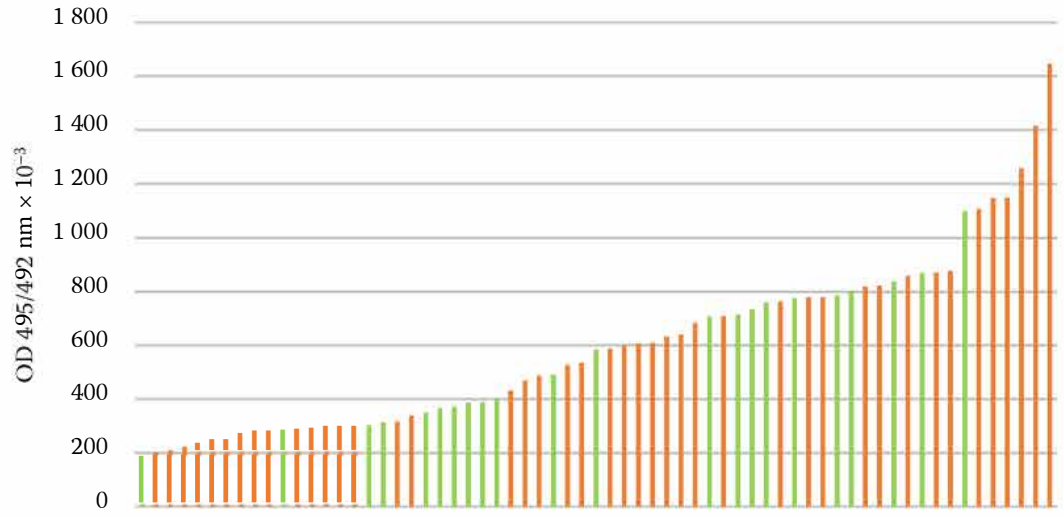

Figure 3. ELISA readings on the symptomatic (orange) or asymptomatic (green) tested grapevine

out symptoms very close to those of the infected plants, but showing obvious symptoms. Negative ELISA readings were recorded both in plants with and without symptoms (Figure 3).

The analysis to identify the grapevine specific viruses revealed the association of GPGV with GFkV (5 cases) and GLRaV-1+3 (2 cases). Three of the plants that were diagnosed with GFkV + GPGV mixed infection had no symptoms.

The widespread abundance of GPGV in the major grape growing regions of the world, caused by the circulation of propagating material, requires rigorous management in the germplasm and propagation sectors. It is necessary to carry out some visual inspection programmes and laboratory diagnoses in order to maintain the high biological value of the propagating material. GPGV should be included in the rules of grapevine certification schemes for the production of virus-free mother plants.

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