SEROLOGICAL DETECTION OF GRAPEVINE FANLEAF VIRUS (GFLV) IN AMPELOGRAPHIC COLLECTION OF USAMV IAŞI, ROMANIA

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Abstract: *Grapevine fanleaf virus (GFLV) is one of the most severe virus diseases in vineyards worldwide. GFLV is a positive ssRNA virus from family Comoviridae, genus Nepovirus. Nepoviruses are isometric particles of about 30 nm in diameter, which possess two single-stranded positive sense genomic RNAs. Virus is transmitted by nematodes and is known as a causative agent of fanleaf degeneration of grapevine. It causes extensive leaf yellowing, stem and leaf deformation, reduced fruit quality, substantial crop loss and shortened longevity of vineyards. In 2011 a sanitary survey was conducted in ampelographic collection belonging to Agricultural Sciences and Veterinary Medicine University from Iaşi (Romania) on 50 genotypes belonging to Vitis spp. Our objective was to determine the presence and distribution of GFLV across the ampelographic collection. Leaf samples were taken during spring season from vines exhibiting virus-like symptoms or general vine decline. Three mature leaves including the petiole from different sections of the vine, keeping between the first and fifth node from the base of the vine were collected in dry, cool weather. Totally, 153 samples of symptomatic leaves from surveyed varieties were collected, put into separate plastic bags, frozen with liquid nitrogen, transported to the laboratory, and stored at −80ºC until analysed. Symptomatic grapevine samples were used for detection of GFLV by a double-antibody sandwich ELISA (DAS ELISA) using monoclonal and polyclonal antibodies. DAS ELISA results were taken as mean absorbance value of three replicates per sample. Positive and negative controls were supplied with the kit. Each value was considered GFLV-positive when the average value was at least three times greater than the mean of healthy control. The results of DAS-ELISA test confirm that virus was present in 11 grapevine varieties (22.0% of total) from the ampelographic collection. Infected vine cultivars with the highest OD (optical density) values were Blauerzweigelt, Newburger, Merlot, Gordan, Cioinic and Galbenă de Odobeşti.*

Key words**:** *Vitis spp., DAS ELISA, GFLV*

INTRODUCTION

Grapevine fanleaf virus (GFLV) is one of the most destructive viral diseases affecting grapevine worldwide. It causes extensive leaf yellowing, stem and leaf deformation, reduced fruit quality, substantial crop loss (up to 80%) and shortened longevity of vineyards (ANDRET-LINK et al., 2004). Among 58 virus species that can infect grapevine, GFLV belongs to the plant virus genus *Nepovirus* of the family *Comoviridae* transmitted by nematode *Xiphinema index* (MARTELLI, 2006). Nepoviruses are isometric particles of about 30 nm in diameter, which possess two single-stranded positive sense genomic RNAs (FRITSCH et al., 1993, WELLINK et al., 2000).

Vineyards viruses control is currently based on preventive measures and cultural practices. Prophylactic measures intend to prevent introduction of diseased vine varieties in healthy vineyards, and cultural practices try to reduce the vectors population (LAIMER, 2009).

The aim of this study was to determine the presence and distribution of GFLV across the ampelographic collection of USAMV Iaşi.

MATERIAL AND METHODS

In 2011 a sanitary survey was conducted in ampelographic collection belonging to USAMV Iaşi (Romania) on 50 genotypes belonging to *Vitis* spp.

Leaf samples were taken during spring season from vines exhibiting virus-like symptoms or general vine decline. Three mature leaves including the petiole from different sections of the vine, keeping between the first and fifth node from the base of the vine were collected in dry, cool weather. Totally, 153 samples of symptomatic leaves from surveyed varieties were collected, put into separate plastic bags, frozen with liquid nitrogen, transported to the laboratory, and stored at −80ºC until analysed.

Symptomatic grapevine samples were used for detection of GFLV by a double-antibody sandwich ELISA (DAS ELISA) using monoclonal and polyclonal antibodies. ELISA was performed with commercial kits (ADGEN Phytodiagnostics, UK), according to the manufacturer recommendation. Crude grapevine extracts were prepared by grinding 1 g leaves in 10 mL of ELISA extraction buffer. Leaf extracts were centrifuged at 2,000 g for 15 min and the supernatant was used as the antigen in DAS ELISA. 100 µL were loaded in each well on microtiter plates (Nunc Immunoplate I, Nunc, Denmark). Incubation steps lasted overnight at 4°C in closed dark boxes. Reactive were preincubated to the plate temperature. Intermediate washings were done with PBS/Tween buffer. Values were recorded measuring absorbance at 405 nm with a microplate reader Sunrise (Tecan, Austria) powered by Magellan data analysis software.

DAS ELISA results were taken as mean absorbance value of three replicates per sample. Positive and negative controls were supplied with the kit. Each value was considered GFLV-positive when the average value was at least three times greater than the mean of healthy control.

RESULTS AND DISCUSSIONS

The incidence of GFLV disease on ampelographic collection of USAMV Iaşi was visually monitored in 2011. Out of the 153 grapevine samples collected from 18 varieties with characteristic symptoms 79 were infected with *Grapevine fanleaf virus* (Figure 1).

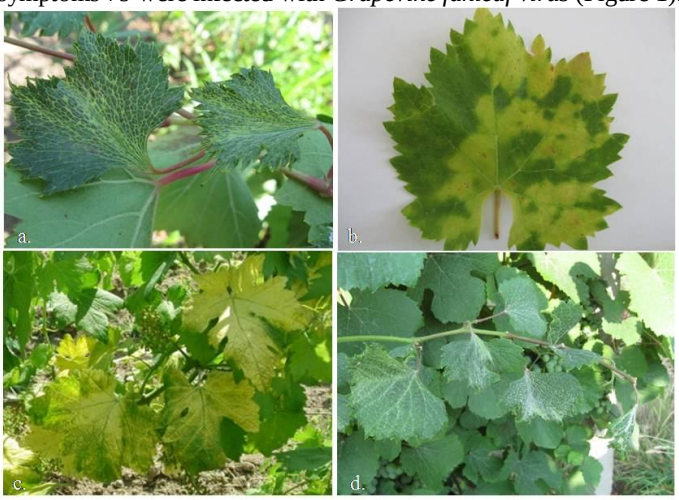

Figure 1: Symptoms associated with GFLV infections on different grapevine varieties: a. Gordan; b. Newburger; c. Merlot; d. Blauerzweigelt.

Serological tests (DAS ELISA) revealed the presence of virus infections in 11 grapevine varieties, where from 108 prevailed samples 78 were positive for GFLV (Table 1). The results show that not all cultivars with virus-like symptoms are caused by GFLV. Leaf yellowing, stem and leaf deformation could be caused by physical injury or some other disorder (fungicide, herbicide, insecticide). GFLV causes the grapevine fanleaf degeneration worldwide and severe losses up to 80%, poor fruit quality and reduced grapevine longevity (Andret-Link et al., 2004).

Table 1

Occurrence of GFLV as determined by DAS ELISA on grapevine samples with symptoms collected from ampelographic collection of USAMV Iaşi in 2011

Grapevine cultivars	No. total of samples	GFLV positives	
		No.	%
Cioinic	8	7	87.5
Frâncuşă	8	7	87.5
Gordan	8	7	87.5
Galbenă de Odobeşti	12	7	58.3
Muscat Ottonel	12	9	75.0
Blauerzweigelt	10	6	60.0
André	10	6	60.0
Andrevit	10	4	40.0
Newburger	8	8	100.0
Mustoasă de Maderat	12	8	66.7
Merlot	10	9	90.0
TOTAL	108	78	73.8

DAS ELISA results confirm that GFLV was present in 11 grapevine varieties, which represent 22.0% of total number of cultivars under observation. Infected foreign cultivars with the highest OD (optical density) values measured at 405 nm were Blauerzweigelt, Merlot and Newburger. Infection with high OD was also confirmed in some indigenous cultivars as Gordan, Cioinic and Galbenă de Odobeşti (Figure 2).

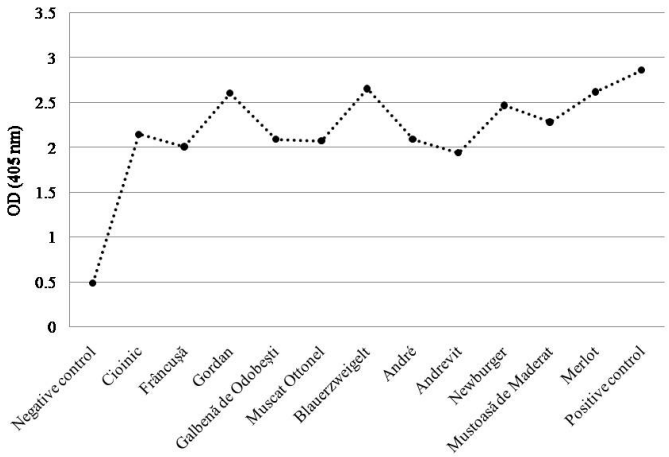

Figure 2: Average values of extinction obtain at 405 nm with DAS ELISA.

Differences in sensitivity to GFLV are known among cultivars of *V. vinifera*; some are resistant to infection and others recover one year after the appearance of symptoms. A fundamental importance in the development of the disease is played by environment and growing area, because the number of infected grapevines in vine plantations will increase dramatically in the presence of infected vineyards.

The results obtained in this work could be used to eliminate the risk of long distance spreading during international exchange of plant material.

CONCLUSIONS

Grapevine cultivars from ampelographic collection of USAMV Iaşi (N-E Romania) have been examined by visual symptom assessment for typical GFLV symptoms in 2011 and serological tests (DAS ELISA) revealed that incidence of GFLV disease ranged 22.0%.

Infected grapevine plants from the 11th varieties should be removed and replaced after reducing of vector populations. Also, the usage of clean planting material and surveying the neighbouring viticulture areas are measures that should be implemented to maintain the disease under control.

ACKNOWLEDGMENTS

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