

Grapevine yellows in Bosnia and Herzegovina: surveys to identify phytoplasmas in grapevine, weeds and insect vectors

Duška DELIĆ¹, Nicoletta CONTALDO², Samanta PALTRINIERI², Biljana LOLIĆ¹, Zorica ĐURIĆ¹, Snježana HRNČIĆ^{1,3}, Assunta BERTACCINI²

¹University of Banjaluka, Faculty of Agriculture, Bulevar vojvode Petra Bojovića 1A, 78000-Banjaluka, Bosnia and Herzegovina

²Dipartimento di Scienze e Tecnologie Agroambientali, Patologia vegetale, Alma Mater Studiorum-University of Bologna, Bologna, Italy

³University of Montenegro, Biotechnological Faculty, Bulevar Mihaila Lalića 1, 81000 Podgorica, Montenegro

Abstract

Presence and diffusion of grapevine yellows phytoplasmas was investigated in 2008 and 2010 in twelve vineyards located in two viticultural areas of Srpska region of Bosnia and Herzegovina. From the same vineyards, grapevine samples from different cultivars and weeds were collected for molecular analyses. In addition, some potential insect vectors were collected in the vineyards of these regions. 'Bois noir' phytoplasma presence was confirmed to be associated with grapevine yellows, while in one *Clematis vitalba* sample phytoplasma from 16SrV-C subgroup was identified. RFLP analysis of the *tuf* gene indicated the presence of the *tuf*-type b of stolbur phytoplasma in the 'bois noir'-infected samples. From collected hemipteran insects *Dictyophara europaea* and *Reptalus cuspidatus* were identified. Molecular analyses did not show phytoplasma presence in the tested insect samples of these species.

Key words: nested-PCR, RFLP analyses, grapevine yellows, 'bois noir', elm yellows phytoplasma group.

Introduction

Investigations on grapevine phytoplasmas associated with yellows diseases (GY) in Bosnia and Herzegovina (B&H) were done through several surveys in 2006, 2008 (Delic *et al.*, 2007; Delic and Lolic, 2010), and in 2010. Aim of the present work was to determine the presence of 'flavescence dorée' (FD) and 'bois noir' (BN) phytoplasmas in grapevine and verify the presence of alternative species that could host the phytoplasmas associated with these diseases as well as to collect potential insect vectors. Furthermore, polymorphisms studies BN strains detected in (B&H) were performed.

Materials and methods

Surveys of vineyards for GY disease presence in grapevine and in alternative host species were conducted in Srpska region of Bosnia and Herzegovina in 2008 and 2010. In the last year the survey was extended for collection potential insect vectors of GY. The monitored area was East Herzegovina (Trebinje, Popovo polje, Mokro polje, Dubljani, Dobromani, Arandelovo) the most important viticulture region of the Srpska. Several vineyards of the western part of the region (Bistrica, Dobo, Gradiška) were also subjected to surveys. Symptomatic samples were collected from 34 different grapevine cultivars (table 1). Weed samples such as bindweed (*Convolvulus arvensis*), *Clematis vitalba*, foxtail (*Setaria* spp.), dogwood (*Cornus* spp.) and vitex (*Vitex agnus castus*) were collected in the same vineyards and in the surrounding meadows. Hemipteran species including leafhoppers and planthoppers were collected by sweep net from *Clematis*, bindweed, vitex and grapevine plants and from

grasses in the meadows. Insects were collected from 3 sites in the East Herzegovina region and from 1 site in western part of the region during July and August 2010.

DNAs were extracted from the leaf midribs of plant samples using the DNeasy Plant Mini kit protocol (Qiagen, USA) with slight modifications. Identified insects were subjected to the DNA extraction following a method described by Maixner *et al.* (2006). DNA samples were then first tested with P1/P7 primer in direct PCR followed with R16F2n/R16R2 in nested-PCR: The latter amplicons were restricted with *Mbo*II and *Hpy*188I enzymes. All phytoplasma positive samples from grapevine were tested in nested PCR with Tuf1f/r/TufAYf/r, and TufINT1f/TufINT4r primer pairs (Botti *et al.*, 2005) followed by RFLP analyses with *Hpa*II to determine the *tuf*-type. All samples were checked also with FD specific primer pairs FD9f2/FD9r in direct PCR and FD9f3/FD9r2 in nested PCR.

Table 1. List of tested grapevine cultivars.

Surveys areas	Grapevine variety
East Herzegovina	Vranac, Smederevka, Kardinal, Afus Ali, Merlo, Cabernet
	Chardonnay, Muskat Galia, Nero, Čabski biser, Kraljica Konisen, Trollinger, Muskat Tolinger, Müller Thurgau, Guledei Wie, Merlo, Frankovka, Italian
West Herzegovina	Riesling, Chardonney, Lasta, Beogradsko rano, Afus Ali, Gročanka, Demi kan, Kardinal, Ribijer, Black Burgundy, Sauvignon, Chardonnay, Petra, Carmen, Muscat Italia, Muscat Hamburg, Rhine
	Riesling, Palatine, Panonija, Trolinger

Table 2. Phytoplasma detection 2008/2010 samples.

Type of samples	Total	BN	FD	year
grapevine	71	35	0	2008
weeds ¹	14	0	0	2008
grapevine	33	5	0	2010
weeds ²	34	0	1	2010

¹*C. vitalba* 7 samples; bindweed 6 samples, foxtail 1 sample; ²*C. vitalba* 9 samples; bindweed 10 samples, vitex 1 sample; dogwood 4 samples.

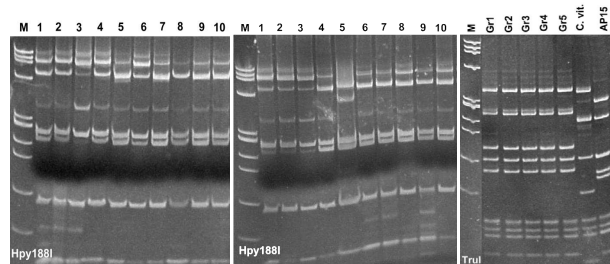


Figure 1. RFLP on R16F2/R2 amplicons. Selected (1 to 10) BN infected grapevine samples. BN (Gr); *C. vitalba* (*C. vit.*), apple proliferation (AP15). M, marker PhiX174 *Hae*III digested. Enzymes: *Hpy188I*, *TruI*.

Results

The presence of BN phytoplasma was detected in 40 out of 57 tested samples from East Herzegovina (Trebinje, Popovo polje, Mokro polje, Dubljani, Dobromani) as well as in two out of 47 tested samples from the western part of the region (Gradiška) (table 2). Restriction profiles obtained on R16F2/R2 amplicons showed some polymorphism that indicates possible presence of different BN strains (figure 1); RFLP analyses of tuf amplicons showed the presence of tuf-type b in all BN positive samples. Molecular analyses of weed samples showed presence of a phytoplasma from the 16SrV-C subgroup in one *C. vitalba* plant collected in August 2010 in Arandelovo (East Herzegovina) (table 2). Among collected hemipteran species in samples from East Herzegovina (Petrovo polje, Dubljani, Arandelovo) *D. europea* was mainly present while, from insects collected in western part of the region (Bistrica) *R. cuspidatus* was identified; nonetheless, phytoplasmas were not detected in the tested specimens of *D. europea* and *R. cuspidatus*.

Discussion

The present work demonstrated that BN phytoplasma is widespread in the main viticulture centres of the inspected regions. Results showed that several cultivars (Vranac, Smederevka, Kardinal, Afus Ali, Merlo, Cabernet, Frankovka) are BN-infected. East Herzegovina is very close with the border of Montenegro where BN infected grapevines were also reported (Radonjić *et al.*, 2009). On the other hand, a phytoplasma belonging to the 16SrV-C subgroup was for the first time detected in the country in a weed sample. FD on grapevine still has not been reported in B&H but it was confirmed to occur

in neighbouring countries such as Croatia (Šeruga Musić *et al.*, 2011), Serbia (Duduk *et al.*, 2004) and Slovenia (Hren *et al.*, 2007) together with its vector *Scaphoideus titanus* Ball. and potential vectors such as *Dictyophara europaea* L. (Filippin *et al.*, 2009). *S. titanus* was only found in East Herzegovina vineyards (Delic *et al.*, 2007).

In order to get clearer view of GY incidence, an extensive epidemiological study should be done monitoring Western Herzegovina vineyards, searching alternative host plants and performing vector transmission trials.

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- Corresponding author:** Duška DELIĆ (e-mail: duskadelic@yahoo.com), University of Banjaluka, Faculty of Agriculture, Banjaluka, Bosnia and Herzegovina.