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ASSESSMENT OF THE HEALTH STATUS OF GRAPEVINE GENETIC RESOURCES IN TERMS OF VIRAL DISEASES

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Abstract: Following requirements of the European Cooperative Programme for Plant Genetic Resources (ECPGR) and the European Genebank Integrated System (AEGIS), a total of 172 accessions from the Agrobioinstitute grapevine genebank including varieties, rootstocks, wild grapes, local autochthonous varieties and new selected forms were periodically checked for their virus health status. The results obtained showed that total of 16.27% of the DAS-ELISA tested accessions were infected by one (89.29%) or more viruses (10.71%). The most of tested plants were infected by *Grapevine leafroll associated virus* 3 (GLRaV-3) (14.53%), followed by *Grapevine leafroll associated virus* 1 (GLRaV-1) (2.91%), while the *Grapevine fanleaf virus* (GFLV) (0.58%) was scarcely represented. Mixed infections (GLRaV-1 + GLRaV-3) were detected in 6 plants (3.48%). All tested genebank accessions for *Grapevine fleck virus* (GFkV) were virus free. Infected plants were quarantined. Additional sanitization work, including tissue culture combined with thermotherapy and treatment with antiviral chemicals was initiated.

INTRODUCTION

The grapevine is an economically important fruit crop worldwide. The germplasm potential in grapevines remains unexplored with a big number of officially registered varieties (about 10,000), from which only 35 account for approximately 70% of the worldwide vineyards (Alleweldt & Dettweiler-Münch, 1994). This negative trend is fully valid for Bulgaria, as a country whose viticulture

is one on the main economically important agricultural sectors. Creation of true to type and disease free stocks (genebanks) contributing directly to increase of the quality of the planting material, increase of the profitability of vineyards, and sustainable development of the grape and wine industry (Gollino et al., 2017). In addition, the core collections play a key role in the preservation and efficient use of the local germplasm biodiversity.

A total of 64 viruses belonging to different genera and families have been reported in grapevines, including some that seriously affect the production and profitability of vineyards. Virus diseases on grapevine have a significant economic impact on plant growth, yield and fruit quality, as they can affect the graft compatibility, rooting capacity, winter hardiness, longevity of the vines and lead to plant mortality of up to 75% of the vineyards (Maliogka et al., 2015). The EC legislation concerning the certification of the grapevine required that the propagating material should be free of five economically important viruses: *Grapevine fanleaf virus* GFLV), *Grapevine leafroll associated virus*-1 (GLRaV-1), *Grapevine leafroll associated virus*-3 (GLRaV-3), *Grapevine fleck virus* (GFkV) and *Arabis mosaic virus* (ArMV). Strategically targeted sanitary selection is necessary to reduce the presence of viruses in propagation material through the maintenance and upgrading of clean stocks (genebanks) from which high-quality (pre-basic) planting material could be derived (Tsvetkov et al., 2005). In cases of high incidence of infectious diseases in breeding vineyards and impossibility to select authentic and disease-free material of a particular variety or rootstock, several therapeutic methodologies have been proposed, such as: thermotherapy (Mannini et al., 1998), chemotherapy (Golino et al., 1993), electrotherapy (Burger, 1989), *in vitro* shoot-tip culture (Duran-Vila et al., 1988), *in vitro* meristem culture (Monis et al., 1997), *in vitro* shoot-tip culture combined with thermotherapy (Fanizza et al., 1988), adventitious organogenesis, somatic embryogenesis (Torregrosa et al., 1993) and somatic embryogenesis combined with thermotherapy (Goussard et al., 1992). Assessment of sanitation-treated material should be carefully carried out. There is need of re-confirmation of the trueness to type and re-indexing for all known diseases. In cases of introduction of material from overseas sources, a long quarantine period and thorough indexing are absolutely necessary (Martelli, 1999; Martelli, 2014).

MATERIALS AND METHODS

The grapevine genebank of Agrobioinstitute was maintained in a greenhouse in the region of Pazardzhik in the period of 11 years (2009-2020) following the technological scheme (Fig. 1), based on the best practices of the European Cooperative Programme for Plant Genetic Resources (ECPGR) and the European Genebank Integrated System (AEGIS) (Weise et al. 2018). The collection consisted of 172 stock accessions and included grape varieties, rootstocks, wild grapes, local autochthonous varieties and new selected forms. All of the genebank

accessions from the Agrobioinstitute grapevine genebank were analyzed during its vegetation in 2015 year, in terms of four economically and certification-important viral diseases.

These plants were tested by double antibody sandwich-enzyme linked immunosorbent assay (DAS ELISA) test for the next 4 viruses: Grapevine fanleaf virus- (GFLV), Grapevine leafroll associated virus-1 (GLRaV-1), Grapevine leafroll associated virus-3 (GLRaV-3) and Grapevine fleck virus) (GFkV) as described by Voller et al. (1976). Old leaves with petioles (before leaf-fall at the end of vegetation) were collected and used as plant samples. Standard protocols for sample preparation and analysis were followed as given by the manufacturer (BIOREBA®) with some modifications. After the addition of 100 mL freshly prepared p-nitrophenylphosphate in substrate buffer (1 mg mL-1) the plates were incubated at room temperature and photometric measurement was done at 405 mn after 2 h. Samples were considered as positive if their absorbance values were more than three times higher than the negative control.

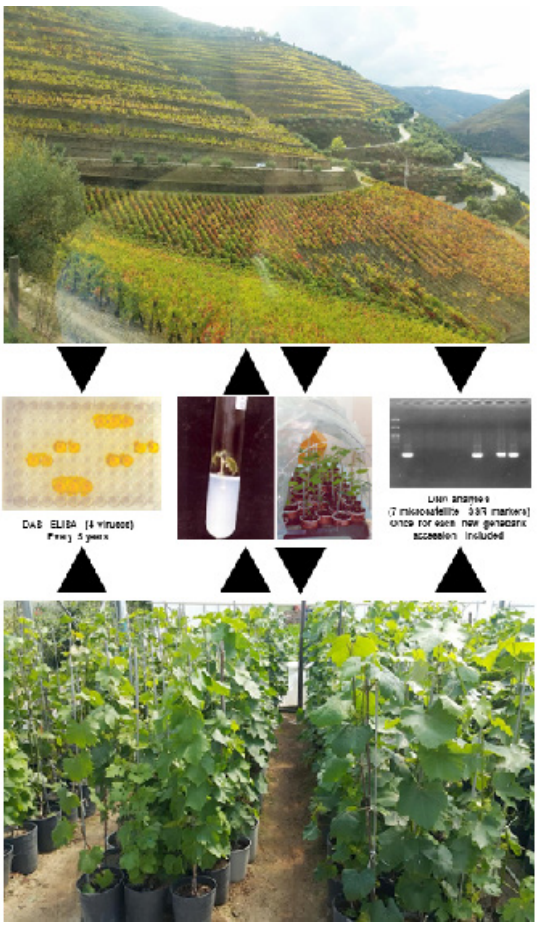

Figure 1: Technological scheme for maintenance, propagation and upgrading of the ABI grapevine genebank

RESULTS AND DISCUSSION

The results obtained (Table 1) showed that a total of 16.27% of the DAS-ELISA tested accessions were infected by one (89.29%) or more viruses (10.71%). The most of the tested plants were infected by *Grapevine leafroll associated virus* 3 (GLRaV-3) (14.53%), followed by *Grapevine leafroll associated virus* 1 (GLRaV-1) (2.91%) while the *Grapevine fanleaf virus* (GFLV) (0.58%) was scarcely represented. Mixed infections (GLRaV-1 + GLRaV-3) were detected in 6 samples (3.48%). All tested genebank accessions for *Grapevine fleck virus* (GFkV) were virus free. The results obtained confirmed the data from the few previous monitoring studies in Bulgaria during the periods 1999- 2003 and 2004-2006 (Yankulova et al., 2007; Kamenova et al., 2007) regarding the relatively high prevalence of *Grapevine leafroll associated virus* 3, *Grapevine leafroll associated virus* 1 and *Grapevine fanleaf virus*. While both authors reported *Grapevine fleck virus* as the most widespread in Bulgaria our results showed its absence in the genebank tested material. This could be explained by the specificity of the tested plant material (pre-basic greenhouse genebank - in our case and basic mother vineyards - in the above mentioned 2 cases). Genotype tolerance to individual viruses and the specificity of plant-pathogen interaction also play a decisive role (Yamakava, 1989; Reynolds, 2017). In this regard, our results showed no viral infection in rootstocks, wild grapes and autochthonous forms. With regard to mixed infections, the GLRaV-1 + GLRaV-3 combination clearly remains the most commonly found in the country. Infected plants were quarantined and additional sanitization work, including tissue culture combined with thermotherapy and treatment with antiviral chemicals was initiated.

CONCLUSION

The results obtained confirmed the data from the few previous monitoring studies in Bulgaria, regarding the relatively high prevalence of *Grapevine leafroll associated virus* 3, *Grapevine leafroll associated virus* 1 and *Grapevine fanleaf virus*.

The absence of a detected *Grapevine fleck virus* (GFkV) infection could be explained by the specificity of the controlled growing conditions of the tested plant material.

Our results showed no viral infection in rootstocks, wild grapes and autochthonous forms. This could be partly explained by the higher tolerance of the mentioned genotypes to viral diseases and also with a reduced presence of virus-transmitting vectors in the sanitary controlled growing conditions in the greenhouse.

The study highlights the needs of continuing of the programs for maintenance and evaluation of the grapevine genetic resources genebanks. This will ensure the conservation and effective use of the grapevine biodiversity as well as the reduction of viral diseases in certified plant propagating material.

Table 1: Incidence of GLRaV-1, GLRaV-3, GFLV and GFkV in the plants of the grapevine genebank accessions

Stock Accessions	Number of genotypes analyzed	Number of virus infected genotypes (%)	Type of infection/Viruses
Bulgarian red wine varieties	26	4 (15.38)	2 single (GLRaV-3) 2 mixed (GLRaV-3+ GLRaV-1)
Introduced red wine varieties	12	-	-
Bulgarian white wine varieties	20	2 (10.0)	2 mixed (GLRaV-3+ GLRaV-1)
Introduced white wine varieties	5	3 (60.0)	3 single (GLRaV-3)
Bulgarian table white seeded varieties	25	7 (28.0)	6 single (GLRaV-3) 1 single (GLRaV-1)
Introduced table white seeded varieties	9	4 (44.4)	4 single (GLRaV-3)
Bulgarian table red seeded varieties	20	4 (20.0)	2 single (GLRaV-3) 2 mixed (GLRaV-3+ GLRaV-1)
Introduced table red seeded varieties	6	3 (50.0)	3 single (GLRaV-3)
Bulgarian table white seedless varieties	11	-	-
Introduced table white seedless varieties	3	-	-
Bulgarian table red seedless varieties	4	-	-
Introduced table red seedless varieties	2	1 (50.0)	1 single (GFLV)
Rootstocks	7	-	-
Wild grapes and autochthonous forms	14	-	-
Bulgarian new breeded forms	8	-	-
Total	**172**	**28**	**20 single (GLRaV-3); 1 single (GFLV); 6 mixed (GLRaV-3 + GLRaV-1)**

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