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Geographical distribution of 'flavescence dorée' phytoplasmas in Croatian grapevines

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Abstract

Seven Croatian grapevine samples found positive for the presence of 'flavescence dorée'(FD) phytoplasmas in 2010 survey confirm the first findings of FD phytoplasma from previous year, as well as the existence of new grapevine yellows heavily affected narrow area west and southwest of Zagreb along the border with Slovenia. Two new locations 100 km east and 60 km north-east of Zagreb, respectively, were found to be affected, widening the potential FD-risk zone and placing the capital in its centre. Grapevine varieties infected with FD-related phytoplasmas encompassed widely known Pinot Noir and Riesling, but also indigenous red Plemenka Crvena and Ružica Crvena, as well as white variety Škrlet. Preliminary molecular characterization results suggest the variability of 16SrV-C identified phytoplasmas, especially from the locations west and southwest of Zagreb.

Key words: 'flavescence dorée', 16SrV-C, PCR, RFLP.

Introduction

The occurrence of 'flavescence dorée' (FD) phytoplasma vector *Scaphoideus titanus* Ball (Budinščak *et al.,* 2005) in Croatia, as well as the occurrence of FD and/or *S. titanus* in the surrounding countries stimulated the efforts to identify the most heavily grapevine yellows (GY) affected areas in Croatia (Šeruga Musić *et al*., 2009). Although it had been expected that these areas would have been under the greatest FD infection pressure, FD-related phytoplasmas were detected in a new GY foci located west and southwest of Zagreb (Šeruga Musić *et al*., 2010).

This study aims to continue investigating grapevine phytoplasma diversity in the country, including the wider Zagreb area with confirmed FD-related phytoplasma occurrence.

Materials and methods

Leaf veins from mature grapevines were sampled and kept on $CaCl_2$ at 4°C for phytoplasma identification. Besides 40 symptomatic grapevines from the 2009 survey (Šeruga Musić *et al*., 2010), 55 grapevines from 2010 survey were included in this study. In addition, 29 *S. titanus* and 5 weed samples were analyzed for phytoplasma presence in 2010.

DNA was extracted (Mikec *et al*., 2006) and phytoplasma 16S rRNA gene was amplified by using generic primers P1/P7 (Deng and Hiruki, 1991; Schneider *et al.*, 1995) in direct PCR, followed by a nested PCR using primer pair R16F2n/R2 (Gundersen and Lee, 1996). Phytoplasma ribosomal group affiliation was determined by restriction fragment length polymorphism (RFLP) analysis of the nested PCR products with enzyme *Tru*1I (Fermentas, Vilnius, Lithuania).

P1/P7 amplicons from samples harbouring phytoplasmas belonging to the ribosomal group 16SrV were submitted to additional nested PCRs primed by group-specific primers R16(V)F1/R1 (Lee *et al*., 1994) and by 16Sr758f/M23Sr (Gibb *et al*., 1995; Padovan *et al*., 1995, respectively). The latter amplicons were digested with *Taq*I (Fermentas, Vilnius, Lithuania) for the RFLP analysis.

Results

Out of 40 grapevines showing typical GY symptoms collected throughout the country for phytoplasma analyses in 2009, 21 were positive for their presence. The majority of samples (19) harboured 'bois noir' (BN) agents, while for the first time two samples from the vicinity of Zagreb were found infected with phytoplasmas belonging to the ribosomal subgroup 16SrV-C (Šeruga Musić *et al*., 2010). The FD phytoplasmas still constituted only a portion of positive samples (9.5%). They were identified from red varieties Pinot Noir (Vivodina) and Plemenka Crvena (Brezje) geographically located in a narrow region west and southwest of Zagreb. These samples came from the highly infected vineyards in a smaller winegrowing region previously not determined to be under high GY infection pressure (Šeruga Musić *et al*., 2009).

In the batch of 55 grapevine samples from 2010, one sample of indigenous white variety Škrlet not analyzed in 2009 survey was also tested. The 25 positive samples detected reveal smaller percentage of infected samples in the 2010 (45.5%) compared to 2009 (52.5%). Yet, the proportion of grapevine samples positive for FD phytoplasmas increased to 28.0% (7 samples). These samples included Škrlet sampled in 2009 from Voloder (about 100 km east of Zagreb), Riesling and Ružica Crvena (indigenous red variety) from Brckovljani, a village about 60 km northeast of Zagreb. The remaining 4 FD phytoplasma positive grapevines were found in the same area as Plemenka Crvena and Pinot Noir from 2009 (figure 1). None of the weeds or *S. titanus* tested in 2010 was found phytoplasma positive in this study.

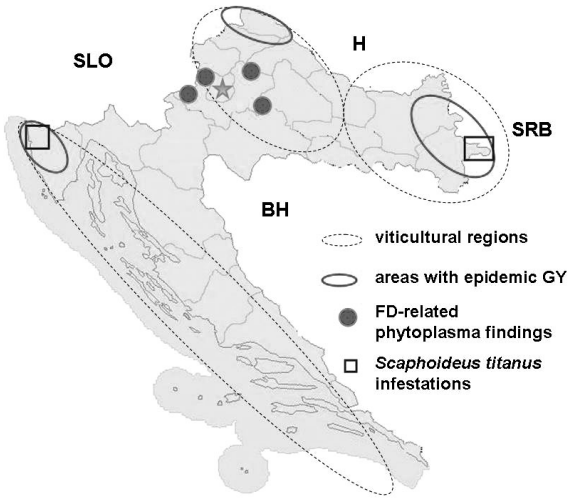

Figure 1. Geographical distribution of grapevines infected with FD phytoplasmas in 2009-2010 in relation to the most heavily GY affected areas in Croatia. The position of Zagreb is marked with a star.

Discussion

BN and FD phytoplasmas are the main GY agents in Euro-Mediterranean area. BN phytoplasmas belong to the ribosomal subgroup 16SrXII-A and they are still the most widespread in the Croatian winegrowing regions (Šeruga Musić *et al*., 2009; 2010). FD phytoplasmas belong to the ribosomal subgroups 16SrV-C and 16SrV-D and despite the finding of principal FD vector in Croatia six years ago (Budinščak *et al.,* 2005), no FD phytoplasmas had been detected before 2009 (Šeruga Musić *et al*., 2010). Interestingly, those phytoplasmas were detected in heavily symptomatic vineyards West (Brezje) and Southwest (Vivodina) of Zagreb in a narrow winegrowing area along the Croatian-Slovenian border. Fifteen *S. titanus* from these two locations were examined in 2010, but none was positive for phytoplasma presence. Nonetheless these initial negative results for the vectors in this area, this highly probable route of natural phytoplasma transmission and it should be further investigated. The finding of FD phytoplasmas in the Škrlet sample from Voloder in year 2009 widens the potential FD-risk area to almost 100 km East of Zagreb, while new FD grapevine positive samples from Brckovljani close this circle to about 60 km northeast placing Zagreb in its centre.

The sequences of the FD phytoplasmas from 2009 revealed affiliation of Vivodina phytoplasma to 16SrV-C ribosomal subgroup, while the other one from Brezje revealed some variability (Šeruga Musić *et al*., 2010). The nested PCR experiments with 16Sr758f/M23Sr primers, also suggested the presence of variability since this primer pair could not amplify the DNA from this sample. Similar difficulties were encountered with samples from the same area collected in 2010. Further analyses are needed to assess the molecular variability and epidemic potential of FD phytoplasmas from Croatian grapevines.

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