DETECTION OF *GRAPEVINE FAN-LEAF VIRUS* IN SOME GRAPEVINE VARIETIES USING ELISA TEST

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Abstract

Grapevine fanleaf virus (GFLV) is responsible for fanleaf degeneration, which is one of the most severe virus diseases of grapevines worldwide. GFLV causes substantial crop losses, reduces fruit quality and shortens the longevity of grapevines in the vineyard. GFLV is transmitted specifically from grapevine to grapevine by the ectoparasitic nematode *Xiphinema index*, and belongs to the genus Nepovirus in the family Comoviridae. ELISA (with DAS-, TAS- and DAS-biotin variants) is the most used method both for diagnosis and studies regarding the sampling strategy for different viruses (detection of the most reliable source of antigen and period of the year in which the analyze is performed). A survey was made to evaluate the sanitary status of grapevine in the ampelographic collection of USAMV Iasi, with regard to occurence of economically important viruses. Propagation material of 36 grapevine varieties was tested for presence of *Grapevine fan-leaf virus*. In the first test, from 14 varieties of grapevine analized, 8 were found to be infected with GFLV. The second test was performed on 22 vines varieties, 7 showed extinction values that exceed the blank value.

Key words: *Grapevine fanleaf virus,* ELISA, grapevine

Grapevine (*Vitis vinifera* L.) is exposed to several biotic stress factors caused by insects, fungi, bacteria, viruses and phytoplasmas responsible for important economic losses worldwide and to encourage the widespread use of agrochemicals. It is one of the most sensitive plants to attack of viruses, with at least 55 species of viruses belonging to 20 genera. [Martelli, 2003].

Among these viruses identified in the vineyards, the most important are those that produce or are in combination with *Grapevine fan-leaf virus*, *Grapevine leafroll virus* and "rugose wood complex" [Lindbo et al., 1993; Baulcombe, 1996 , Prins, 2003].

The control of phytoplasma and viruses encountered in vines, is currently based on preventive measures and cultural practices. Prophylactic measures intended to prevent introduction of diseased vine varieties in healthy vineyards, and cultural practices aimed the reduction of vectors of viruses populations. [M. Laimer, 2009].

Grapevine fanleaf virus (*GFLV*) is the virus responsible for causing a serious degenerative diseases in grapevine, fan-leaf disease. *GFLV* lead to substantial losses of crops, reduced fruit quality and shortens the life of the vine plant. The virus is transmitted by the nematode *Xiphinema index* ectoparasites belonging to the genus Nepovirus, Comoviridae.

MATERIAL AND METHOD

Visual observations were made in the field concerning the symptoms of fan leaf virus infections on grapevine. Were sampled from 36 varieties from Ampelographic Collection of USAMV Iasi, which were studied in the laboratory using DAS-ELISA technique to diagnose the presence of virus in the plant.

Sample preparation is done by weighing one gram of each sample, grind them in a mortar with pestle previously sterilized. After grinding is added 10 ml of extraction buffer, mixing the sample, decanting and use the supernatant for ELISA test. After preparing the samples, the positive control together with negative controls are added to

ELISA plate, each carefully pipetting 100µl in each well. Cover plate with aluminum foil and put to incubate overnight at 4 ° C.

For the reaction between thr two components, antigen and antibodies were used 96 wells plates from Neogen Europe firm in UK, and exctinction values were measured using plate reader Tecan Sunrise, at a wavelength of 405 nm. The color intensity is directly proportional to the concentration of

antigen in the wells and can be used as a measure in assessing the antigen-antibody reaction through plate reader - TECAN.
For qualitative analysis and diagnosis of GFLV infection of plant material fallowing six steps which are outlined below:

The main stages of work in DAS-ELISA:

- Plate layer with studied sample, positive and negative control (100 ml/well) and incubate them overnight at 4 ° C;
- Washing the plate with washing buffer PBS / Tween;
- Conjugate addition on the plate (100 ml / well) and incubation at 37 ° C for 2 hours;
- Addition of enzyme substrate (100 ml / well) and incubation at 37 ° C for 1 hour in the dark;
- Washing the plate with washing buffer PBS / Tween;
- Measuring the extinction values at 405 nm after 60 min.

RESULTS AND DISCUSSIONS

Serological tests of the used material, revealed the presence of viral infections with *Grapevine fanleaf virus* in some analyzed varieties.

To optimizarării this technique for each sample the test was performed in duplicate, the principle of the method consists in forming an immune complex, the antigen is bound to antibodies and conjugate, as a "sandwich" and by adding the enzyme substrate apears a yellow colour, the intensity of colour is directly proportional to the concentration of antigen.

In *table 1* were analyzed 14 varieties of grapevines, of which varieties Armaş, Frâncuşă, Newburger, Traminer Roz, Galbenă de Odobeşti, Gordan, Dimiat and Cioinic showed high extinction values, in excess of the negative control (M-) which explains the presence of virus in samples taken for analysis. In *table 2* were analyzed 22 varieties of the same Ampelographic collections, among them were identified as having a positive reaction to the presence of virus following varieties: Blauerzweigelt, Grasă de Cotnari, Bastard de Magaraci, Merlot, Riesling Aromat, Napoca and Chasselas Doré with values obtained exceeding the blank values.

After measuring the values of extinction was concluded that can be used in DAS-ELISA the conjugate in low concentration (1:20) which reduce the costs of test performance.Extinction values were measured at 60 min interval according to the certificate of kit's performance.

Table 1

Detection of *Grapevine fanleaf virus* using serological method ELISA

Sample	Variety	Extinction values after 60 min.	
	Positive control	1.584	1.404
	Negative control	0.380	0.456
1.	Pinot Gris	0.066	0.034
2.	Bătută Neagră	0.180	0.130
3.	Dimiat	1.360	1.280
4.	Muscat de Pöloskei	0.114	0.268
5.	Traminer Roz	1.336	1.232
6.	Victoria	0.006	0.042
7.	Gordan	1.320	1.404
8.	Armaş	1.480	1.232
9.	Perlă de Csaba	0.160	0.320
10.	Frâncuşă	1.448	1.264
11.	Newburger	1.284	1.408
12.	Sultanina	0.078	0.120
13.	Galbenă de Odobeşti	1.372	1.192
14.	Cioinic	1.464	1.100

Table 2

Detection of *Grapevine fanleaf virus* using serological method ELISA

Sample	Variety	Extinction values after 60 min.	
	Positive control	1.288	1.566
	Negative control	0.340	0.405
1.	Zghihară de Huşi	0.264	0.368
2.	Chardonnay	0.276	0.164
3.	Chasselas Doré	0.284	0.316
4.	Blauerzweigelt	1.436	1.176
5.	Busuioacă de Bohotin	0.092	0.060
6.	Pinot Noir	0.092	0.002
7.	Grasă de Cotnari	1.504	2.028
8.	Bastard de Magaraci	0.908	1.432
9.	Afuz Ali	0.180	0.184
10.	Merlot	1.176	1.816
11.	Riesling Aromat	1.444	1.400
12.	Napoca	1.256	1.380
13.	Aligoté	0.208	0.172
14.	R6/ Chasselas Doré	1.644	1.448
15.	Sauvignon Gros	0.372	0.236
16.	Coarna Neagra	0.300	0.244
17.	Fetească Regală	0.304	0.268
18.	Astra	0.280	0.380
19.	Chasselas roze	0.328	0.224
20.	Gamay Beaujolais	0.396	0.256
21.	Ardeleanca	0.416	0.336
22.	Superbizibo	0.280	0.116

Infected		Healthy	
60 min	1.263	60 min	0.006

Legend: = positive reaction ;
negative reaction ;
blank

According to the certificate of quality control performed at 405 nm, using substrate ADGEN Yellow is:

Grapevine fan leaf virus infection has adverse effects on plant growth and quality of their products with a major economic impact on the culture of grapevine.

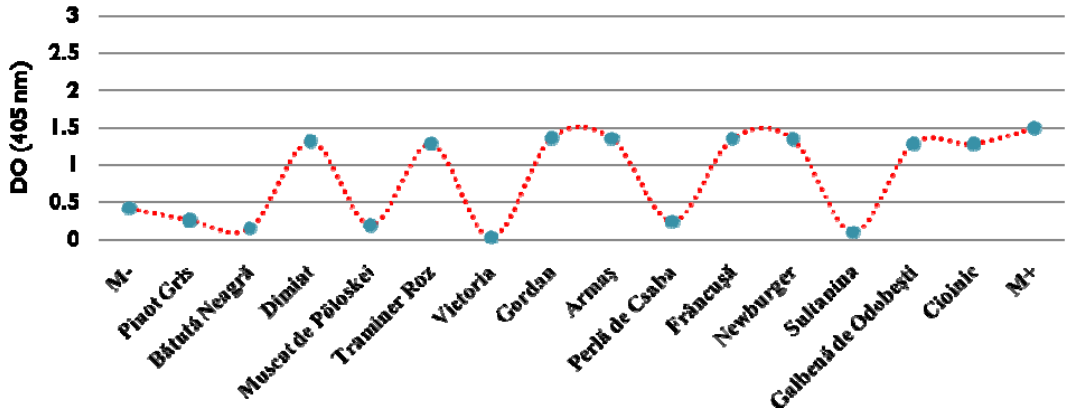

Figure 1 **Averages values of extinction at 405 nm in DAS-ELISA**

Following serological tests on the material, the presence of virus was confirmed in eight of 14 species analyzed (*figure 1*).
Varieties as: Dimiat, Traminer Roz, Gordan, Armaş, Frâncuşă, Newburger, Galbenă de Odobeşti and Cioinic had values of extinction that exceeded the blank value.

The material tested in *figure 2*, summing 22 varieties of table grapes and wine grapes. The presence of GFLV virus was confirmed in seven varieties, apox. 32% of the analyzed varieties.

Varieties as: Blauerzweigelt, Grasă de Cotnari, Bastard de Magaraci, Merlot, Riesling Aromat, Napoca and Chasselas Doré.

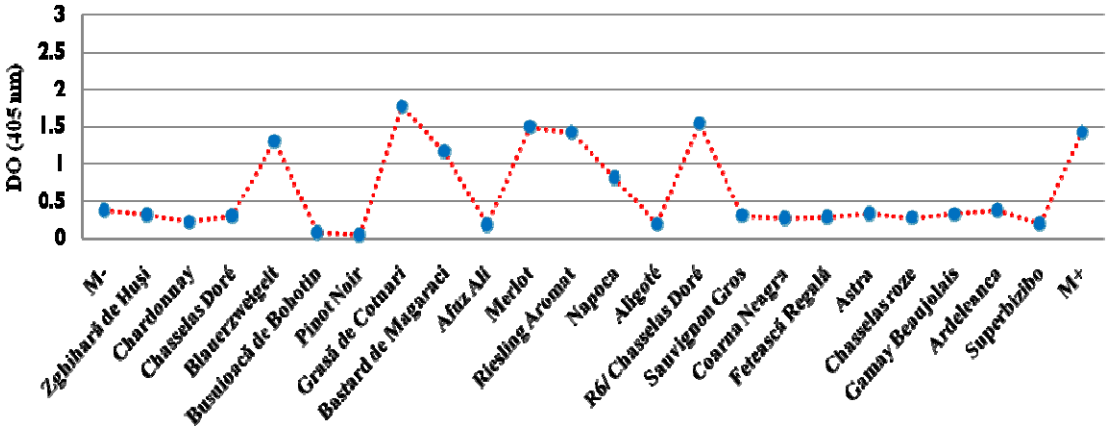

Figure 2 **Averages values of extinction at 405 in DAS-ELISA**

CONCLUSIONS

The presence of virus was confirmed in eight of 14 species analyzed. Varieties as: Dimiat, Traminer Roz, Gordan, Armaş, Frâncuşă, Newburger, Galbenă de Odobeşti and Cioinic had values of extinction that exceeded the blank value.

In second test, from 22 analyzed varieties, presence of GFLV virus was confirmed in seven varieties, apox. 32%. Varieties as: Blauerzweigelt, Grasă de Cotnari, Bastard de Magaraci, Merlot, Riesling Aromat, Napoca and Chasselas Doré.

3. It can be used in DAS-ELISA tests, the conjugate in low concentration (1:20) which reduce the costs of test performance.

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