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Research Note

Survey for grapevine yellows phytoplasmas in diverse European countries and Israel

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S u m m a r y : Phytoplasmas of a large number of GY-affected grapevines from several European countries and Israel were characterized using PCR-RFLP of a 16S rDNA region. Only phytoplasmas in the EY group and the stolbur group were found. The latter was the most wideley spread.

K e y w o r d s : phytoplasma, grapevine yellows, characterisation, flavescence dorée, bois noir.

Introduction: The term grapevine yellows (GY) refers to a group of diseases of *Vitis vinifera* with similar symptoms which are caused by phytoplasmas (previously mycoplasma-like organisms). Recently it has been shown, mainly on the basis of 16S rDNA analysis, that the phytoplasmas associated with GY belong to different groups (Daire *et al.* 1993 a; Prince *et al.* 1993; Seemüller *et al.* 1994). The flavescence dorée (FD) phytoplasma in Southern France was assigned in the elm yellows (EY) group as well as some GY phytoplasmas detected in Northern Italy (Daire *et al.* 1993 a, b, Bertaccini *et al.* 1995) and Germany (Maixner *et al.* 1995) while the bois noir disease and the Vergilbungskrankheit phytoplasmas, detected in Northern France (Daire *et al.* 1993 b) and Germany (Maixner *et al.* 1994), belong to the stolbur (STOL) group, as well as the GY phytoplasmas detected in Italy, Israel (Daire *et al.* 1993 a) and in Cataloña (Laviña *et al.* 1995). By other authors the latter group is referred to as Aster yellows subgroup I-g (Bertaccini *et al.* 1995). In addition, grapevine phytoplasma members of the X-disease group were found in Northern Italy and in Northern America (Prince *et al.* 1993, Daire *et al.* 1993 a) and more recently an Australian GY-associated phytoplasma could be classified in the Aster yellows group (Padovan *et al.* 1995). The aim of the present study is to provide further insight into GY etiology in Europe and Israel.

Material and methods: From 1992 to 1995 GY-affected *V. vinifera* were sampled in 19 regions in France, Switzerland, Italy, Spain and Israel (Table). Altogether 306 samples were tested.

The phytoplasmas were detected by PCR amplification of a region of 16S rDNA. The procedure was essentially the same as previously described (Daire *et al.* 1993 b) except that we used the phytoplasma specific primer pair fU5/rU3 (Lorenz *et al.* 1995) which spans a region of about 860 bp. The phytoplasmas were subsequently classified in different groups according to the *Tru*9I restriction profile of the rDNA as illustrated in the Figure.

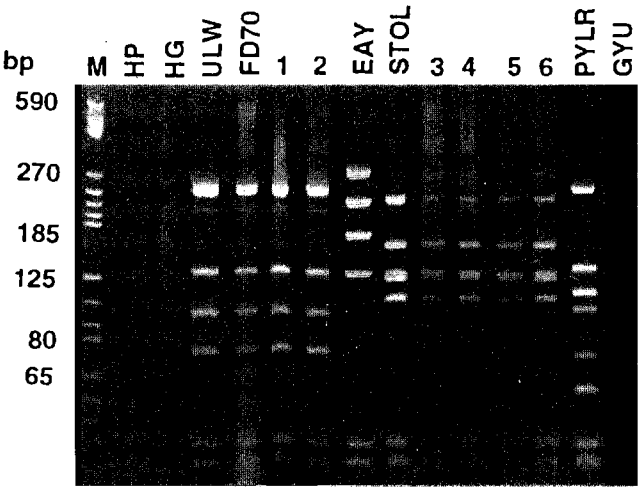

Figure: PAGE of *Tru*9I-digested 16S rDNA amplified from phytoplasmas in GY-diseased grapevines collected in various regions. 1, Cabernet Sauvignon from Gironde (France). 2, Garganega from Friuli-Venezia Giulia (Italy). 3, Grenache from Aude (France). 4, Chardonnay from Friuli-Venezia Giulia. 5, Chardonnay from Switzerland. 6, Chardonnay from Israel. Phytoplasma reference strains maintained in periwinkle and members of the EY, aster yellows (AY), STOL and X-disease (XD) groups were joined for comparison. ULW, elm yellows. FD70, flavescence dorée (EY group). EAY, European aster yellows (AY group). STOL, stolbur of tomato strain F (STOL group). PYLR, peach yellow leaf roll. GYU, grapevine yellows from Udine (XD group). M, molecular weight marker (pBR322/*Hae*III). HP, healthy periwinkle. HG, healthy Chardonnay.

Results and Discussion: The results indicate that only phytoplasmas belonging to the EY or the STOL group were detected (Table). Concerning the EY group phytoplasmas, one should point out that the *Tru*9I restriction analysis of the rDNA region analysed does not allow to distinguish between EY and FD phytoplasmas. However, the EY group phytoplasmas detected in this study were actual FD phytoplasmas as shown subsequently by successful amplification of their DNA using FD specific primer pairs (Daire unpubl. data). This is also corroborated by the presence of *Scaphoideus titanus*, the FD vector, in vineyards where such phytoplasmas are present. The occurrence of EY group phytoplasmas, i. e. FD phytoplasma, in the Languedoc and Roussillon areas and in Friuli-Venezia Giulia have already been reported (Daire *et al.* 1993 b) and in Armagnac it corresponds to the FD disease known from the 1950s. Conversely, for the first time a FD phytoplasma was detected in the Bordeaux area where in several vineyards a severe outbreak of the disease occurred in 1994.

In the survey a STOL phytoplasma was identified in 17 out of 19 regions for the first time (exceptions: Bourgogne, Vallée du Rhône-Ardèche and Israel). These findings, along with previous ones, suggest that this phytoplasma in Europe, and possibly in Israel, is more frequently associated with GY than others. Maixner *et al.* (1994) in

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Table

Detection and identification of GY phytoplasmas by PCR-RFLP of rDNA

Country	Viticultural area	phytoplasma group EY[a]	STOL[a]
France	Southern Champagne (Haute Marne)		4/5
	Bourgogne		27/40
	Franche Comté		19/31
	Alsace		1/2
	Savoie		8/8
	Diois		1/1
	Vallée du Rhône (Ardèche)		7/10
	Vallée du Rhône (Vaucluse)		15/18
	Anjou		5/5
	Poitou		3/3
	Bordeaux	8/13	
	Armagnac	5/6	
	Languedoc	36/46	4/46
	Roussillon	28/47	7/47
Switzerland	Valais		4/4
Italy	Trentino		10/10
	Friul-Venezia Giulia	18/37	8/37
Spain	Navarra		3/4
Israel	Golan		15/16

[a] number of positive samples *vs.* total tested samples

Germany and SFORZA *et al.* (1996) in France, demonstrated that the planthopper *Hyalesthes obsoletus* could be a vector of a STOL phytoplasma to grapevines. The significance of this and other potential vectors of a STOL phytoplasma is currently under investigation. The fact that FD and STOL phytoplasmas can be present in the same area should make possible mixed infections, however, so far, we have not detected such occurrence. Although investigations should be continued, the absence of detection of a phytoplasma belonging to the X-disease group in this study suggests that it plays a secondary role in GY epidemics in Europe.

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