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Geographical distribution of "bois noir" phytoplasmas infecting grapevines in the Republic of Macedonia

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Abstract

The main viticultural production areas in the Republic of Macedonia were surveyed in 2006 for the presence of grapevine yellows. PCR and RFLP analyses were used to detect and identify phytoplasmas infecting grapevines. Only phytoplasmas associated with "bois noir" disease (ribosomal subgroup 16SrXII-A or stolbur) were detected. Molecular analyses showed that all phytoplasmas identified belonged to tuf-type II (VKII).

Key words: Phytoplasmas, "bois noir", grapevine yellows, PCR, RFLP.

Introduction

Grapevine yellows (GY) are serious diseases occurring in the most important viticulture areas in the world. They are caused by phytoplasmas, unculturable bacterial plant pathogens, which belong to different ribosomal groups including among several others: aster yellows (16SrI group), X-disease (16SrIII group), elm yellows (16SrV group) and stolbur (16SrXII group). "Bois noir" (BN) is the most widespread GY in Europe and in the Mediterranean basin and it is caused by pathogens of the stolbur group.

The presence of BN phytoplasmas in Macedonia was reported for the first time in 2003, in a survey limited to a small viticultural region, *i.e.* Veles and Skopje areas (Šeruga *et al.,* 2003). The aim of this work was to check the presence of GY phytoplasmas and their geographical distribution in the main viticultural production areas in Macedonia.

Materials and methods

During summer 2006, leaf samples were collected from 29 grapevines showing suspected GY symptoms. The most important and wider vineyards in Macedonia were chosen for this preliminary survey, in the areas of Negotino, Kavadarci, Strumica, Veles, Stip and Radovis (table 1). Data concerning variety, year of plantation, number and position of sampled plants in vineyards were noted, together with labelling and photographic report of symptomatic plants.

All the vine samples were analysed by means of molecular methods. DNA was extracted from all samples according to reported protocol (Angelini *et al.*, 2001). Nested PCR assays were carried out with phytoplasma universal and specific primer pairs, targeting ribosomal and non ribosomal gene fragments. The following primer pairs were used: P1/P7 (direct) (Deng and Hiruki, 1991; Schneider *et al*., 1995) and M1/B6 or R16(I)F1/R1 (nested) (Martini *et al*., 1999); ftuf1-rtuf1 (direct) and ftufAY-rtufAY (nested) (Langer and Maixner, 2004).

PCR reaction products were digested by restriction enzymes *Taq*I*, Tru9*I *and Hpa*II. The AAY and STOLC phytoplasma strains in periwinkle, belonging to the 16SrI-B and 16SrXII-A subgroups, respectively (E. Boudon-Padieu, Dijon, France), were used as a positive control in the PCR and RFLP analyses.

Results

Phytoplasma symptoms

The infected plants showed discolouration of leaves from light green to yellow or reddening, downward rolling of leaves and reduces quality and quantity of fruit. The most affected plants had small, rolling, yellow leaves with necrosis in white cultivars and reddish leaf coloration in red cultivars.

Phytoplasma detection

Thirteen grapevine samples out of 29 were found infected with GY phytoplasmas by means of the molecular analyses (table 1). Results of analyses with ribosomal and non ribosomal primer pairs were always in agreement, confirming that the 13 samples were infected with BN phytoplasma.

Restriction profiles obtained from all the amplified products indicated the presence of identical RFLP patterns in all the samples, resembling that of STOLC phytoplasma, which belongs to the 16SrXII-A subgroup. RFLP digestion of amplicons in the *tuf* gene showed that all the positive samples were infected with tuf-type II (VKII), one of the strains associated with BN disease in Europe.

Discussion

This survey included the most important and bigger vineyards, in the areas of Negotino, Kavadarci, Strumica, Veles, Stip and Radovis and it is the first report on the geographic distribution of GY in Macedonia.

Table 1. Results of nested PCR assays in grapevine samples from viticultural regions in Macedonia.

Region	Vineyard	Cultivar	PCR results M1B6 / ftuf/rtuf AY
Negotino	Ilo Vilarov	Italian Riesling	-/-
		Smederevka	+/+
		Vranec	+/+
		Grenache blanc	+/+
		Rkatsiteli	-/-
		Zilavka	+/+
Kavadarci	Ljubas	Italian Muscat	-/-
		Afus ali	-/-
		Smederevka	-/-
		Chardonnay	+/+
Strumica	Hamzali	Grenache blanc	+/+
		Vranec	-/-
		Rkatsiteli	-/-
		Smederevka	-/-
Radovis	Dobridol	Plovdina	-/-
		Smederevka	+/+
		Rhein Riesling	-/-
		Vranec	+/+
Stip	Kavaklija	Rhein Riesling	-/-
		Pinot noir	-/-
		Afus ali	-/-
	Ezovo	Afus ali	-/-
		Vranec	+/+
	Tri Cesmi	Smederevka	+/+
		Pinot noir	-/-
		Rhein Riesling	+/+
	Vrsakovo	Vranec	+/+
Veles	Sopot	Chardonnay	+/+
		Afus ali	-/-

The results of the molecular analyses on samples collected in summer 2006 showed the presence of "bois noir" phytoplasmas. These results confirmed previous studies carried out in a limited area of Macedonia (Seruga *et al.*, 2003). All grapevines were infected with tuf-type II (VKII) isolate, the BN strain found also in convolvulus (Langer and Maixner, 2004). Further studies to verify the presence of BN vectors in affected areas, in order to control the spread of the disease are in progress.

"Bois noir" disease presence was reported around Macedonia: Bulgaria, Serbia, Albania and Greece (Davis *et al.*, 1997; Duduk *et al.*, 2003; 2004; EPPO, 2006). On the opposite, FD phytoplasma and its vector, *Scaphoideus titanus*, have been reported only in Serbia so far (Magud and Toševski, 2004). FD phytoplasma was not found in grapevine collected during this survey; however, more extensive investigations are needed to check the presence of FD phytoplasma and of its vector in Macedonia, considering that they are widespread in the neighboring Serbia. In addition to that, the presence of FD phytoplasma was reported in clematis plants collected from Macedonia (Filippin *et al.*, 2007).

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