Bulletin of Insectology ***60*** *(2): 335-336, 2007*
ISSN 1721-8861

Distribution and molecular characterization of apple proliferation phytoplasma in Turkey

Didem CANIK, Filiz ERTUNC
Ankara University, Faculty of Agriculture, Plant Protection Department, Ankara, Turkey

Abstract

During summer and autumn in the years 2005 and 2006, extensive surveys were carried out in important pome fruit production provinces Isparta, Yalova and Ankara in order to determine presence and diffusion of '*Candidatus* Phytoplasma mali' in Turkey. 201 symptomatic and nonsymptomatic samples were taken from apple orchards and analyzed by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA), 4'-6'-diamino-2-phenyleindole (DAPI) staining, and polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP). Universal and AP group specific primers were used in PCR. RFLP analysis was performed with *Rsa*I and *Ssp*I enzymes on the positive PCR products. According to the results, 8 apple trees were found '*Ca.* P. mali' infected and RFLP results show that there were no differences between the isolates. Best of our knowledge, this is the fist report of '*Ca.* P. mali' in Turkey.

Key Words: '*Candidatus* Phytoplasma mali', DAS-ELISA, DAPI, molecular characterization.

Introduction

Apple proliferation (AP) is the most important phytoplasma disease of *Malus* species in many pome fruit growing areas in Europe (Kunze, 1989; Jarausch *et al*.; 2000). It was first reported in Italy (Rui *et al*., 1950). The pathogen, '*Candidatus* Phytoplasma mali' (Seemüller and Schneider 2004), belongs to the 16SrX group (apple proliferation) and is phylogenetically related to phytoplasmas causing pear decline and European stone fruit yellows.

Pome fruit production is commercially important in Turkey with 3 million tons production, and apple production has an important portion with 2.5 million tons annually. Because of the increase of the use of imported fruit trees, symptoms related to '*Ca.* P. mali' have increased in apple orchards. Small fruits, enlarged stipules, rosette form growing on shoots and autumn blossom have been observed at apple orchards recently. The objectives of this study were to verify the presence and to characterize '*Ca.* P. mali' in Turkey.

Materials and methods

During 2005 and 2006 extensive surveys were carried out in summer and autumn in commercial and experimental apple orchards in the important pome fruit production provinces of Isparta, Yalova and Ankara in order to verify '*Ca.* P. mali' presence. Shoot and leaf samples were taken from a total of 201 apple trees which showed enlarged stipules, rosette formed growth of shoots, small fruits and autumn blossom symptoms. Samples were chosen from local and common varieties. DAS-ELISA, DAPI staining and PCR analyses were performed to all plant samples for the detection and identification of '*Ca.* P. mali'. DAS-ELISA was performed with a commercial monoclonal anti IgG kit to fresh midribs according to the manufacturer instructions. ELISA plates were analyzed in an ELISA reader at 405 nm. DAPI staining was applied to 3-5 μm thick fresh midribs and wood sections of samples according to the procedure of Seemüller and Kirkpatrick (1996). Stained tissues were analyzed under fluorescence microscope.

Nucleic acid was extracted following the procedure developed by Lefort *et al.* (1998). After nucleic acid extraction from phloem tissue of midribs and the tissues under the bark, direct PCR with ribosomal universal primer pair P1/P7 (Deng and Hiruki, 1991, Smart *et al*., 1996) and nested PCR with 16F2/R2 (Lee *et al.,* 1995) and fO1/rO1 (Lorenz *et al.,* 1995) AP group specific primer pairs were performed. RFLP analysis was done with *Rsa*I and *Ssp*I enzymes on the positive PCR products. An Italian '*Ca.* P. mali' isolate was used as positive control.

Results

No positive reaction was found after DAS-ELISA, except for the positive control of the kit. After DAPI staining, phloem tissues from 8 samples were observed shiny under fluorescence microscope.

Results of the PCR and RFLP analyses were analyzed by electrophoresis and visualized on image analysis system. The universal and group specific primer pairs amplified expected-size PCR products in 8 apple samples. RFLP analysis of these 8 positive PCR products revealed the same restriction pattern as the positive control. Out of 201 samples, 193 samples gave negative results with all methods performed. Starkrimson, Krimson, Starking, Granysmith and Gloster were found as infected varieties. These positive samples were collected in Isparta and Ankara provinces. However, '*Ca.* P. mali' was not observed in the samples from Yalova.

Discussion

In this study, apple trees showing phytoplasma disease symptoms in Turkey were analyzed for '*Ca.* P. mali' presence. The results show that AP infection in Turkey is limited and the local varieties are free from the infection. Phytoplasmas isolated from the infected trees showed the same restriction patterns as the positive control of '*Ca.* P. mali'. The lower incidence of '*Ca.* P. mali' in Turkey might be due to the preferential use of local cultivars. The causal agent spreads by vectors and grafting. There is no report or knowledge about insect vectors in Turkey yet. The pathogen probably has been introduced by infected imported plant material. The typical symptoms of infected trees were autumn blossom, rosette growing of shoots and enlarged stipules. The AP like symptoms of healthy trees might be caused by nutrition deficiency. It is known that zinc deficiency cause rosette growing on shoots. According to the results obtained with the different detection methods, the commercial AP phytoplasma IgG kit is not suitable to detect the Turkish '*Ca.* P. mali' isolates. DAPI staining is a fast and easy method to detect the phytoplasma in infected samples. We found that the most reliable method was PCR-RFLP to detect the infection and identify the pathogen as well.

Acknowledgements

The authors thank A. Bertaccini for supplying positive control of AP and Bioreba for supplying ApP IgG kit.

References

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

JARAUSCH W., SAILLARD C., HELIOT B., GARNIER M., DOSBA F., 2000.- Genetic variability of apple proliferation phytoplasmas as determined by PCR-RFLP and sequencing of a non-ribosomal fragment.- *Molecular and Cellular Probes*, 14: 17-24.

KUNZE L., 1989.- Apple proliferation, pp. 99-113. In: *Virus and virus-like diseases of pome fruits and simulating noninfectious disorders* (FRIDLUND P. R., Ed.).- Washington State University.

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.

LEFORT F., LALLY M., THAMPSON D., DOUGLAD G. C., 1998.- Morphological traits microsatellite fingerprinting and genetic relatedness a standard of elite oaks (*Q. rubur*) at Tuallynally, Ireland.- *Silvae Genetica*, 47: 5-6.

LORENZ K-H., SCHNEIDER B., AHRENS U., SEEMÜLLER E., 1995.- Detection of the apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and nonribosomal DNA.- *Phytopathology*, 85: 771-776.

RUI D., CIFERRI R., REFATTI E., 1950.- La virosi degli "scopazzi del melo" nel Veronese.- *Notiziario delle Malattie delle Piante*, 13: 7-11.

SEEMÜLLER E., KIRKPATRICK B. C., 1996.- Detection of phytoplasma infections in plants, pp. 299-311. In: *Molecular and Diagnostic Procedures in Mycoplasmology, Vol. 2*.

SEEMÜLLER E., SCHNEIDER B., 2004.- '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma pyri' and '*Candidatus* Phytoplasma prunorum', the casual agents of Apple Proliferation, Pear Decline and European Stone Fruit Yellows, respectively.- *International Journal of Systematic Bacteriology and Evolutionary Microbiology*, 54: 1217-1226.

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

Corresponding author: Didem CANIK (e-mail: dcanik@agri.ankara.edu.tr), Ankara University, Faculty of Agriculture, Plant Protection Department, 06110 Ankara, Turkey.