

Molecular identification of 16SrIII and 16SrXII phytoplasma groups in *Chenopodium album* in Czech Republic

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Abstract

Chenopodium album L. plants showing symptoms of leaf yellowing, small leaves development, proliferation, plant and inflorescence stunting were examined for phytoplasma infection using PCR with universal phytoplasma primers R16F1/R0 and fU5/rU3. Only in two samples out of the 38 tested, the phytoplasma infection was confirmed. RFLP and sequence analyses based on partial 16S rDNA fragment confirmed that *C. album* is harbouring phytoplasmas belonging to 16SrIII (X-disease) group. This species has been also found infected with phytoplasmas belonging to 16SrXII group in Czech Republic.

Key words: weed, lambsquarters, phylogeny, X-disease, stolbur, phytoplasma.

Introduction

There are only a few data concerning the phytoplasma occurrence in *Chenopodium* species. Firstly the phytoplasma infection of *Chenopodium album* L. was reported by Seruga *et al.* (2003) during survey for phytoplasma detection in Croatian vineyards; phytoplasmas were identified by the use of nested PCR and RFLP analyses as belonging to the 16SrIII group. Tolu *et al.* (2006) obtained one positive out of seven *Chenopodium* spp. samples collected during the vineyards surveys in Italy and classified this phytoplasma into the subgroup 16SrII-E by RFLP analyses. *Chenopodium murale* L. was also identified as an alternative phytoplasma reservoir for the 16SrII phytoplasma associated with a lime decline disease in Saudi Arabia (Alhudaib *et al.*, 2009).

The aim of this work was to characterise phytoplasmas detected in *C. album* collected within tomato and pepper fields in the Czech Republic.

Materials and methods

Since the beginning of vegetation period from 2008 to 2010, 38 samples from *C. album* plants were collected in the horticultural region of south Moravia (Lednice) within tomato and pepper fields. The plants exhibited leaf yellowing, small leaves development, proliferation, plant and inflorescence stunting (figure 1).

The total DNA extraction was performed according to an enrichment procedure (Ahrens and Seemüller, 1992). DNA samples were subjected to PCR analysis with phytoplasma primer pairs in 16S rRNA gene. Nested-PCR assays were performed with primers pairs R16F1/R0 followed by fU5/rU3 (Lee *et al.*, 1995; Lorenz *et al.*, 1995). Identification of phytoplasmas was carried out by RFLP analyses using *AluI*, *MseI*, and *RsaI* according to Lee *et al.* (1998).

PCR products were directly cloned using pGEM-T (Promega) cloning kit, following the instructions of the manufacturer. Nucleotide sequences were obtained on automated ABIPRISM 3130 Genetic analyzer (Perkin

Elmer Applied Biosystems, Lincoln). Sequence data were analyzed by DNASTAR programme (Lasergene). The sequences were compared with those available in the GenBank using BLAST algorithm version 2.2.25.

Results

In two out of the 38 examined samples the presence and identity of phytoplasmas was confirmed by PCR/RFLP and sequence analysis. Products of expected length were obtained in fU5/rU3 PCR (879 bp). No amplification was observed in samples of healthy plant and water controls. The samples showed two distinct profiles in RFLP analyses, one corresponding to phytoplasmas belonging to 16SrIII and the second to 16SrXII ribosomal groups.

The identity of phytoplasma strains was confirmed by sequence analysis of fU5/rU3 PCR fragments. Strain number 2692 showed the 99% identity with stolbur phytoplasmas from grapevine from Italy, CH-1 (Acc. No. HQ589193), from potatoes from Russia, RuS93 (GU004375) and from tomato from Italy, PTV (GU004374); strain number 2909 showed 99% identity with X-disease phytoplasmas - *Cirsium* strain (FN298626) and *Taraxacum* strain (FN298621) from Finland and clover phyllody phytoplasma (HQ589196) from Italy.

Discussion

These findings agree with published results where the *Chenopodium* spp. is described as a sporadic host of different phytoplasmas. Up to date *Chenopodium* plants infected by 16SrIII group phytoplasmas were only in vineyards in Croatia (Seruga *et al.*, 2003). Other *Chenopodium* spp. showing yellowing and stunting associated with phytoplasmas belonging to 16SrII group have been reported from vineyard in Italy (Tolu *et al.*, 2006) and in case *C. murale* from citrus farm in Saudi Arabia (Alhudaib *et al.*, 2009). Although Özdemir *et al.* (2009) reported *C. album* L. as an alternative host of

tomato phytoplasmas, our results represents the first molecular identification of phytoplasma belonging to 16SrXII group in lambsquarters. The sporadic detection of phytoplasma infected lambsquarters plants in the locality with mass occurrence of phytoplasma diseases (Navrátil *et al.*, 2009) is probably connected with the low attractivity of weed plants for insect vectors compared to pepper and tomato plants.

Occurrence of phytoplasmas belonging to 16SrIII group was previously described in Czech Republic in *Trifolium* spp. (Fránová *et al.*, 2004), where it is not very common. Phytoplasmas belonging to the 16SrXII group are more common, causing the local epidemics in solanaceous crops (Fránová *et al.*, 2009; Navrátil *et al.*, 2009).



Figure 1. *C. album* plants. A. Infected plants showing leaf yellowing, small leaves development, proliferation, plant and inflorescence stunting. B. healthy plant.

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References

- AHRENS U., SEEMÜLLER E., 1992.- Detection of DNA of plant pathogenic mycoplasma-like organisms by a polymerase chain reaction that amplifies a sequence of the 16S rRNA gene.- *Phytopathology*, 82: 828-832.
- ALHUDAIB K., AROCHA Y., WILSON M., JONES P., 2009.- Molecular identification, potential vectors and alternative hosts of the phytoplasma associated with a lime decline disease in Saudi Arabia.- *Crop Protection*, 28: 13-18.
- FRÁNOVÁ J., PALTRINIERI S., BOTTI S., ŠIMKOVÁ M., BERTACCINI A., 2004.- Association of phytoplasmas and viruses with malformed clovers.- *Folia Microbiologica*, 49: 617-624.
- LEE I-M., BERTACCINI A., VIBIO M., GUNDERSEN D. E., 1995.- Detection of multiple phytoplasmas in perennial fruit trees with decline symptoms in Italy.- *Phytopathology*, 85: 728-735.
- LEE I-M., GUNDERSEN-RINDAL D. E., DAVIS R. E., BARTOSZYK I. M., 1998.- Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein genes sequences.- *International Journal of Systematic Bacteriology*, 48: 1153-1169.
- LORENZ K. H. B., SCHNEIDER U., AHRENS E., SEEMÜLLER E., 1995.- Detection of the apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and non ribosomal DNA.- *Phytopathology*, 85: 771-776.
- NAVRÁTIL M., VÁLOVÁ P., FIALOVÁ R., LAUTERER P., ŠAFÁŘOVÁ D., STARÝ M., 2009.- Incidence of stolbur disease in vegetable crops in South Moravia (Czech Republic) and consequences of yield losses.- *Crop Protection*, 28: 898-904.
- ÖZDEMİR N., SAYGILI H., SAHİN F., KARSAVURAN Y., BAYRAK O. F., ORAL B., 2009.- Host range and genetic characterization of a phytoplasma causing tomato stolbur disease in Turkey.- *Acta Horticulturae*, 808: 255-262.
- SERUGA M., SKORIC D., KOZINA B., KRAJACIC M., CURKOVIC-PERICICA M., 2003.- Epidemiologija zutica vinove loze u Hrvatskoj.- *Zbornik sazetaka Osmog hrvatskog biologskog kongresa*, 27.09-02.10 2003, Zagreb, Hrvatska.
- TOLU G., BOTTI S., GARAU R., PROTA V., SECHI A., PROTA U., BERTACCINI A., 2006.- Identification of a 16SrII-E phytoplasma in *Calendula arvensis*, *Solanum nigrum* and *Chenopodium* spp.- *Plant Disease*, 90: 325-330.

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