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Presence of phytoplasmas in hemipterans in Czech vineyards

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Abstract

A survey to monitor the presence of stolbur phytoplasma in its putative vectors was conducted in infected vineyards of South Moravia. Collected hemipterans were analysed using polymerase chain reaction and detected phytoplasmas were identified after restriction fragment length polymorphism of 16S rRNA gene. In the majority of the samples tested, phytoplasmas of the aster yellows group were detected.

Key words: phytoplasma, vectors, hemipterans, nested PCR, RFLP.

Introduction

The preliminary survey of hemipterans as putative vectors of phytoplasmas was conducted in 2008-2009 in the South Moravian region of the Czech Republic, in vineyards infected with Potato stolbur phytoplasma.

The most important vector of stolbur phytoplasma is the planthopper *Hyalesthes obsoletus* Signoret (Sforza *et al*., 1998). Other reported stolbur phytoplasma vectors occurring in the Czech Republic are *Reptalus panzeri* (P. Low), *Euscelis incisus* (Kirschbaum), *Macrosteles laevis* (Ribaut), *Macrosteles quadripunctulatus* (Kirschbaum), *Macrosteles cristatus* (Ribaut), *Macrosteles viridigriseus* (Edwards), *Speudotettix subfusculus* (Fallen), *Anoscopus albifrons* L., *Aphrodes bicinctus* (Schrank) and true bugs of the genus *Lygus* Hahn (Neklyudova and Dikit, 1973; Palermo *et al*., 2004). Vectors of aster yellows disease are leafhoppers (family Cicadellidae).

The hemipterans tested for phytoplasma presence by PCR were members of Cixiidae, Delphacidae, Cicadellidae, Aphrophoridae and Miridae family.

Materials and methods

In infected vineyards, the two groups of hemipterans, polyphagous and insects preferring Poaceae family plants, were collected from grapevine plants, weeds, and cover crop between vine rows, see table 1.

Collected insect material was determined and sorted by sex, development stadium and locality of finding and divided to 157 laboratory samples. Each sample consisted of one to ten individuals and was freeze-stored in absolute ethanol.

The total DNA was extracted using High Pure PCR template Preparation Kit (Roche) according to the manufacturer´s protocol for the isolation of nucleic acids from mammalian tissue. Purified DNA was eluted twice with 50 µl of elution buffer.

Presence of phytoplasma was analysed using PCR with R16R0/R16F1 primer pair followed by nested amplification of 16S rRNA phytoplasma gene with R16F2n/R16R2 primers (Gundersen and Lee, 1996). Positive samples were further analysed using restriction fragment length polymorphism of PCR product to identify the detected phytoplasma. Moreover, the stolbur phytoplasma positive samples were characterised by analysis of non-ribosomal tuf gene sequence amplified using PCR with primer pair fTufAY/rTufAY (Schneider *et al*., 1997) followed by nested PCR with fTufAY2/rTufAY2 primers in combination with restriction analysis (Pasquini *et al*., 2007).

Results

Out of 157 samples tested, 39 resulted phytoplasma positive using 16S rRNA nested-PCR. Although all insect samples were collected in stolbur phytoplasma infected vineyards, the phytoplasma was detected only in one sample after restriction fragment length polymorphism analysis with *Alu*I, *Fsp*BI, *Hae*III, *Hha*I, *Hinf*I, *Mse*I, *Msp*I, *Rsa*I and *Sau*3AI enzymes (Lee *et al*., 1998). *Tuf* gene sequence analysis revealed the detected stolbur phytoplasma to belong to the tuf-type b (data not shown).

Digestion of PCR products of the remaining 38 phytoplasma positive samples with the same nine enzymes revealed that 81% of samples contained aster yellows phytoplasmas, subgroups B, C and F according to Marcone *et al*. (2000). Eight percent was identified as X-disease phytoplasma, subgroup B and 3% of samples belonged to the Bermuda grass white leaf phytoplasma ('*Candidatus* Phytoplasma cynodontis') according to Lee *et al*. (1998). Three percent revealed mixed infection of aster yellows and X-disease phytoplasma and five percent of samples resulted in unknown restriction profiles. Overview of the results is shown in table 1.

Discussion and conclusions

The obtained results indicate that some of the collected hemipterans species might be putative vectors of phytoplasmas in Czech Republic. Although the vineyards were stolbur infected, the stolbur phytoplasma was detected only in *H. obsoletus*. In most of the positive samples were found phytoplasmas of the aster yellows group, primarily in *Jassargus obtusivalvis*, *Euscelis incisus* and *Javesella pellucida*.

Table 1. Summary of hemipteran species and phytoplasmas detected in them according with sex or stadium of development. Polyphagous insects are marked with *.

Phytoplasma identified	Species tested	No. of samples		
		male	female	larvae
Aster yellows 16SrI-B	*Macrosteles quadripunctulatus* (Kirschbaum)*	1	0	0
Aster yellows 16SrI-C	*Hardya tenuis* (Germar)*	1	0	0
	Jassargus obtusivalvis (Kirschbaum)	5	2	0
	Javesella pellucida (F.)	1	0	0
	Laodelphax striatella (Fallen)	0	1	0
Aster yellows 16SrI-F	*Aphrodes bicinctus* (Schrank)*	1	0	0
	Errastunus ocellaris (Fallen)*	1	1	0
	Euscelidius variegatus (Kirschbaum)*	0	1	0
	Euscelis incisus (Kirschbaum)*	2	3	1
	Hardya tenuis (Germar)*	0	1	0
	Jassargus obtusivalvis (Kirschbaum)	3	0	1
	Javesella pellucida (F.)	2	1	0
	Laodelphax striatella (Fallen)	1	0	0
	Philaenus spumarius (L.)*	0	1	0
X-disease 16SrIII-B	*Euscelis incisus* (Kirschbaum)*	1	0	0
	Jassargus obtusivalvis (Kirschbaum)	0	1	0
	Lygus rugulipennis (Poppius)*	0	0	1
X-disease + aster yellows 16SrIII-B + 16SrI-F	*Euscelis incisus* (Kirschbaum)*	0	1	0
Bermudagrass white leaf 16SrXIV	*Jassargus obtusivalvis* (Kirschbaum)	1	0	0
Stolbur phytoplasma 16SrXII	*Hyalesthes obsoletus* Signoret*	1	0	0
Unidentified	*Laodelphax striatella* (Fallen)	1	1	0

The tuf-type b of stolbur phytoplasma correspond to the type earlier reported in Czech vineyards (Fialová *et al*., 2009).

Up to now, the presence of bermudagrass white leaf phytoplasma as well as the aster yellows phytoplasma, subgroup 16SrI-F was not reported in Czech Republic, and their presence in plant hosts needs to be confirmed.

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