Bulletin of Insectology ***64*** *(Supplement): S65-S66, 2011*
ISSN 1721-8861

Association of aster yellows subgroup 16SrI-C phytoplasmas with a disease of *Ribes rubrum*

Jaroslava PŘIBYLOVÁ, KAREL PETRZIK, JOSEF ŠPAK
Biology Centre of the Academy of Sciences, Institute of Plant Molecular Biology, České Budějovice, Czech Republic

Abstract

A *Ribes rubrum* plant showing malformation and twisting of branches was found in a private garden in South Bohemia. Observation of ultrathin sections of tissues from symptomatic shoots revealed the presence of phytoplasma-like bodies. Different primer sets were used for amplification of the 16S-23S ribosomal gene segment. RFLP analysis and sequencing for phytoplasma identification classified the detected phytoplasma in the aster yellows group, subgroup 16SrI-C. Successful transmission of detected phytoplasma by dodder (*Cuscuta campestris* Yuncker) to periwinkle (*Catharanthus roseus* L.) was confirmed by detection of numerous phytoplasma bodies in ultrathin sections of *C. roseus* and by PCR from dodder and periwinkle. RFLP analyses of PCR products as well as nucleotide sequences of the currant plant and symptomatic periwinkles were identical. Sequenced data obtained from both currant and indicator plant, were aligned and sequences of 1,613 bp were found to be identical. Transmissions of phytoplasma by grafting to healthy currant rootstocks were unsuccessful.

Key words: PCR, RFLP analysis, sequencing, transmission, red currant.

Introduction

Many fruit species including small fruits are affected by phytoplasma infection. The most common symptoms in these hosts are proliferation, color changes of leaves, leaf roll, and flower malformations. There are only a few conclusive data concerning the phytoplasma occurrence in *Ribes* sp. For the first time, phytoplasma bodies were localized by electron microscopy in the phloem tissue of fruit stalks of red currant that exhibited full blossom symptoms by Rakús *et al*. (1974). Navrátil *et al*. (2004) detected phytoplasmas of aster yellows and apple proliferation groups in *Ribes rubrum* using nested PCR and RFLP analysis. Špak *et al.* (2004) firstly detected the presence of phytoplasma in black currant with symptoms of the severe form of blackcurrant reversion disease.

Materials and methods

A currant bush showing severe twisting of shoots (figure 1) and producing poor yield of berries was found in a private garden in southern Bohemia. The symptoms appeared on new growing shoots and persisted throughout the life of the plant.

For transmission electron microscopy (TEM) ultrathin sections from phloem tissue of affected currant shoots and from petioles of symptomatic periwinkles were used. Samples were prepared as described (Navrátil *et al.*, 2004).

Cuttings from infected shoots were rooted and maintained in an insect proof glasshouse for symptom observation. In subsequent years, dodder transmission by *Cuscuta campestris* of the possibly infectious agent to 2 plants of *Catharanthus roseus* (all plants grown from seed) was carried out. Some cuttings were grafted onto healthy currant rootstock material, 3 plants cv. Jonkheer van Tets and 3 plants cv. Baldwin, all raised from seeds.

DNA was extracted from phloem tissues of currant symptomatic shoots, leaves of symptomatic periwinkles and dodder plants after transmission tests, leaf midribs of rooted planted cuttings and grafted rootstock plants according to Lee *et al*. (1991). The primer pairs: P1 (Deng and Hiruki, 1991), P7 (Schneider *et al*., 1995); R16F2n/R2 (Gundersen and Lee, 1996) and R16(I)F1/R1 (Lee *et al*., 1994) were used to amplify 16S-23S rRNA genes of the phytoplasma genome. PCR reactions were carried out according to Špak *et al*. (2004). About 6 µl of positive nested-PCR products were digested from R16F2/R2 amplicons obtained from the original currant and symptomatic vinca. Digestions were carried out with 2.5 U of *Alu*I, *Hha*I, *Mse*I, *Kpn*I, *Rsa*I restriction enzymes for at least 16 h. The restriction patterns were compared after electrophoresis on a 10% polyacrylamide gel.

Figure 1. Symptoms of twisting and deformation of shoots from affected currant plant.

Results

The same twisted branches and shoot malformations were observed on planted rooted cuttings in the subsequent year. Dodder transmission tests continued for 3 months, until dodder bridge was broken. Symptom of green flower petals, as the most pronounced symptom, appeared on both periwinkles 4 months after. Grafted rootstock material did not show any symptoms for 3 years. Examination of tissue samples by TEM revealed some ovoid or spherical phytoplasma-like bodies in phloem tissue of currant shoots. Phloem cells filled with these bodies were observed in samples from symptomatic periwinkles. PCR products were obtained with mentioned primer pairs from the original currant plant, rooted planted cuttings, both symptomatic periwinkles and from dodder. No PCR products were obtained from symptomless grafted rootstock plants. The digestion of R16F2/R2 amplified fragments of the currant and periwinkle with restriction enzymes showed the same patterns of the strain 16SrI-C according to Lee *et al*. (1998). The nucleotide sequences of phytoplasma from currant and periwinkle were identical. The 16S partial sequence from currant of 1,613 bp was deposited in the GenBank under accession no. AY669063. Comparison with available sequences revealed a close relationship with sequences of the 16SrI-C subgroup.

Discussion

Similar symptoms of shoot deformation on red currant were observed in Germany on cv. Casa (Dr. E. Kruger, personal communication). Similar symptoms of twisting and branch deformation were reported in apple trees in the Czech Republic where phytoplasmas were detected by PCR assays and by electron microscopy. Using RFLP analyses these phytoplasmas were classified in the aster yellows group, subgroup 16SrI-C, 16SrI-B and in the apple proliferation group, subgroup 16SrX-A (Fránová, 2005). The presence of phytoplasma bodies in sieve elements of the affected currant plant supports the phytoplasma aetiology of the disease. In currant phloem tissue we found phytoplasma bodies in small numbers, but numerous bodies were observed in *C. roseus* after dodder transmission. *C. roseus* is a much better source of phytoplasmal nucleic acids than currants. In *C. roseus*, the phytoplasma numbers are usually higher and phytoplasma components are easier to obtain (Marcone *et al*., 1997). This is probably a reason why phytoplasma transmission by grafting into rootstock material was unsuccessful. So far we did not observe twisted branches and shoot malformations in black currants (Špak *et al*., 2009).

Acknowledgements

This research was funded by projects QH91224 of the National Agency for Agricultural Research and AV0Z50510513 of the Academy of Sciences of the Czech Republic.

References

DENG S., HIRUKI C., 1991.- Amplification of 16S rRNA genes from culturable and non-culturable mollicutes.- *Journal of Microbiological Methods*, 14: 53-61.

FRÁNOVÁ J., 2005.- The occurence of phytoplasmas in apple trees showing branch twisting.- *Journal of Phytopatology,* 153: 384-388.

GUNDERSEN D. E., LEE I-M., 1996.- Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs.- *Phytopathologia Mediterranea*, 35: 144-151.

LEE I-M., DAVIS R. E., HIRUKI C., 1991.- Genetic relatedness among clover proliferation Mycoplasma-like organisms (MLOs) and other MLOs investigated by nucleic acid hybridization and restriction fragment length polymorphism analyses.- *Applied and Environmental Microbiology*, 57: 3565-3569.

LEE I-M., GUNDERSEN D. E., HAMMOND R. W., DAVIS R. E., 1994.- Use of mycoplasma-like organisms (MLOs) group specific oligonucleotide primers for nested PCR assays to detect mixed-MLO infections in a single host plant.- *Phytopathology*, 84: 559-566.

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 gene sequences.- *International Journal of Systematic Bacteriology*, 48: 1153-1169.

MARCONE C., RAGOZZINO A., SEEMÜLLER E., 1997.- Dodder transmission of alder yellows phytoplasma to the experimental host *Catharanthus roseus* (periwinkle).- *European Journal of Forest Pathology*, 27: 347-350.

NAVRÁTIL M., VÁLOVÁ P., FIALOVÁ R., PŘIBYLOVÁ J., ŠPAK J., KUBELKOVÁ D., KAREŠOVÁ R., 2004.- Occurrence of phytoplasmas in red and white currant in the Czech Republic.- *Acta Horticulturae*, 656: 119-124.

RAKÚS D., KRÁLÍK O., BRČÁK J., 1974.- Mycoplasma-like Organisms in *Ribes houghtonianum* Jancz. infected with a yellows disease.- *Ochrana rostlin*, 10: 307-309.

SCHNEIDER B., SEEMÜLLER E., SMART C. D., KIRKPATRICK B. C., 1995.- Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas, pp. 369-380. In: *Molecular and Diagnostic Procedures in Mycoplasmology, Vol. I* (RAZIN S., TULLY, J. G., Eds).- Academic Press, San Diego, CA, USA.

ŠPAK J., PŘIBYLOVÁ J., KUBELKOVÁ D., ŠPAKOVÁ V., 2004.- The presence of phytoplasma in black currant infected with the black currant reversion disease.- *Journal of Phytopathology*, 152: 600-605.

ŠPAK J., KUBELKOVÁ D., PŘIBYLOVÁ J., ŠPAKOVÁ V., PETRZIK K., 2009.- Elucidation of the roles of *Blackcurrant reversionn virus* and phytoplasma in the etiology of full blossom disease in currants.- *Plant Disease*, 93(8): 832-838.

Corresponding author: Jaroslava PŘIBYLOVÁ (e-mail: pribyl@umbr.cas.cz), Biology Centre of the ASCR, v.v.i., Institute of Plant Molecular Biology, Branišovská 1160/31, 370 05 České Budějovice, Czech Republic.