DETECTION OF PATHOGEN *FLAVESCENCE DORÉE PHYTOPLASMA* IN SOME GRAPEVINE VARIETIES USING ELISA TEST

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Abstract

Flavescence dorée (FD) is an economically important quarantine disease of grapevine in Europe. It is caused by different strains of phytoplasma belonging to the 16S ribosomal group V. They are transmitted from one grapevine to another by Scaphoideus titanus, a leafhopper of american origin widespread in European vineyars. 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). The aim of this study was to identify the presence of FD in various varieties of vines from Ampelographic collection of USAMV Iasi. Propagation material of 30 grapevine varieties was tested for presence of Flavescence dorée phytoplasma. The test was performed on 30 grapevines varieties, only 6 showed infections with Grapevine Flavescence doreé phytoplasma, extinction values that exceed the blank value.

Key words: Flavescence dorée, ELISA, grapevine

Grapevine yellows (GY) are associated with several different phytoplasmas worldwide but cannot be identified on the basis of symptoms. Phytoplasmas are obligate parasitic phloem restricted bacteria, which are transmitted by insect vectors and propagated by vegetative multiplication of plant material.

Flavescence dorée (FD) is the more dangerous GY and FD phytoplasma is a quarantine organism in the European Community (EC directive Nr 77/1993 modified 92/103), because it is transmitted by a vine-feeding leafhopper vector, *Scaphoideus titanus Ball* (Schvester et al. 1963) spread out in many parts of the Western Mediterranean area (Boudon-Padieu, 2002). FD phytoplasma belongs to the Elm yellows (EY) group (Daire et al. 1992, 1993, 1997 a; Seddas et al. 1996) or 16SrV group, according to the classification of Seemüller et al. (1998) and Lee et al. (1998), respectively. International exchange of plant material increases the risk of long distance spreading of phytoplasma and of introduction of new vector insects. *Scaphoideus titanus* Ball (Schvester, 1969) belongs to Cicadellidae family, group Deltocephalinae. Is a hemimetabolic insect that feeds with vine phloem and present an economical importance as *Flavescence doreé phytoplasma* vector,which is a persisting disease in vigneyards.

The vine-feeding leafhopper has an inability to travel long distance, adults do not move outside plantation and the movement is strongly influenced by plantation area [Lessio and Alma 2004, 2006]. The FD epidemics began from the border of vineyard, where symptomatic grapevines were concentrated, only later spreding out to the remaining grapevines (Borgo and Angelini, 2002).

The identification of these reservoirs could prove to be very important in protecting vineyards from FD disease, which has been declared a quarantine disease in Europe.

Scaphoideus titanus Ball. the leafhopper vector of FD, is present in all vineyards . Thus the occurrence of FD was kept under a constant watch because the leafhopper species is a highly efficient vector of FD, which in turn is a very dangerous disease because of its quick spreading when the vector is present. So far, none of the FD *sesu stricto* isolated has ever been identifiied in other wild or cultivated plants except grapevine.

The aim of this study was to identify the grapevine varieties from Ampelographical collection of USAMV Iasi, that harbour phytoplasma which cause FD disease.

MATERIAL AND METHOD

Visual observations were made in the field concerning the symptoms of FD on grapevine. Were sampled from 30 varieties from Ampelographic Collection of USAMV Iasi, which were studied in the laboratory using TAS-ELISA technique to diagnose the presence of phytoplasma in vine plants.

ELISA with polyclonal and monoclonal antibodies have been used for detection of *Flavescence dorée* in vector [Boudon-Padieu et

al., 1989] and grapevine [Caudwell & Kuszala, 1992; Kuszala et al., 1993; Kuszala, 1996], the method relies on availability of antibodies which are sold commercially.

Were collected three mature leaves from each block that prezent symptoms characteristic of phytoplasma infection, the leaves were taken from the area between first and fifth node. They were placed in plastic bags, labeled, keept away from sunlight and transported to the laboratory for analysis.

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 *FD* infection of plant material fallowing six steps which are outlined below:

The main stages of work in TAS-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;
- Addition of antibody on plate (100 µl/godeu) and incubate at 37°C for 2 hours;
- Washing the plate with washing buffer PBS / Tween;
- Conjugate addition on the plate (100 ml / well) and incubation at 37 ° C for 2 hours;
- Washing the plate with washing buffer PBS / Tween;
- 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 Flavescence doreé phytoplasma* in some analyzed varieties.

To optimize 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.

Table 1

Detection of pathogen *Grapevine Flavescence doreé phytoplasma* using ELISA test

Prove	Variety	Values of extinction after 60 min	
	Positive control	1.086	1.128
	Negative control	0.350	0.341
1.	Bastard de Magaraci	0.074	0.096
2.	Coarna alba	0.119	0.121
3.	Milcov	0.082	0.105
4.	Gamay Beaujolais	0.118	0.122
5.	Alicante Bouschet	0.123	0.098
6.	Tavriz	0.121	0.103
7.	Regina viilor	0.087	0.096
8.	Cetatuia	0.098	0.086
9.	Blauerzweigelt	0.131	0.105
10.	Chasselas Doré	0.096	0.107
11.	Aligoté	0.129	0.120
12.	Galbenă de Odobeşti	0.126	0.151
13.	Cioinic	0.321	0.395
14.	Roz Românesc	0.125	0.148
15.	Moldova	0.243	0.239
16.	Pinot noir	0.145	0.162
17.	Mustoasa de Maderat	0.075	0.076
18.	Balada	0.066	0.071
19.	Perlette	0.110	0.125
20.	Aromat de Iasi	0.087	0.092
21.	Dodrelabi	0.493	0.671
22.	André	0.296	0.215
23.	Feteasca regala	1.045	1.125
24.	Pinot gris	0.305	0.425
25.	Silvania	0.217	0.238
26.	Grasa de Cotnari	0.147	0.156
27.	Napoca	0.156	0.177
28.	Chardonnay	0.458	0.532
29.	Cruciuliţa	0.603	0.867
30.	Zghihară de Huşi	0.090	0.097

Legend: = positive reaction ;
= negative reaction ;
= blank.

In *table 1* were analyzed 30 varieties of grapevines, table grapes and wine grapes varieties, of which only six of them to identify the presence of *Grapevine Flavescence doreé phytoplasma*. Varieties as Cioinic, Dodrelabi, Feteasca regala, Pinot Noir, Chardonnay and Cruciuliţa showed values of exctinction that exceeded the negative control, maximum values were obtained in varieties: Feteasca regala, Cruciuliţa.

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.

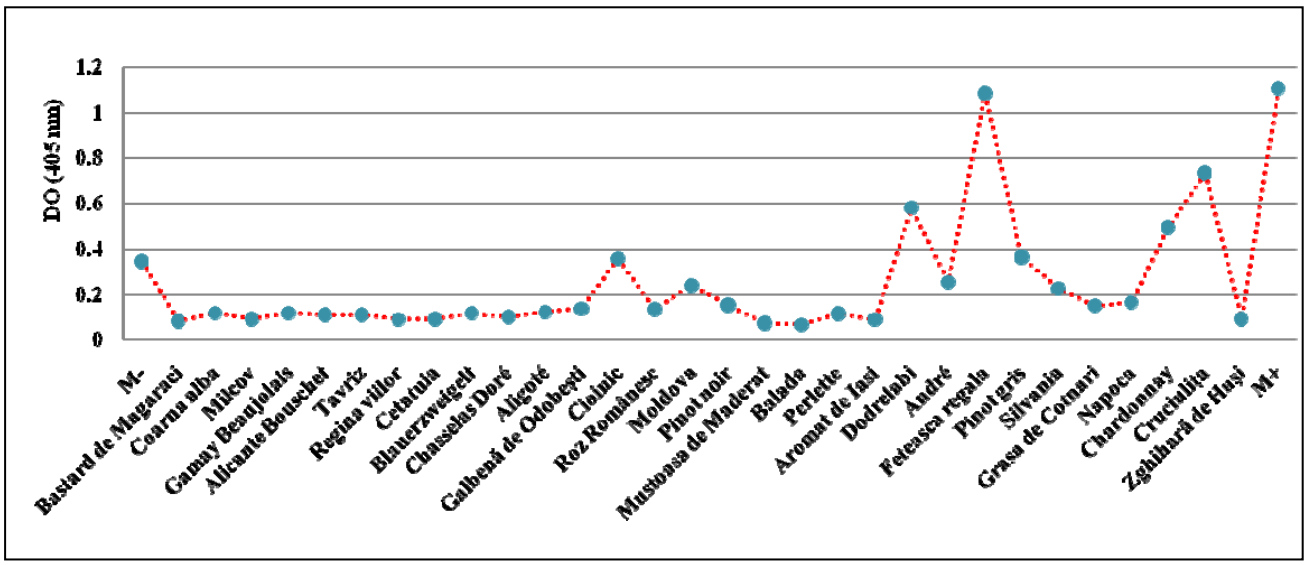

Figure 1 **Average values of exctinction obtain at 405 nm in TAS-ELISA**

Selection of suitable planting material can be used to control the disease. Differences in sensitivity are known among cultivars of *V. vinifera*, some being resistant to infection and others recovering completely the year after the appearance of symptoms. Control of the insect vector is facilitated by the fact that, in Europe, *S. titanus* is confined to grapevine as a host and has just a single generation per year (Caudwell et al., 1987).

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

Infected		Healthy	
60 min	0.258	60 min	0.091

CONCLUSIONS

Were analyzed 30 varieties of grapevines, of which only six of them to identify the presence of *Grapevine Flavescence doreé phytoplasma*. Varieties as Cioinic, Dodrelabi, Feteasca regala, Pinot Noir, Chardonnay and Cruciuliţa showed values of exctinction that exceeded the negative control, maximum values were obtained in varieties: Feteasca regala, Cruciuliţa.

The occurrence of the disease in Ampelographical Collection underlines the importance of starting control measures and to limited the leafhopper vector populations.

The use of clean planting material (Caudwell et al., 1997) and the control of new foci of the disease by surveying GY in neighbouring viticulture areas are measures that should be implemented to contain the disease.

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