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Surveys for grapevine yellows phytoplasmas in Bosnia and Herzegovina

Duška Delić[1], Gabrijel Seljak[2], Marta Martini[3], Paolo Ermacora[3], Luigi Carraro[3], Arben Myrta[4], Gordana Đurić[1]
[1]*University of Banja Luka, Faculty of Agriculture, Banja Luka, Bosnia and Herzegovina*
[2]*Kmetijsko gozdarski zavod Nova Gorica, Nova Gorica, Slovenija*
[3]*Dipartimento di Biologia applicata alla Difesa delle Piante, Università di Udine, Udine, Italy*
[4]*Istituto Agronomico Mediterraneo, Valenzano, Italy*

Abstract

Surveys were conducting in autumn 2004, 2006 and summer 2005 and 2007 in the traditional areas dedicated to grapevine cultivation in Bosnia and Herzegovina. The aim is to monitor the presence and distribution of grapevine yellows phytoplasmas diseases and asses the presence and distribution of *Scaphoideus titanus* vector of '*Candidatus* phytoplasma vitis'. The presence of phytoplasmas in plant samples and their identification was carried out by symptom observations in the field, nested polymerase chain reaction (nested-PCR) and restriction fragment length polymorphism (RFLP) analyses. The first laboratory analyses showed the presence of phytoplasma belonging to 16SrXII group, subgroup A. Presence of *S. titanus* was confirmed in the southern district of the country.

Key words: grapevine yellows, phytoplasma, vector, Bosnia and Herzegovina.

Introduction

Grapevine is important culture in Bosnia and Herzegovina (B&H) covering about 4000 ha. Grapevine industry mostly concentrated in the southern parts of the country where climatic conditions are favorable.

Presence of the grapevine yellows (GY) phytoplasmas for many years has not been investigated, mainly due to the absence of phytoplasma expertise and detection facilities available locally. During the last years, presence of several grapevine yellows phytoplasmas and their vectors have been reported in neighboring countries (Duduk *et al*., 2004; Magud and Tosevski, 2003; Skoric *et* al., 1998; Seruga *et al*., 2003). Moreover, plant propagation material (rootstocks, scions, and seedlings) are frequently imported from Serbia, Slovenia and Italy (Martini *et al*., 2002) where quarantine "flavescence dorée", FD phytoplasma and its vector are present and widely distributed.

The surveys mainly addressed to the symptomatic plants, the purpose is to monitor the presence of grapevine yellows phytoplasmas and asses the presence and distribution of *Scaphoideus titanus* Ball in B&H vineyards. The aim of the work is to improve knowledge of epidemiology of the phytoplasmas diseases in the country.

Materials and methods

Field surveys

Surveys were carried out in autumn (October 2004 and 2006) and summer (August 2005, and June 2007). The symptoms considered were leaf yellowing or reddening of leaf veins and blades, lack of lignifications. Commercial vineyards in the north-western (Banjaluka) and southern (Mostar, Stolac, Čapljina and Trebinje) areas of Bosnia and Herzegovina were inspected (figure 1). Samples were taken from symptomatic and symptomaless plants of both local and imported cultivars.

Some carrot (*Daucus carota*) and bindweed (*Convolvulus arvense*) showing phytoplasma symptoms were collected at random.

Figure 1. Map of surveyed locations in B&H for GYs and *S. titanus*.
(In colour at www.bulletinofinsectology.org).

From the June 2007, surveys for assessing the presence of *S. titanus* vector of "flavescence dorée" are carrying out. Yellow sticky traps were used for the capturing of the insect adults. The same vineyards in the southern (Trebinje) and northern-western (Banjaluka) district, were selected for collection of adults. From the beginning of July yellow sticky traps were changed every two weeks and send for identification at Kmetijsko gozdarski zavod Nova Gorica.

PCR/RFLP analyses

Samples collected during the survey 2004 and 2005 were tested by nested PCR method. The phytoplasma enrichment procedure modified by Malisano *et al.* (1996) was adopted for DNA extraction from leaves. Universal primers P1/P7 followed by R16F2n/R16R2 were used for the detection of phytoplasmas in the samples. The R16F2n/R2 amplicons were then digested with *Mse*I restriction enzyme. The results were double-checked by another nested PCR procedure using stolbur specific primer pair fStol/rStol (Maixner *et al*., 1995).

Results

Field surveys

Phytoplasma symptoms were observed in southern and north-western areas. In some vineyards located at the south, incidence of symptomatic plants was in range from 30 to 50%.

Several individuals of *S. titanus* identified on the traps from vineyards in Trebinje. The traps were exposed from the middle of July.

PCR/RFLP analyses

Ten samples out of 33 tested (collected during the surveys 2004 and 2005) were phytoplasma positive in a nested PCR with the universal primer pair. The 16S rDNA fragments amplified in the nested PCR from phytoplasmas detected in grapevine samples were subjected to RFLP analyses with the restriction enzyme *Mse*I. Restriction profiles obtained with all amplified products indicate the presence of "bois noir", BN belonging to the stolbur 16SrXII group, subgroup A.

This identification was confirmed in a second nested PCR with 16SrXII group-specific primer pair fStol/rStol.

Phytoplasmas of 16SrXII-A subgroup was also identified in a few samples of herbaceous plants (bindweed and carrot). Amplicons were only obtained from symptomatic samples from southern areas (Mostar and Trebinje).

Discussion

The results of the first survey for grapevine yellows phytoplasmas in B&H showed the presence of BN phytoplasma. Stolbur infection find in native and foreign cultivars indicates the probable role of *H. obsoletus* in the natural dissemination of the disease. In the past stolbur phytoplasma has been reported on vegetable plants (Aleksic *et al*., 1969).

The laboratory examination of the traps from southern districts (Trebinje) prove the presence of *Scaphoideus titanus* that represents first record of the vector in Bosnia and Herzegovina.

The frequent importation of uncertified plant material could easily lead to outbreaks of FD phytoplasma. Therefore, these results encourage us to extended monitoring of vineyards and preventing of FD outbreak.

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Corresponding author: Duška DELIĆ (e-mail: duskadelic@yahoo.com), University of Banjaluka, Faculty of Agriculture, Bulevar Vojvode Petra Bojovića 1A, Banjaluka, Bosnia and Herzegovina.