

## Detection and incidence of FD and BN phytoplasmas in vineyards of different grapevine cultivars in Serbia

Slobodan KUZMANOVIC<sup>1</sup>, Marta MARTINI<sup>2</sup>, Zarko IVANOVIC<sup>1</sup>, Dragana JOSIC<sup>3</sup>, Svetlana ŽIVKOVIC<sup>1</sup>, Mira STAROVIC<sup>1</sup>

<sup>1</sup>Institute for Plant Protection and Environment, Belgrade, Serbia

<sup>2</sup>Department of 'Biologia Applicata alla Difesa delle Piante', University of Udine, Udine, Italy

<sup>3</sup>Institute for Soil Science, Belgrade, Serbia

### Abstract

Presence and diffusion of grapevine yellows (GY) phytoplasmas was investigated from 2003 to 2005 in seventeen vineyards located in five of the most important viticultural regions of Serbia. From the same vineyards, symptomatic grapevine samples representing ten most important cultivars in those regions were collected (2003-2006) for molecular analyses. Both "flavescence dorée" (FD) and "bois noir" (BN) phytoplasmas were confirmed to be associated with grapevine yellows in Serbia.

Field inspections showed as GY epidemics have been spreading very fast in the monitored vineyards, especially in those vineyards in which only the presence of FD phytoplasma was detected. The highest incidences of FD phytoplasma were found in vineyards of Plovdina, a local variety that appeared to be extremely sensitive to FD.

**Key words:** nested-PCR, RFLP analyses, grapevine yellows, elm yellows group.

### Introduction

The first investigations on grapevine phytoplasmas in Serbia have been carried out during the last six years, even though the first symptoms of grapevine yellows (GY) were observed twenty years ago. Phytoplasma infection in vines showing GY symptoms was proved by electron microscopy observations (Kusmanović *et al.*, 2003). Molecular analyses demonstrated that "flavescence dorée" (FD) and "bois noir" (BN) are the most important phytoplasmas in Serbia (Duduk *et al.*, 2003; 2004; Kuzmanović *et al.*, 2004).

FD is associated with different phytoplasma strains belonging to the elm yellows group (16SrV), subgroups 16SrV-C and -D, and is present particularly in France, Italy and Spain. BN is associated to a stolbur phytoplasma (16SrXII-A) and is present in Asia Minor, the Mediterranean and European countries (Maixner *et al.*, 2006).

Aim of the present work was to determine the presence of FD and BN phytoplasmas in different grapevine cultivars and to monitor their incidence in vineyards over three years period in Serbia.

### Materials and methods

During 2003-2005 the incidence of GY was monitored in seventeen vineyards in five of the most important viticultural regions of Serbia: Župsko, Sićeavačko, Kutinsko, Vršačko and Deliblatska peščara. From the same vineyards symptomatic grapevine samples were collected during 2003-2006 from the 10 most important grapevine cultivars in these regions: Plovdina, Smederevka, Prokupac, Black Burgunder, Italian Riesling, and Župski bojadisler, Frankovka, Reinische Riesling, Chardonnay and Župljanka.

Total DNA was extracted from plant material following a modified enrichment CTAB extraction method. Nested-PCR with phytoplasma universal primers was adopted for amplification of 16S rRNA gene: P1/P7 primer pair in direct PCR followed by R16F2n/R16R2 in nested-PCR. RFLP analysis of nested-PCR products were carried out with restriction enzymes *TruI* and *AluI* (only for samples tested in 2005-2006) and the RFLP patterns were compared with profiles of reference strains EY1, ALY, RuS, FDC and FDD and with those previously published (Lee *et al.*, 1998).

Grapevine samples infected by FD phytoplasma were further analysed by RFLP analyses with *TaqI* of P1A/P7A products obtained in nested-PCR following direct-PCR with P1/P7 primer pair.

### Results

GY incidence in the inspected vineyards was registered as number of symptomatic plants per total number of plants of each variety, converted in % in table 1. As shown in table 1 the average incidence almost doubled from year to year. In 2005 in Plovdina vineyards the disease incidence was over 90%, even in those vineyards in which the incidence was less than 5% at the beginning of observations.

The majority (about 88%) of tested grapevine samples were positive in nested-PCR assay using P1/P7 primer pair followed by R16F2n/R16R2. RFLP analysis permitted to identify FD phytoplasma in the samples collected in Sićeavačko region, on the other hand in Vršačko, Kutinsko and Deliblatska peščara regions the phytoplasma involved was BN (table 1). In the samples from Župsko both phytoplasmas were found to be present (table 1). RFLP analyses with *TaqI* endonuclease of P1A/P7A nested-PCR products permitted to classify the

**Table 1.** Incidence (%) of GY symptomatic grapevines during 2003-2005 and phytoplasma identification (P.) in vineyards located in five of the most important grape-growing regions of Serbia.

Region/ Vineyard	Grapevine variety	% of symptomatic grapevines			P.
		03	04	05	
Župsko	1 Black Burgunder	7	9	11	FD
"	" Italian Riesling	2	3	4	FD
"	2 Plovdina	78	96	100	FD
"	" Smederevka	13	27	42	FD
"	3 Župski bojadiser	19	34	59	FD
"	" Frankovka	5	12	29	FD BN
"	4 Plovdina	16	57	93	FD
"	5 Plovdina	70	92	100	FD
"	6 Plovdina	12	84	100	FD
"	" Prokupac	4	9	16	FD
"	7 Reinische Riesling	6	18	37	BN
Sićevačko	8 Plovdina	90	97	100	FD
"	9 Frankovka	16	35	42	FD
"	10 Plovdina	2	14	98	FD
"	11 Plovdina	3	20	91	FD
"	12 Plovdina	2	13	93	FD
"	13 Plovdina	4	33	92	FD
"	14 Plovdina	4	27	97	FD
Kutinsko	15 Chardonnay	8	12	15	BN
Vršačko	16 Chardonnay	9	12	18	BN
"	" Frankovka	29	34	35	BN
"	" Župljanka	5	7	11	BN
Deliblatska peščara	17 Župljanka	6	8	14	BN
Average %		18	33	56	

FD phytoplasmas as belonging to subgroup 16SrV-C. All ten examined cultivars were affected by GY (table 1). In particular, the FD phytoplasma was found in grapevines of Plovdina, Italian Riesling, Black Burgunder, Smederevka, Župski Bojadiser, Prokupac and Frankovka cvs. in Župsko, as well as in samples of Plovdina and Frankovka collected in Sićevačko. BN phytoplasma was detected in grapevine samples of Chardonnay, Frankovka, Župljanka and Reinische Riesling cvs. growing in Vršačko, Deliblatska peščara, Kutinsko and Župsko.

## Discussion

The present work confirms that phytoplasmas are widespread in all five inspected viticultural regions in Serbia and that two phytoplasma types, FD (subgroup 16SrV-C) and BN, are associated with grapevine yellows in Serbia (Duduk *et al.*, 2004; Kuzmanović *et al.*, 2004). Our results showed that all ten different cultivars were affected by GY. FD phytoplasma was detected in vine-

yards of central and eastern Serbia (Župsko and Sićevačko) in seven different cultivars. Considering this and previous works (Duduk *et al.*, 2004; 2006; Kuzmanović *et al.*, 2004) BN is present in all the most important grape growing regions of Serbia except Sićevačko.

Field inspections for determining GY incidence, showed as GY epidemics have been spreading very fast in the monitored vineyards, especially in those vineyards in which only the presence of FD phytoplasma was detected. For this reason effective control strategies should be adopted. The highest incidences of FD phytoplasma were found in vineyards of Plovdina, a local variety that appeared to be extremely sensitive to FD. It has been confirmed that Chardonnay is sensitive to BN. On the other hand, in the regions where FD phytoplasma was detected, symptomatic Chardonnay plants were not found. Frankovka, a cultivar imported from Croatia, was shown to be sensitive to both phytoplasmas (Kusmanović *et al.*, 2007).

## References

- DUDUK B., BOTTI S., IVANOVIC M., DUKIC N., BERTACCINI A., 2003.- Molecular characterization of a "flavescence dorée" phytoplasma infecting grapevine in Serbia, pp. 91-92. In: *Extended Abstracts 14th Meeting of ICVG*, Locorotondo (Bari), Italy, 12-17 September 2003.
- DUDUK B., BOTTI S., IVANOVIĆ M., KRSTIĆ B., DUKIĆ N., BERTACCINI A. 2004.- Identification of phytoplasmas associated with grapevine yellows in Serbia.- *Journal of Phytopathology*, 152: 575-579.
- DUDUK B., BOTTI S., IVANOVIĆ M., BERTACCINI A., 2006.- Status of grapevine yellows in Serbia.- pp. 193-194. In: *Extended Abstracts 15th Meeting of ICVG*, Stellenbosch, South Africa, 3-7 April 2006.
- KUZMANOVIĆ S., STAROVIĆ M., TOŠIĆ M., STOJANOVIĆ S., TOMIĆ T., 2003.- Phytoplasmas on grapevine in Serbia, pp. 93-95. In: *Extended Abstracts 14th Meeting of ICVG*, Locorotondo (Bari), Italy, 12-17 September 2003.
- KUZMANOVIĆ S., MARTINI M., FERRINI F., ERMACORA P., STAROVIĆ M., TOŠIĆ M., OSLER R., 2004.- Stolbur and "flavescence dorée" phytoplasmas present in grapevine in Serbia, pp. 138-139. In: *Book of Abstracts V Congress of Plant Protection* (VUKŠA P., Ed.), Zlatibor, Serbia, 22-26 November 2004.
- KUZMANOVIĆ S., MARTINI M., ERMACORA P., FERRINI F., STAROVIĆ M., TOŠIĆ M., CARRARO L., OSLER R., 2007.- Incidence and molecular characterization of "flavescence dorée" and stolbur phytoplasmas in grapevine cultivars from different viticultural areas of Serbia.- *Vitis*, submitted.
- LEE I.-M., GUNDERSEN-RINDAL D. E., DAVIS R. E., BARTOSZYK I. M., 1998.- Revised classification scheme of phytoplasmas based on RFLP analyses of 16SrRNA and ribosomal protein gene sequences.- *International Journal of Systematic Bacteriology*, 48: 1153-1169.
- MAIXNER M., LANGER M. GERHARD Y., 2006.- Grapevine yellows – current developments and unsolved questions, pp. addendum. In: *Extended Abstracts 15th Meeting of ICVG*, Stellenbosch, South Africa, 3-7 April 2006.

**Corresponding author:** Slobodan KUZMANOVIC (e-mail: kuzmanovic@beotel.yu), Institute for Plant Protection and Environment, Teodora Drajzera 9, Belgrade, Serbia.