Bulletin of Insectology ***60*** *(2): 283-284, 2007*
ISSN 1721-8861

A new phytoplasma associated with a zigzag line pattern in leaves of *Lilium* spp. in Mexico

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Abstract

Since 2005, symptoms of zigzag lines between midribs of leaves, dwarfism, gigantic stems or with shortened internodes were observed on *Lilium* spp. plants, in several producer plots of Mexico State, grown from imported bulbs. The 16S rDNA analysis showed that the symptoms were associated to a phytoplasma related to '*Candidatus* phytoplasma asteris'; it belongs to a different phylogenetic lineage from lily fasciation phytoplasma. The current Mexican normative (NOM-007-FITO-1995) does not include specifications for phytoplasmas in this ornamental crop, and it will be necessary to create a normative that will prevent the introduction of these pathogens into Mexico, as well as their distribution to production areas.

Key words: PCR, phylogenetic analyses, lily, phytoplasmas.

Introduction

In recent years *Lilium* spp. has becoming an important ornamental crop in regions of Mexico such as Santiago Tetla, Tlaxcala State, Orizaba and Fortín de las Flores, Veracruz State, and several localities of Mexico State. Since 2005, symptoms of zigzag lines between veins of leaves, dwarfism, gigantic stems or with shortened internodes, death of little roots, air bulbils, leaves without petioles born from the bulb, fall of flowers, and some flower buttons atrophied, were observed on *Lilium* spp. plants grown from imported bulbs, in several producer plots of Mexico State and greenhouses of SENASICA, symptoms suggestive of a phytoplasma pathogen. There are no specifications for phytoplasmas in this ornamental crop in the current Mexican normative (NOM-007-FITO-1995), and so no control to prevent their introduction.

For this reason, the aim of the present study was to identify the causal agent of this disease, as well as to provide data for the development of control of these diseases in ornamental crops in a new Mexican normative for the import of propagative material such as bulbs.

Materials and methods

During inspections of *Lilium* spp. plantations grown from imported bulbs, in several producer plots of San Pablo Ixayo, Boyeros and Tequexquinauac, Mexico State and greenhouses of SENASICA, plants showing similar symptoms caused by organism-like-phytoplasma were collected.

Total DNA was extracted from leaves and stems of 12 symptomatic and asymptomatic plants obtained from the different producer plots by the method of Dellaporta (Dellaporta *et al.,* 1983). DNA samples were analyzed for phytoplasma by a nested PCR using specific primers P1/P7 (Smart *et al*., 1996) followed by R16F2n/R2 (Lee *et al*., 1998). For PCR amplification, 30 cycles were conducted in an automated themocycler (Techne® TC-412). PCR was performed in mixtures containing 20 ng of nucleic acid, 200 µM dNTP mix, PCR Buffer 1X, MgCl2 1.5 mM and 20 *p*moles each primer. The following conditions were used: denaturation at 94 ºC for 1 min, (9 ºC for 5 min for the first cycle), annealing for 54 ºC during 50 s (53 °C for second amplification in nested PCR), and primer extension for 2 min at 72 ºC (5 min in the final cycle), for second amplification in nested PCR 72 °C for 1 min 20 s and 10 min in the final cycle. The PCR products (5-10 µL) were analysed by electrophoresis on a 1.6% agarose gel followed by staining with ethidium bromide and visualization of the DNA bands with a UV transilluminator.

PCR products were sequencing and compared with reported sequences in GenBank using BLAST program. To edit the sequences the programs FinchTv version 1.4.0, and BioEdit Sequence Aligment Editor were used. A phylogenetic tree was constructed by Mega v. 3.1 using maximum parsimony.

Results and discussion

A fragment of approximately 1.2 kb was amplified from DNA of all symptomatic plants only, from both, leaves and stems. Phytoplasma identity was determined by sequencing the nested PCR products, three different sequences were obtained and deposited in Genbank (Accession Nos. EF421158, EF421159 and EF421160). Nucleotide analysis of the three partial 16s rDNA sequences revealed that this phytoplasma belonged to the '*Candidatus* Phytoplasma asteris' group (AY-16SrI) (IRPCM, 2004; Lee *et al*., 1998). The sequences were 99.9-99.6% similar to those of onion yellows phytoplasma (AP006628), and *Oenothera* phytoplasma (M30790), respectively.

A 16SrI phytoplasma associated with fasciation in lilies has been reported in the Czech Republic (Bertaccini *et al*., 2005); even though, these two lily phytoplasmas

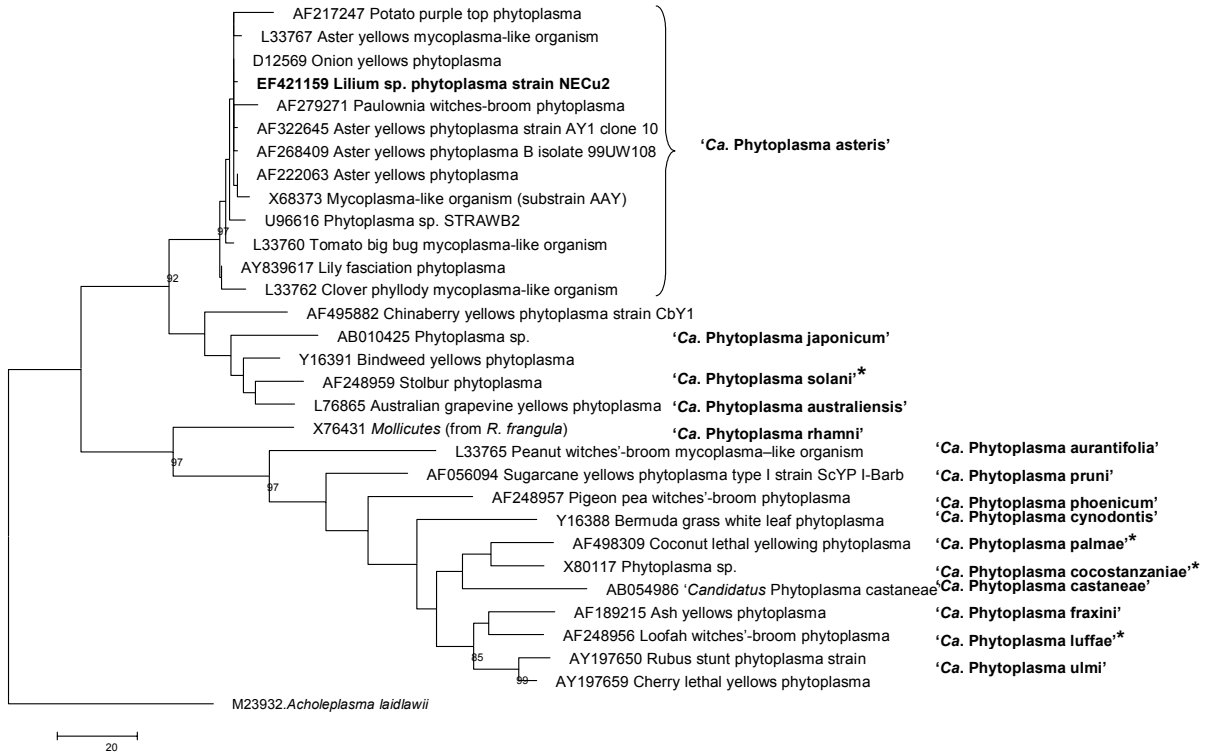

Figure 1. Phylogenetic tree constructed by maximum parsimony analysis of partial 16S rRNA sequences, with a bootstrap of 5,000 replicates. * '*Candidatus* species' not yet formally published.

belong to the same ribosomal group, related to '*Ca*. P. asteris', they correspond to two different phylogenetic lineages of the six reported by Lee *et al*. (2004). The lines in zigzag between midribs of leaves phytoplasma belongs to the clade that groups 16SrI-B, 16SrI-D, 16SrI-L, 16SrI-M and 16SrI-N, and the lily fasciation phytoplasma to the clade of 16srI-C (figure 1), this could explain the difference in symptoms both of them displayed on the plants.

As was mentioned above, the present Mexican normative do not have pathogen specifications for phytoplasmas in imported bulb crops and it will be necessary to create one that avoids the distribution of these pathogens to the producer areas, given the demand this crop has in the national market.

Acknowledgements

To Consejo Nacional de Ciancia y Tecnología (CONACYT). Also, to Consejo Mexiquence de Ciencia y Tecnologia (COMECYT).

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