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Is a phytoplasma responsible for fig mosaic disease?

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Abstract

The fig mosaic disease (FMD) is a wide-spread disease in fig growing countries. This disease can be transmitted by vegetative propagation material and eriophyd mite, *Aceria ficus* Cotte. Because of the unknown etiology of this disease the possible role of phytoplasmas were studied in this work. Both EM and PCR methods were used. According to PCR analysis, one fig sample found infected by phytoplasma when universal P1/P7 and R16F2/R2 primers were used. This test was repeated and the same result was observed. EM works also showed that two samples one of which was cv. Yediveren and other one was experimentally infected seedling, included phytoplasma like bodies. This is the first record for phytoplasma infection of fig trees for our knowledge but it is still uncertain if it was an accidental companion or real agent of the disorder.

Key words: Fig, phytoplasma, universal primers, electron microscopy.

Introduction

The fig mosaic disease, described first by Condit and Horne (1933), is a wide-spread disease in fig growing countries. This disease can be transmitted by vegetative propagation material and eriophyd mite, *Aceria ficus* Cotte (Flock and Wallace, 1955), but not by seed (Martelli *et al*., 1993). The disease was thought to be of viral origin until ultrastructural observations showed the occurrence of intracytoplasmic enveloped spherical bodies in infected fig cells (Bradfute *et al*., 1970; Plasvic and Milicic, 1980; Appiano *et al*., 1990). Following years the agent of the disease was called "disease-associated bodies" (DABs) which differed in shape and size (Martelli *et al*., 1993). Despite the agent of the disease was assumed as a member of potyvirus family (Brunt *et al*., 1996), it was not prooved by Spanish working group. Their data showed that it is not related with the family of *Potyviridae* but beside some DABs and 720 nm rod-shaped virus, which might responsible for the disease, showing a tail of 230 nm might be the agents of the disease (Serrano *et al*., 2004). Like other diseases with similar characteristics, it has been always pointed out that its causal agent could be a virus but there is no work done on the possibility of phytoplasma origin. The aim of this study is to explain if phytoplasmas have any responsibility for fig mosaic disease.

Materials and methods

In the study the fig plantations of Antakya-Hatay, located in east Mediterranean region of Turkey, were randomly surveyed during 2006 and 2007 and samples were collected from only symptomatic trees of most common local cultivars like Sari Zeybek, Gok lop, Sari lop, Bursa siyahi, Morguz, Yesilguz and Yediveren. Some of the experimentally infected fig seedlings were also used for EM and PCR.

The samples were processed for EM following a standard process and observed under a EM 910 (Zeiss) twice. Small pieces (1-2 x 5-10 mm) of leaves were processed for standard EM observation following Roland and Vian (1991) and Medina *et al*. (2003) and embedded in Araldite resin. Serial semithin (1-2 μm) and ultrathin (70-90 nm) sections were cut in an ultramicrotome (Reichert Ultracut). Firstly, semithin sections were deposited on slides, stained with Richardson's blue dye, mounted with cover slips and DPX (EMS) and observed under an optical microscope. Ultrathin sections were collected on formvar and carbon coated copper grids, stained with uranyl acetate and Reynold's lead citrate, and observed in a EM 910 (Zeiss) transmission electron microscope. For resin block (two tissue fragments per block) per every sample (young or older leaf) were sectioned and analyzed.

For PCR analyses one gram of fresh leaf midribs were used to extract nucleic acid according to Prince *et al*. 1993. Samples were resuspended in TE buffer [10 mM Tris-HCl, 1 mM EDTA (pH 8.0)]. DNA and dried leaf materials of European stone fruit yellows (ESFY) (16 SrX-B) (Nemeth *et al*., 2001) isolates were employed as positive controls. Healthy GF-305 leaves were used as a negative control. In order to compare apricot and fig samples, four apricot samples, possibly infected by ESFY phytoplasma, were also tested. P1/P7 (Deng and Hiruki, 1991; Smart *et al*. 1996) and R16F2n/R2 universal primers (Lee *et al*. 1993) were used in PCR. 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.

Results

During EM investigations some polymorphic particles resembling phytoplasmas were observed in the sieve tubes infected with FMD (figure 1). These bodies were not present in the healthy control material. These particles were found in cv. Yediveren which was showing very typical mosaic symptoms and a fig seedling which was experimentally infected by eriophyd mite, *Aceria ficus* Cotte (Caglayan *et al*., Unpublished data).

PCR analysis of three fig samples revealed that one of them was infected by a phytoplasma. This fig sample was experimentally infected plant and yielded around at expected band size (~1.2 kb). As far as fig samples 4 apricot samples were tested together. Among apricot samples 2 samples were found infected (figure 2).

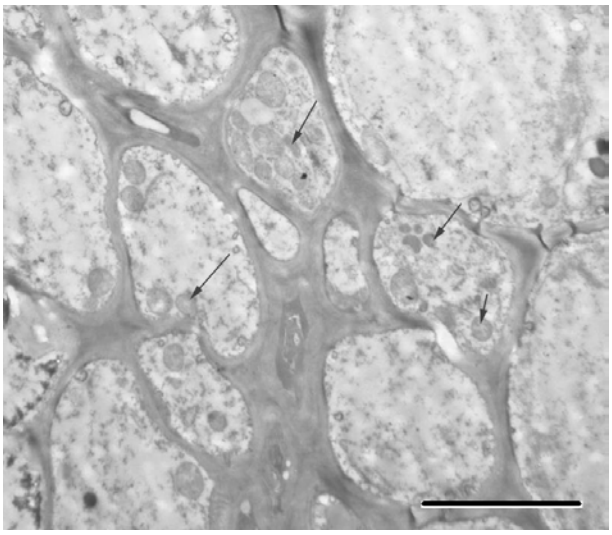

Figure 1. Phytoplasma-like structures in sieve elements of fig leaves cv. Yediveren. Bar=3.63µ.

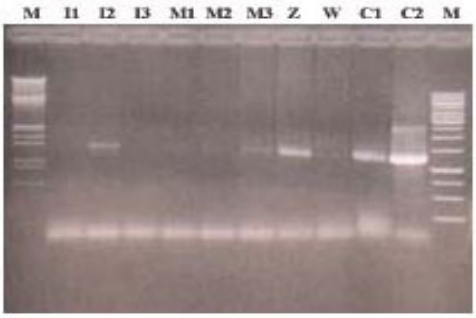

Figure 2. Agarose gel electrophoresis of PCR products (1.2 kb) from: I1, I2 and I3 are fig samples, M1, M2, M3 and Z are apricot samples, W: water control, C1 and C2 are ESFY positive control, M is marker.

Discussion

FM is widespread in the Mediterranean basin and the different type of symptoms can be commonly seen in fig plantations. The cytopathological patterns observed in many studies are double membrane bodies, polymorphic particles or different type of viruses (Bradfute *et al*., 1970; Martelli *et al*., 1993; Serrano *et al*., 2004). In this study first time phytoplasmas were detected in infected fig leaves both by EM observations and PCR analysis. Because of very common symptoms on both commercial fig plantations and also on seedlings in the field, it resembles effective mite or other unknown vector transmission. It is interesting data in this study that phytoplasma like bodies (PLBs) have seen in a seedling, which is infected by mite, collected from infected fig trees. Experimental transmission tests of FMD by mites have been already done by different workers (Caglayan *et al*., Unpublished data; Credi, Unpublished data). However this study doesn't show directly that mites can include PLBs in figs, but it was indirectly prooved because of the phytoplasma detection in experimentally infected fig seedling both by EM and PCR analysis.

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References

APPIANO A., CONTI M., LOVISOLO O., 1990.- Mosaico del fico: stato attuale dele conoscenze e nuove osservazioni.- *Agricoltura e Ricerca,* 112/113: 109-112.

BRADFUTE O. E., WHITMOYER R. E., NAULT R. L., 1970.- Ultrastructure of plant leaf tissue infected with mite-born viral-like pathogens.- *Electron Microscopy Society of America*, 28: 178-179.

CONDIT I. J., HORNE W. T., 1993.- A mosaic of the fig in California.- *Phytopathology*, 23: 887-896.

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.

FLOCK R. A., WALLACE J. M., 1955.- Transmission of fig mosaic by the eriophyid mite *Aceria ficus*.- *Phytopathology*, 45: 52-54.

KUMAR P., JONES A. T., REDDY D. V. R., 2003.- A novel mite-transmitted virus with a divided RNA genome closely associated with pigeon-pea sterility mosaic disease.- *Phytopathology*, 93: 71-81.

LEE I.-M., DAVIS R. E., SINCLAIR W. A., DE WITT N. D., CONTI M., 1993.- Genetic relatedness of mycoplasma-like organisms detected in *Ulmus* spp. in USA and Italy by means of DNA probes and polymerase chain reactions.- *Phytopathology*, 83: 829-833.

MARTELLI G. P., CASTELLANO M. A., LAFORTEZZA R., 1993.- An ultrastructural study of fig mosaic.- *Phytopathologia Mediterranea,* 32: 33-43.

MEDINA V., RODRIGO G., TIAN T., JUAREZ M., DOLÍA V. V., ACHON A., FALK B. W., 2003.- Comparative cytopathology of Crinivirus infections in different plant hosts.- *Annals of applied Biology*, 143: 99-110.

NÉMETH M., EMBER I., KRIZBAI L., KÖLBER M., HANGYÁL R., BOZSICS G. Y., 2001.- Detection and identification of phytoplasmas in peach based on woody indexing and molecular methods.- *International Journal of Horticultural Science*, 7: 37-41.

PLAVSIC B., MILICIC D., 1980.- Intracellular changes in trees infected with fig mosaic.- *Acta Horticulturae*, 110: 281-286.

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.

ROLAND J. C., VIAN B., 1991.- General preparation and staining of thin sections, pp. 1-66. In: *Electron microscopy of plant cells* (HALL J. L., HAWES C., Eds).- Academic Press, New York, USA.

SERRANO L., RAMON J., SEGARRA J., MEDINA V., ACHON M. A., LOPEZ M., 2004.- New approach in the identification of the causal agent of fig mosaic disease.- *Acta Horticulturae*, 657: 559-566.

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

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