Bulletin of Insectology ***64*** *(Supplement): S111-S112, 2011*
ISSN 1721-8861

Detection of stolbur phytoplasma in willow in Spain

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Abstract

Preliminary results of nested-PCR indicated that phytoplasmas were detected in willow (*Salix babylonica* Linn) showing yellows, ball-like structures and small leaves symptoms collected in Valencia Province (Eastern Spain). RFLP analyses showed that the phytoplasmas belonged to the stolbur group (16SrXII).

Key words: willow, witches' broom, phytoplasma, nested-PCR, RFLP, stolbur.

Introduction

Willow (*Salix babylonica* Linn) is a traditional tree normally grown in urban areas in Valencia Province (Eastern Spain). For several years, symptoms characteristic of diseases potentially associated with phytoplasmas presence have been observed in willows in Valencia. Affected trees showed yellows, thin leaves and ball-like structures (figure 1).

The main objective of the present work was to verify phytoplasma presence in some affected willow tree showing abnormal symptoms and to identify the phytoplasma group present in those samples. The results presented here are preliminary and the work is in progress.

Materials and methods

Samples from different willow trees with phytoplasmas-like symptoms were collected in 2003, 2010 and 2011.

Healthy samples of willow, positive samples for stolbur and for '*Ca.* P. asteris' were also included in the assay as negative and positive controls, respectively. Total DNA was extracted as described Green *et al.* (1999).

A nested-PCR was performed using the universal phytoplasma primers P1/P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995) in the first amplification followed by R16F2n/R16R2 (Gundersen and Lee, 1996) in the second amplification to detect phytoplasmas in the affected trees.

The PCR products were analysed in 1.2% agarose in TAE buffer gels, stained with ethidium bromide and visualized with a UV transilluminator.

Restriction fragment length polymorphism (RFLP) analyses of the nested-PCR products (1.2 kb 16S rDNA fragments) were used for identification of the putative phytoplasma detected (Lee *et al.,* 1998) with *Hha*I, *Mse*I, *Rsa*I and *Taq*I endonucleases (Fermentas, Vilnius, Lithuania) in 5% polyacrylamide gels.

Results and discussion

Fragments of the expected size (1.2 kb) were only amplified from symptomatic samples and positive controls. No amplification was produced from healthy samples or water controls. The RFLP profiles when compared with control phytoplasma profiles (figure 2) and with profiles of other phytoplasma 16S rRNA groups described by Lee *et al.* (1998) indicated that the phytoplasmas present in willow trees belong to the stolbur group, 16SrXII.

During 2008, aster yellows group 16SrI (subgroup 16SrI-C) phytoplasma was associated with a yellows-type disease of willows in China (Wei *et al*., 2009); to our knowledge, this work represents the first report of phytoplasmas in willow trees in Spain.

Figure 1. Willow branch showing symptoms of ball-like structures.
(In colour at www.bulletinofinsectology.org)

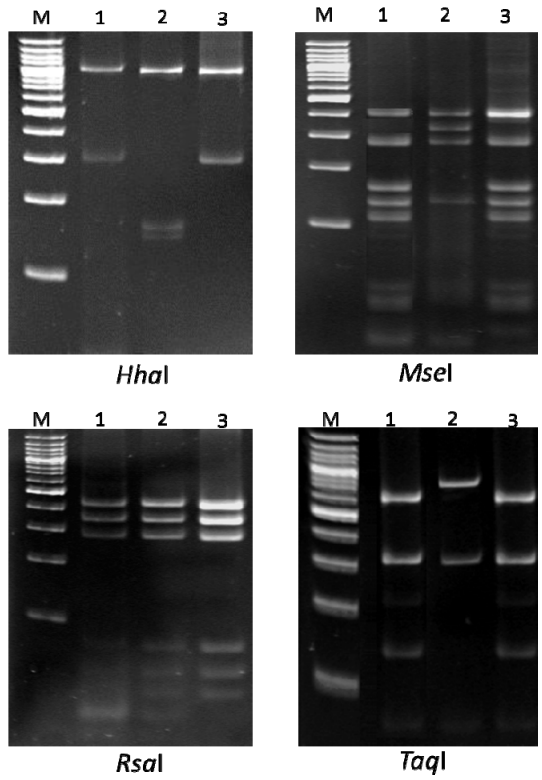

Figure 2. Polyacrylamide 5% gel of the RFLP analyses of 16S rDNAs (nested-PCR products amplified with primers R16F2n/R16R2) using endonucleases *Hha*I, *Mse*I, *Rsa*I and *Taq*I of willow sample (lane 1), positive control aster yellows phytoplasma (lane 2) and positive control stolbur phytoplasma. Lane M, 100 bp DNA marker (Fermentas, Vilnius, Lithuania).

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