Weed Hosts of Phytoplasmas in the Czech Republic

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Abstract

The research was focused on wild plants that represent possible sources of phytoplasma infection. Thirteen species of wild herbaceous plants with symptoms of shoot proliferation, phyllody, virescence, sterility or yellowing have been collected from different locations of the Czech Republic. To detect and identify phytoplasmas, plants were subjected to electron microscopy, fluorescence microscopy, PCR, and RFLP analysis. There were phytoplasma positive species: *Matricaria chamomilla, Plantago lanceolata, Plantago media, Silene latifolia* ssp. *alba, Stellaria media, Taraxacum officinale, Thlaspi arvense, Tragopogon pratensis, Trifolium pratense, Trifolium repens, Trifolium hybridum, Tripleurospermum maritimum*, and *Verbascum densiflorum*. All the phytoplasmas detected were identified as belonging to the aster yellows group (subgroup 16SrI-B and 16SrI-C). With regard to a relatively high incidence of positively tested plants from several locations, *Trifolium* species are considered to be one of the most important natural reservoirs of aster yellows phytoplasma within the Czech Republic.

Keywords: *Matricaria chamomilla*; *Plantago lanceolata; Plantago media; Silene latifolia* ssp. *alba; Stellaria media; Taraxacum officinale; Thlaspi arvense; Tragopogon pratensis; Trifolium pratense; Trifolium repens; Trifolium hybridum; Tripleurospermum maritimum; Verbascum densiflorum*; aster yellows phytoplasma; PCR; RFLP; TEM; DAPI staining

INTRODUCTION

Phytoplasmas represent an important group of pathogens that cause diseases in several hundred plant species including crop-plants. It is assumed, that perennial wild herbaceous plants represent possible sources of phytoplasmas infection. Phytoplasmas are often monitored on vineyard weeds in grapevine growing countries (ARZONE *et al*. 1995; MAIXNER *et al*. 1995; ŠKORIC *et al*. 1998). Wild herbaceous plants of different species infected with phytoplasmas have also been studied on a material originating from Italy and Germany (MARCONE *et al*. 1997a,b; SCHNEIDER *et al*. 1997). In former Czechoslovakia, symptoms indicating phytoplasma disease etiology have been described in many wild plant species by Bojňanský, Blattný, Valenta, and Musil (see KLINKOWSKI 1977). However, the current situation of phytoplasma occurrence in wild plants in the Czech Republic is poorly understood. In our study, the combined employment of microscopy and molecular techniques was used to detect and identify phytoplasmas infecting weeds in the Czech Republic.

MATERIAL AND METHODS

Plants. Plants of thirteen wild herbaceous species with symptoms attributable to phytoplasma infection (phyllody, virescence, proliferation, yellowing, and stunting) were collected in different locations in the Czech Republic from 1997 to 2001 during the growing seasons. There were: *Matricaria chamomilla* S. F. Gray (Jamné locality – south Bohemia), *Plantago lanceolata* L. (Jamné locality), *P. media* L. (Jamné locality), *Silene latifolia* ssp. *alba* (Miller) Greuter et Burdet (Olomouc locality – central Moravia), *Stellaria*

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media (L.) Vill. (Jamné locality), *Taraxacum officinale* Web. (Jamné locality), *Thlaspi arvense* L. (Olomouc locality), *Tragopogon pratensis* L. (Litoměřice locality – north Bohemia), *Trifolium pratense* L. (2 localities: Jamné, Hladké Životice – north Moravia), *T. repens* L. (3 localities: Jamné, Olomouc, Hradec nad Svitavou – east Bohemia), *T. hybridum* L. (Jamné locality), *Tripleurospermum maritimum* (L.) Schultz (2 localities: Olomouc, Jamné), and *Verbascum densi orum* Bertol. (Louka locality – south Bohemia). Healthy plants of the species were included in the study as controls.

Fluorescence microscopy. Segments of stem and petiole parts of diseased plants were fixed, then cut into 18 μm thin tangential sections, stained in a solution of 4',6-diamidino-2-phenylindole.2HCl (DAPI) and examined with a fluorescence microscope.

Electron microscopy. Segments of stem and petiole parts were post-fixed in 1% osmium tetraoxide, dehydrated by washing in solutions with increasing ethanol content and embedded in Durcupan (Fluka, Busch, Switzerland) resin. Ultra-thin sections were stained with uranyl acetate in 70% ethanol and lead citrate and examined with a Philips 420 electron microscope.

DNA extraction. The isolation of DNA was performed according to the procedure of AHRENS and SEEMÜLLER (1992) using fresh shoots.

Detection of phytoplasmas by PCR. Universal primers derived from 16S rRNA gene were employed for detection of phytoplasmas. PCR assays were carried out using the primer pair R16F1/R0 (LEE *et al*. 1995). Products were diluted (1:5) with sterile deionized water, and re-amplified with the primer pair R16F2/R2 (LEE *et al*. 1995) in nested-PCR. PCR products were analysed by electrophoresis through a 1.5% agarose gel and stained with ethidium bromide. DNA bands were visualized with an UV transilluminator.

Identification of phytoplasmas by RFLP analysis. Nested-PCR products obtained with the primer pair R16F2/R2 were subjected to digestion with selected restriction endonucleases. 18 μl of each PCR product was digested separately with *Mse*I (New England Biolabs, USA) and *Alu*I (Promega, USA) according to the instruction of the manufacturer. The restriction products were separated by electrophoresis through 3% MetaPhor agarose (FMC, USA) gel stained with ethidium bromide and visualized with an UV transilluminator.

RESULTS

Phytoplasmas were detected in all symptomatic plants of 13 species using at least two methods (see Table 1). PCR-amplification of phytoplasma specific DNA was achieved with template prepared from all symptomatic plants examined. No amplification was obtained from any healthy plants. Products of nested-PCR primed by R16F2/R2 were subjected to RFLP analysis. The results confirmed the presence of phytoplasmas belonging to the aster yellows group

Table 1. Results of phytoplasma detection and identification in symptomatic weeds from the Czech Republic

Species (numbers of analysed plants)	Symptoms	TEM	DAPI staining	PCR	RFLP pattern
Matricaria chamomilla (1)	V, Ph, P	+	nt	+	16SrI-B
Plantago lanceolata (1)	V	+	nt	+	16SrI-B
Plantago media (1)	V	+	nt	+	16SrI-B
Silene latifolia ssp. *alba* (2)	V	nt	+	+	16SrI-B
Stellaria media (6)	V, P	+	nt	+	16SrI-B
Taraxacum officinale (4)	Fm	+	nt	+	16SrI-B
Thlaspi arvense (2)	V, P	+	+	+	16SrI-B
Tragopogon pratensis (1)	V	nt	+	+	16SrI-C
Trifolium pratense (9)	Ph, V, Ye	+	+	+	16SrI-B, 16SrI-C
Trifolium hybridum (6)	Ph, Ye	+	nt	+	16SrI-C
Trifolium repens (4)	Ph, V, Ye	+	+	+	16SrI-B, 16SrI-C
Tripleurospermum maritimum (8)	V, P	+	+	+	16SrI-B
Verbascum densiflorum (3)	P	+	+	+	16SrI-B

V – symptoms of virescence; Ph – symptom of phyllody; Ye – yellowing; P – proliferation; Fm – flower malformation; + positive detection; nt – not tested

(16SrI). Except *Trifolium pratense*, *T. repens* and *Tragopogon pratensis*, all diseased plants of the examined species were associated with phytoplasmas of 16SrI-B subgroup. In a diseased plant of *Tragopogon pratensi*s, phytoplasmas of 16SrI-C subgroup were found. Phytoplasmas of both subgroups were separately detected in *Trifolium pratense* and *T. repens* samples. No mixed infection of 16SrI-B and 16SrI-C subgroups was found. In view of the frequent occurrence of symptomatic plants, *Trifolium* species are considered to be the most important natural reservoirs of aster yellows phytoplasma within common weed in the Czech Republic.

Tragopogon pratensis and *Verbascum densiflorum* represent new phytoplasma host species.

In the weed species of *Matricaria chamomilla, Silene latifolia* ssp. *alba, Thlaspi arvense*, and *Tripleurospermum maritimum* this is the first molecular phytoplasma identification.

DISCUSSION

In naturally diseased plants of several weed species originated from the Czech Republic, only aster yellows phytoplasma has been identified. Weeds tested positively may represent reservoirs of aster yellows phytoplasma and thus a potential risk for crops (NAVRÁTIL *et al*. 1999). Within the species analysed, *Trifolium* species are considered to be the most important source of phytoplasma infection, especially in the case of *Trifolium repens*, the infected populations of which have been persistent for five years within studied localities. In addition, this species was also found to host phytoplasmas of the 16SrI-C subgroup, however, the most plants of the other species harboured phytoplasmas of the 16SrI-B subgroup. Although, virescence, phyllody and other disease symptoms on *Trifolium* species were known, this is the first molecular identification of phytoplasmas in symptomatic material from the Czech Republic. In general, there are several reports of molecular identification of phytoplasmas infecting *Trifolium* species from Europe. Phytoplasmas with very high similarity with those belonging to the group 16SrIII are reported in *Trifolium pratense* and *T. repens* from Italy (FIRRAO *et al*. 1996). Clover phyllody phytoplasma (subgroup 16SrI-C) was stated in *T. repens* from Germany (SCHNEIDER *et al*. 1997). In Lithuania, clover phyllody-diseased plants were infected by a subgroup 16SrI-C (STANIULIS *et al*. 2000). These authors also mentioned a mixed phytoplasma infection (16SrI-C and 16SrIII-B) in clover plants with dwarf symptoms.

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