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Short communication

Detection of *Flavescence Dorée* phytoplasma in ampelographic collection at Iaşi, Romania

N. Irimia, E. Ulea and F.D. Lipşa*

Department of Plant Sciences, University of Agricultural Sciences and Veterinary Medicine Iaşi, Mihail Sadoveanu Alley 3, 700490 Iaşi, Romania

ABSTRACT

***Flavescence dorée* phytoplasma (FDP) is a quarantine pathogen in the European Union, where it causes serious damage and has a negative effect on grapevine production. This phytoplasma belongs to the 16SrV ribosomal group (syn. elm yellows group) and is transmitted by the leafhopper *Scaphoideus titanus* Ball. During 2010 and 2011 a sanitary survey was conducted in ampelographic collection of University of Agricultural Sciences and Veterinary Medicine (USAMV) from Iaşi (Romania) on 170 cultivars belonging to *Vitis* spp. The results of TAS ELISA test confirm that FDP was present in 19 grapevine cultivars (11.2%) from the total ampelographic collection. Infected foreign cultivars with the highest OD (optical density) values were Chardonnay, André, Michele Palieri, Pinot Gris and Bastarde de Magaraci. Infection was also confirmed in some indigenous cultivars as Moldova, Negru de Căuşani, Feteasca neagră, Muscat Ottonel and Vulpe.**

Key words: *Vitis* spp., *Flavescence dorée* phytoplasma, detection, TAS ELISA.

Flavescence dorée (FD) is the most dangerous yellows disease of grapevine (*Vitis vinifera* L.) in temperate areas of Europe, North-America, Asia Minor and Australia, where affects dramatically large vineyards areas and has a negative effect on grapevine production (Boudon-Padieu, 1). It is associated with *Flavescence dorée* phytoplasma (FDP) belongs to the 16SrV-C and 16SrV-D subgroups from ribosomal group 16SrV or Elm Yellows group (Seemüller *et al.*, 12). FDP is obligate parasitic phloem restricted bacteria, which is transmitted by leafhopper *Scaphoideus titanus* Ball. (*Homoptera*, *Cicadellidae*). The symptoms associated with FDP infections are similar and include: rolling and bright red discoloration of leaves, vein chlorosis and necrosis, withering of flowers and bunches, uneven or absence of lignifications and drooping canes (Mori *et al.*, 9).

In Romania, yellows symptoms have been observed in grapevine cv. Regina de Puglia since 1967 (Rafailă and Costache, 10), but the presence of leafhopper *Scaphoideus titanus* (Schvester *et al.*, 11) was confirmed first in 2009 on commercial vineyards from Murfaltlar and Blaj and one untreated vineyard plot near Bucharest (Chireceanu *et al.*, 5). FDP is a quarantine pathogen in Romania, were vineyards are occupying, 175,953 ha (FAOSTAT, 6). A sanitary survey was conducted from 2010 to 2011 in ampelographic collection of University of Agricultural Sciences and Veterinary Medicine (USAMV) from Iaşi (north-east Romania) on 170 cultivars belonging to *Vitis* spp. in order to register the symptoms referable to FD disease. Surveys were carried out three times a year in July, August and September. Samples of grapevine were collected from plants showing leaf roll, leaf redness, vein chlorosis, necrosis and absence of lignification. Totally, 378 samples of symptomatic leaves from 37 cultivars were collected. Leaves and petioles for each sample were detached with a scalpel from different sections of the vine, keeping between the first and fifth node from the base of the vine. Samples were collected in dry weather, put into separate plastic bags, frozen with liquid nitrogen, transported to the laboratory, and stored at -80°C until analysed.

Grapevines samples were used for detection of FDP by a double-antibody sandwich ELISA (TAS ELISA) using monoclonal and polyclonal antibodies (Caudwell and Kuszala, 3; Zimmermann *et al.*, 14). ELISA was performed with commercial kits (ADGEN Phytodiagnostics, UK), according to the manufacturer's 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. (Zimmermann *et al*., 13) and the supernatant was used as the antigen in TAS 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 after 60 min. incubation at room temperature with a microplate

* Corresponding author' E-mail: flipsa@uaiasi.ro

† All authors contributed equally to this work

reader (Tecan, Austria) powered by Magellan data analysis software. TAS 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 FDP-positive when the average value was at least three times greater than the mean of healthy control (Boudon-Padieu *et al*., 2). Statistic was performed with Microsoft Office Excel 2007. Analysis of variance was performed with the use of One-Way ANOVA test.

The incidence of FD disease on ampelographic collection of USAMV Iaşi was visually monitored during 2010 and 2011. Out of the 378 grapevine samples collected from 37 cultivars with characteristic symptoms 118 were infected with *Flavescence dorée* phytoplasma (Fig. 1). Serological tests (TAS ELISA) revealed the presence of phytoplasma infections in 19 grapevine cultivars. The results show that not all cultivars with phytoplasma-like symptoms are caused by FDP (Table 1). Rolling and bright red discoloration of leaves could be caused by crown gall, physical injury or some other disorder. FDP causes a chronic infection which will lead to a reduction of production and premature aging of grapevine.

TAS ELISA results confirmed that FDP was present in 19 grapevine cultivars, which represent 11.2% of total number of cultivars from ampelographic collection. This method for FDP detection is reliably, and even more sensitive as real-time PCR (Fiore *et al.*, 7). Infected foreign cultivars with the highest OD (optical density) values measured at 405 nm were Chardonnay, André, Michele Palieri, Pinot Gris and Bastarde de Magaraci. Infection with high OD was also confirmed in some indigenous cultivars as Moldova, Negru de Căuşani, Fetească neagră, Muscat Ottonel and Vulpe. These findings are in concordance with the previous data published by Irimia *et al.* (8) about the presence of FDP in ampelographic collection of USAMV Iaşi (N-E Romania). ANOVA did not reveal statistical differences among the mean values of extinction in FDP-infected cultivars during 2010 and 2011 (data not shown).

Fig. 1. Symptoms associated with FDP infections on some grapevine cultivars. (a) Moldova; (b) Fetească regală; (c) Pinot Gris; (d) Tavriz.

Table 1. Occurrence of FDP as determined by TAS ELISA on grapevine genotypes collected from ampelographic collection of USAMV Iaşi.

Cultivar	Total No. of samples	FDP positive	
		No.	%
Cioinic	12	7	58.3
Coarnă Neagră	15	9	60.0
Fetească Neagră	10	5	50.0
Fetească Regală	15	7	46.7
Milcov	10	3	30.0
Moldova	17	15	88.2
Muscat Ottonel	12	10	83.3
Negru de Căuşani	10	4	40.0
Purpuriu	12	6	50.0
Vulpe	12	6	50.0
André	10	4	40.0
Bastarde de Magaraci	8	4	50.0
Blauerzweigelt	10	6	60.0
Chardonnay	8	7	87.5
Dodrelabi	10	3	30.0
Decabriski	12	7	58.3
Michele Palieri	15	5	33.3
Pinot Gris	12	10	83.3
Tavriz	17	5	29.4
Total	227	118	51.9

Infected grapevine plants from each of the above mentioned 19 cultivars will be removed and replaced after insecticide usage against the leafhopper *Scaphoideus titanus*. Differences in sensitivity to FDP are known among cultivars of *V. vinifera*; some are resistant to infection and others recover completely one year after the appearance of symptoms. Insect vector control is facilitated in Europe, because *S. titanus* is restricted to grapevine as host and has a single generation per year (Caudwell *et al.*, 4). 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. Identification of infected cultivars was very important in protecting vineyards against FD disease, which has been declared a quarantine disease in Europe.

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REFERENCES

1. Boudon-Padieu, E. 2002. *Flavescence dorée* of the grapevine: Knowledge and new developments in epidemiology, etiology and diagnosis. *ATTI Giornate Fitopatologiche*, **1**: 15-34.

2. Boudon-Padieu, E., Larrue, J. and Caudwell, A. 1989. ELISA and Dot-Blot detection of *Flavescence doree*-MLO in individual leafhopper vectors during latency and inoculative state. *Curr. Microbiol.* **19**: 357-64.

3. Caudwell, A. and Kuszala, C. 1992. Mise au point d'un test ELISA sur les tissus de vignes atteintes de *Flavescence dorée*. *Res. Microbiol.* **143**: 791-806.

4. Caudwell, A., Boudon-Padieu, E., Kuzsala, C. and Larrue, J. 1987. Biologie et étiologie de la flavescence dorée. Recherches sur son diagnostic et sur les méthodes de lute. **In**: *Atti del Convegno*

sulla flavescenza dorata delle vite, Vicenza-Verona, Italy, pp. 175-200.

5. Chireceanu, C., Ploaie, P.G., Gutue, M., Nicolae, I., Stan, C. and Comsa, M. 2011. Detection of the *Auchenorrhyncha* fauna associated with grapevine displaying yellows symptoms in Romania. *Acta Phytopath. Entom. Hungarica*, **46**: 253-60.

6. FAOSTAT, 2010. *FAO Statistical Databases*, Food and Agricultural Organization of the United Nations online at http://faostat.fao.org/.

7. Fiore, N., Prodan, S. and Pino, A.M. 2009. Monitoring grapevine viruses by ELISA and RT-PCR throughout the year. *J. Plant Pathol.* **91**: 489-93.

8. Irimia, N., Ulea, E. and Bălău, M. 2010. Detection of *Flavescence dorée* phytoplasma grapevines infection using Elisa Test. *Lucr. şt. Seria Agronomie*, **53**: 187-91.

9. Mori, N., Bressan, A., Martini, M., Guadagnini, M., Girolami, V. and Bertaccini, A. 2002. Experimental transmission by *Scaphoideus titanus* Ball of two *Flavescence dorée*-type phytoplasmas. *Vitis*, **41**: 99-102.

10. Rafailă, C. and Costache, M. 1970. The golden Flavescence (*Flavescence dorée*), a new disease of grapevine in Romania. *Inst. Cercet. Protectia Plant.* **6**: 151-56.

11. Schvester, D., Carle, P. and Moutous, G. 1961. Sur la transmission de la Flavescence dorée des vignes par une cicadelle. *C. R. Séances Acad. Agr. France*, **47**: 1021-24.

12. Seemüller, E., Marcone, C., Lauer, U., Ragozzino, A. and Göschl, M. 1998. Current status of molecular classification of the phytoplasmas. *J. Plant Pathol.* **80**: 3-26.

13. Zimmermann, D., Sommemryer, B., Walter, B. and Van Regenmortel, M.H.V. 1990b. Production and characterization of monoclonal antibodies specific to closterovirus-like particles associated with grapevine leafroll disease. *J. Phytopathol.* **130**: 277-88.

14. Zimmermann, D., Walter, B. and Le Gall, O., 1988. Purification de particules virales associees a l'enroulement de la vigne et mise au point d'un protocole ELISA permettant leur detection. *Agronomie*, **8**: 731-41.

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