Phytoplasmas Associated to Diseases of Ornamental Cacti in Mexico

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Abstract: This study was aimed to elucidate the putative causal agents. Healthy and diseased Echinopsis sp. and Opuntia sp. plants were collected from several Mexican nurseries. DNA was extracted from proliferating buds or stems and used in Polymerase Chain Reactions (PCR) to detect phytoplasmas. Two universal phytoplasma primer pairs were tested in nested PCR, initially with primer pair P1/70nt followed by primer pair R16F2/R16R2 in a sequential test. The amplified DNA fragments were cloned and sequenced. Two different 16S rDNA partial operons were determined. One of present sequences was always associated to yellow mosaics in Echinopsis while the other to witches-broom syndromes in Opuntia. The comparative analysis of the sequences against the GenBank indicated that they were highly but not 100% homologous to phytoplasmas of group 16SrII. This is of major importance since this is the first report of finding representatives of this group affecting cacti in the Americas; all other reports of this kind of phytoplasmas have been detected in mainly in Asia.

Key words: PCR, witches broom, mosaics, Mollicutes, fastidious prokaryotes, cladode distortion

INTRODUCTION

America is the center of origin of cacti plants being Mexico a rich region in their biodiversity. In the last years stems and fruits are used as nutritional source but its demand as ornamental plants has been grown substantially. Recently, an increase in field diseases has been observed and a number of new varieties with unusual anatomical characteristics are offered to cacti collectors in national nurseries. These cacti plants show abnormal growth with excessive proliferation of buds, yellow stems, yellow mosaics and purple colorations (Fig. 1). Some of these syndromes have been previously associated to phytoplasmas by present group in scientific meetings (Leyva-López et al., 1999).

Phytoplasmas are nonculturable degenerate gram-positive prokaryotes related with mycoplasmas and spiroplasmas. Lee et al. (2000) have classified these pathogens in 14 main groups according to restriction enzyme patterns of their 16SrRNA genomic operons (16SrI-XIV). These pathogenic agents are usually transmitted by insect vectors and are associated to more than 700 diseases worldwide (Weintraub and Beanland, 2006; Bertaccini, 2007).

Phytoplasmas are associated to a high number of plant diseases in Mexico, being the so-called potato purple top the most notorious due to its financial impact on the Mexican potato industry. Present studies led to conclude that two different phytoplasmas were involved in the potato purple top syndrome (Leyva-López et al., 2002). One of them was identified as potato hair sprouts phytoplasma and is a member of the group 16SrII, being this parasite the first one found in Mexico, since all the reported members have been described affecting plants in Asia. Recently, Lee et al. (2006) reported the presence of another member of this group that might be the same phytoplasma reported in 2002.

The potato hair sprouts phytoplasma was found infecting cacti growing as weed or barriers in the potato fields as well (Leyva-López et al., 2002). Very few phytoplasmas associated to cacti species have been described so it was of interest to investigate its presence in other succulent plants.

It is important to emphasize that these pathogens confer unusual characteristics that make cacti plants attractive in commercial nurseries. Objectives of this

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study included the detection and molecular analysis of any phytoplasma related to the observed syndromes affecting ornamental cacti being commercialized in Mexican nurseries.

**MATERIALS AND METHODS**

**DNA isolation:** Cacti samples of affected plants were collected from plant nurseries located in the Mexican States of Guanajuato and Colima during 2006-2007. Fresh tissue in active growth was collected and used for DNA isolation and processing using the method reported by Lopes and Larkins (1993).

**PCR assays:** Universal primer pairs were used for the amplification of the 16S rRNA gene and the 16S/23S spacer region. The universal phytoplasma primers Pl (5'-aaggtttgatctctgctaggatt-3') and tint (5'-tcacctcgttgaacctac-3') were used to amplify the 16S rRNA and tRNA operon region in a first PCR (Smart et al., 1996). PCR was performed in a 25 µL total volume of reaction, containing 100 ng of total DNA, 10 pmol of each primer, 1x of buffer solution for PCR, 2 mM MgCl₂, 200 mM of dNTPs and 2.5 units of Taq DNA polymerase (PROMEGA Corp, Madison WI). PCR rounds were 3 min to 90°C followed by 30 cycles of three steps: 1 min to 90°C, 1 min at 55°C and 1 min at 72°C. Products obtained in the first amplification were used to make 1:20 dilutions and 1 µL was taken from each dilution like DNA template to make the second amplification for samples that could have low titer of phytoplasmas, where the pair of internal initiators R16F2 (5'-catgcatctctgctaggatt-3')/R16R2 (5'-tagccgctgaacctac-3') (Lee et al., 1994) were used with the conditions of reaction mentioned previously. Products were visualized in agarose 1% gels. PCR amplification was conducted in an automated thermocycler (PT-100, MJ Research, San Francisco CA, USA).

**Cloning and nucleotide sequencing:** RFLP-PCR and DNA sequences analysis were used for molecular characterization. PCR products were digested with Kpn I, Hinf I and Alu I (Promega Corporation, Madison, WI, USA) and visualized in agarose 1.5% gels for enzymatic restriction analysis. Intact amplified products were purified with Wizard PCR prep Kit and inserted into pGEM-T vector Easy Vector System II (Promega, Madison, WI) later used to transform *Escherichia coli* DH5alpha cells. Plasmids containing the expected sizes were chosen for automatic sequencing using an ABI PRISM 377 PERKIN-ELMER DNA sequencer.

**Sequence alignment and phylogenetic analysis:** Restriction site maps of the 16S rRNA gene of the phytoplasmas found here were generated using the CLC Free Workbench 4.5.1 program for the Macintosh operating system (Cambridge, MA, USA). DNA sequences were compared to each other and with the existing sequences at the GenBank database using the same software and the NCBI Blast program (Zhang et al., 2000).

**RESULTS AND DISCUSSION**

PCR assays using the Pl/Tint pair allowed the amplification of a 1650 bp fragment in a first reaction, followed by the R16F2/R16R2 pair that yielded the a 1250 bp amplified fragment. PCR analysis demonstrated
Thus, the phytoplasmas named here as Opuntia sp. mosaic-inducing phytoplasma (DQ535899) and Echinopsis sp. yellow patch phytoplasma (DQ535900) belong to the 16SrII group. It is remarkable to emphasize that cacti species that are alternating hosts for phytoplasma are common in commercial nurseries and they have a great demand because of the unusual characteristics that make them more attractive may involve involuntary risk of dispersion of diseases to crops of agronomic importance.

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