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Detection of '*Candidatus* Phytoplasma pyri' in Turkey

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Abstract

Typical '*Candidatus* Phytoplasma pyri' (Pear Decline, PD) symptoms were first observed in 2005 in pear orchards of Bursa province, which is an important pear growing area in Turkey. The present report resulted from a survey of pear varieties to investigate the infection level of PD in Bursa in 2006. The observed symptoms were foliar reddening in late summer and fall, leaf roll, leaf curl, poor growth and slow or quick decline. The presence of PD phytoplasma in pear trees was investigated by polymerase chain reaction using universal P1/P7 and R16F2/R2 primers and the identification was performed by RFLP analyses. A total of 116 pear trees were tested and 61 samples were found infected by the phytoplasma. '*Ca.* P. pyri' was identified using *Rsa*I, *Ssp*I and *Mse*I restriction enzymes. PD is widely distributed in pear trees with a high incidence and it is a big threat for pear production in Turkey.

Key words: Pear decline, PCR, RFLP, Bursa, Turkey.

Introduction

Pear (*Pyrus communis*) is in many countries severely affected by '*Candidatus* Phytoplasma pyri' (Pear decline, PD) phytoplasma. This phytoplasma belongs to the Apple proliferation group (16SrX) (Seemüller *et al*., 1998), and is transmitted by pear psyllids [*Cacopsylla pyricola* (Foerster), *Cacopsylla pyrisuga* (Foerster), *Cacopsylla pyri* (L.)]. Because phytoplasmas can be persistently spread by phloem–feeding insects (Lee *et al*., 2000), great efforts have been done to control these vectors. The spread of PD cannot be totally prevented by insecticide application because of difficult psyllid control. PD induces serious symptoms and frequently cause decline of the infected trees in a few months (Seemüller, 1989; Nemeth, 1986). As there are no practically available curative treatments for phytoplasma diseased plants, the use of healthy propagating material, as well as the control of vectors is important preventive control measures.

The first suspicious symptoms for the presence of PD in Bursa province located in Marmara Region of Turkey were observed in 2005. Until that time, growers usually assigned the disease to some other biotic or abiotic factor because of the lack of knowledge and detection techniques. The main observed symptoms in Bursa pear orchards were small, leathery leaves with up-rolled margins. Infected leaves become abnormally red in the autumn and drop prematurely. The presence of PD has been already confirmed by PCR and RFLP analyses in Bursa province (Ulubaş Serçe *et al*., 2006) and in the East Mediterranean region of Turkey (Sertkaya *et al*., 2005). In both studies a few plant samples were tested and the incidence of this disease in Turkey was not clarified. The objective of the present study was to determine the incidence of '*Ca.* P. pyri' in Bursa pear orchards by nested PCR/RFLP.

Materials and methods

Surveys were carried out during autumn (September 2006) in the commercial pear orchards of Bursa province located in the Marmara region of Turkey. Symptoms recognized as '*Ca*. P. Pyri' infection in pear trees were small, leathery leaves with up-rolled margins, abnormal leaf reddening and prematurely leaf drop.

DNA and dried leaf materials from AP (16SrX-A), PD (16SrX-C), European stone fruit yellows (ESFY) (16SrX-B) (kindly provided by I. Ember, Budapest, Hungary) were employed as positive controls.

One gram of fresh, frozen or dry leaf midribs and phloem tissue of branch were used to extract nucleic acid by a chloroform/phenol procedure (Prince *et al*., 1993). Samples were resuspended in TE buffer and diluted to adjust the final concentration of 20 ng/µl.

The universal primers for phytoplasma detection, P1/P7 (Deng and Hiruki, 1991; Smart *et al*., 1996) were used in the first step, amplifying one fragment of about 1,800 bp in length. The second step was performed with the R16F2/R2 primers (Lee *et al*., 1995). The following PCR amplification conditions were used: for the first cycle 94 °C for 2 min; 35 cycles 94 °C for 1 min (30 sec for nested-PCR) denaturation, 55 °C for 2 min (30 sec for nested-PCR) annealing, 72 °C for 3 min (1 min for nested-PCR) extension and the final cycle 72 °C for 5 min (Lee *et al*., 1998). Reaction mixture without DNA templates was used as negative control. Nested-PCR products were analyzed by electrophoresis in 1xTAE buffer using 1.5% agarose gel and DNA bands were visualized with a UV transilluminator after staining the gels with ethidium bromide.

Nested PCR products including reference isolates (1,200 bp) of phytoplasma 16S rDNA sequence were subjected to RFLP analysis. Five µl aliquots of each PCR products were separately digested overnight at 37 °C with restriction endonucleases *Rsa*I, *Ssp*I (MBI, Fermentas, Lithuania; GmbH, Germany). The digested

products were analyzed by electrophoresis using 2% agarose gel and stained with ethidium bromide, DNA bands were photographed under UV light.

Results

In the last 3 to 4 years the rapid spread of PD disease in Bursa province represents a serious outbreak with high levels of infection.

In 2006 a survey for estimating PD spread in Bursa on leading pear varieties in this province that are Deveci, a local pear variety, Santa Maria, William and Comice, foreign varieties, was carried out. Symptoms observed during this study on pear trees were typical for phytoplasma infection: vigour of trees was reduced; leaves were rolled upward and became red. In the late summer the whole tree leaves became red and dropped prematurely. Fruit number and size in symptomatic trees were decreased. Slow or quick decline symptoms were also observed.

Samples from pear trees with typical '*Ca*. P. pyri' symptoms usually gave a positive reaction in PCR. In addition, positive tests were obtained from samples of trees without visual symptoms for all tested cultivars. All positive samples from pear trees yielded a nested PCR product of the appropriate size using R16F2/R2 universal primers. No amplicons was obtained from the reaction mixture devoid of nucleic acid template. RFLP profiles obtained by using *Rsa*I, *Ssp*I restriction enzymes on R16F2/R2 PCR products showed that phytoplasmas belonging to the apple proliferation group (16SrX) were present.

Out of the 116 tested pear samples, 52.58% were found to be infected with PD. The incidences of the disease in Deveci, Santa Maria, Comice and William pear varieties were 58.06, 48.57, 44.44 and 40.00%, respectively.

Discussion

This limited survey demonstrated the presence of '*Ca*. P. pyri' in various varieties of pear in Bursa province of Turkey. However, to have a comprehensive picture for '*Ca*. P. pyri' distribution in the country, more surveys are needed. The results of field surveys and laboratory assays revealed that this phytoplasma is one of the most serious agents of disease for pear.

Heavy infestations of *Cacopsylla pyri,* the most frequent psyllid in Bursa pear orchards, induce in pear trees to repeated phytoplasma infections resulting in a progressive weakening of the tree. We hypothesize that a high percentage of PD with an important number of potential insect vectors that acquire the pathogen from infected trees is responsible for the high presence of PD in Turkey.

In order to control a further spread of the disease the uprooting of all infected trees and an efficient vector control are necessary. In view of the economical importance of PD, further investigations concerning epidemiological aspects of the disease and activity of vectors are needed.

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References

DENG S., HIRUKI C., 1991.- Genetic relatedness between two non-culturable mycoplasmalike organisms revealed by nucleic acid hybridization and polymerase chain reaction.- *Phytopathology,* 81: 1475-1479.

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 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.

LEE I.-M., DAVIS R. E., GUNDERSEN-RINDAL D. E., 2000.- Phytoplasma: Phytopathogenic mollicutes.- *Annual Review of Microbiology,* 54: 221-255.

NEMETH M., 1986.- *Mycoplasma and Rickettsia Diseases of Fruit Trees*.- Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

PRINCE J. P., DAVIS R. E., WOLF T. K., LEE I.-M., MOGEN B. D., DALLY E. L., BERTACCINI A., CREDI R., BARBA M., 1993.- Molecular detection of diverse mycoplasmalike organisms (MLOs) associated with grapevine yellows and their classification with aster yellows, X-disease, and elm yellows MLOs.- *Phytopathology*, 83: 1130-1137.

SEEMÜLLER E., 1989.- Pear decline, pp. 188-201. In: *Virus and Virus like Diseases of Pome Fruits and Simulating Nonimnfectious Disorders* (FRIDLUND P. R., Ed.).- Wash. State Univ. Coop. Ext. Spec. Publ. SP0003.

SEEMÜLLER E., MARCONE C., LAUER U., RAGOZZINO A., GÖSCHL M., 1998.- Current status of molecular classification of the phytoplasmas.- *Journal of Plant Pathology,* 80: 3-26.

SERTKAYA G., MARTINI M., ERMACORA P., MUSETTI R., OSLER R., 2005.- Detection and characterization of phytoplasmas in diseased stone fruits and pear by PCR-RFLP analysis in Turkey.- *Phytoparasitica,* 33: 380-390.

SMART C. D., SCHNEIDER B., BLOMQUIST C. L., GUERRA L. J., HARRISON N. A., AHRENS U., LORENZ K. H., SEEMULLER E., KIRKPATRICK B. C., 1996.- Phytoplasma-specific PCR primers based on sequences of 16S-23SrRNA spacer region.- *Applied Environmental Microbiology*, 62: 2988-2993.

ULUBAŞ SERÇE Ç., GAZEL M., ÇAGLAYAN K., BAŞ M., SON L., 2006.- Phytoplasma diseases of fruit trees in germplasm and commercial orchards in Turkey.- *Journal of Plant Pathology,* 88 (2): 179-185.

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