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Detection of phytoplasmas in mixed infection with begomoviruses: a case study of tomato and pepper in Mexico

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Abstract

Scanning electron microscopy (SEM) and molecular techniques were employed to investigate the possible causal agents of yellow-type diseases in tomato and pepper in Baja California Sur (BCS) state of Mexico. Mixed infection of phytoplasmas (16SrIII, X-disease group) and two different begomoviruses (TYLCV and ToChLPV) was identified in pepper. Phytoplasma infection was also confirmed in tomato by SEM and nested PCR assays and along with mixed infection by two begomoviruses (TYLCV and PepGMV).

Key words: phytoplasma, begomoviruses, SEM, nested-PCR, RFLP, tomato, pepper, BCS.

Introduction

In the Mexican state of BCS the occurrence of phytoplasmas associated with yellow type diseases of tomatoes and peppers was obtained by SEM analysis during 2005-2008 in field and greenhouse-growing crops from principal agricultural areas (Lebsky and Poghosyan, 2007, Poghosyan *et al*., 2008). Some symptoms in pepper and tomato plants associated with phytoplasma infection are similar to those provoked by begomovirus group (*Geminiviridae*). Thus and accurate disease diagnosis is necessary to prove the disease aetiology and the possible presence of both pathogens.

The study was initiated in 2008 to determine the origin of yellow type disease in tomato and pepper in two experimental plots of CIBNOR in El Comitan (tomato of Japanese selection) and in El Carrizal (chile ancho), where the plantings were established. A very high incidence of disease (up to 80-90%), was recorded on both crops. The yellows-type symptoms may be indicative of both phytoplasma and begomovirus presence and the presence of large whiteflies populations in the crops also confirm the possibility of mixed infection. To verify this hypothesis scanning electron microscopy (SEM) and molecular techniques were applied.

Materials and methods

Symptoms in diseased plants were strong reduction, chlorosis and foliar malformations in apical and internodal leaves, more differently and extensively expressed in tomato plants (spoon-like lamina and claw-like apex), discoloration and necrotic lesions on old leaves, shortened internodes, distortion and thickness of leafstalks and stems, dried and aborted flowers and fruits, deformed and reduced in size and quantity.

The symptoms of disease were transmitted by grafting from field plants to tomato and pepper test plants in greenhouse conditions. Samples from both field and test plants were processed for SEM (S-3000N Hitachi) analysis of phytoplasmas as reported earlier (Lebsky and Poghosyan, 2007), and for molecular probes. Micropropagated plants (phytoplasma-free) were used as controls. For molecular detection of phytoplasmas total DNA was extracted from the same plants by Zhang *et al.* (1998). Nested-PCR assay was performed with phytoplasma-specific primer pairs P1/P7 and R16F2n/R16R2 (Gundersen and Lee, 1996). PCR products were analysed as discribed by Lee *et al*., (1998), cloned in vector pGEM-T-Easy vector, sequenced (Genwiz, USA).

To detect virus, total DNA from symptomatic samples was obtained by a modified Dellaporta method and analyzed by nested PCR using the begomovirus universal primers (Mauricio-Castillo *et a*l., 2007, Wyatt and Brown, 1996). Amplicons of ~1.4 kb were cloned and digested by enzymes *Eco*RI and *Hinf*I. Sequence of genomic component A of viruses was determined by PCR amplification of viral DNA with degenerate primers (Mauricio Castillo *et al*., 2007). Cloning, sequencing and subsequent analysis using GenBank database (BlastN and ClustalV alignment method) were employed.

Results

Observations of field and greenhouse indexed samples by SEM revealed the presence of phytoplasma cells ranging from 400 to 1500 nm in the phloem of diseased plants: in leafstalks, leaf midribs, stems floral parts and roots (figure 1). Some asymptomatic field samples also had a low concentration of phytoplasmas in their phloem tissue.

No pathogen was detected in healthy micropropagated plants. In phloem tissue of some samples along with phytoplasmas some rod-shaped bacteria and groups of twinned particles caracteristic of geminiviruses (*Geminiviridae*) were detected (figure 2).

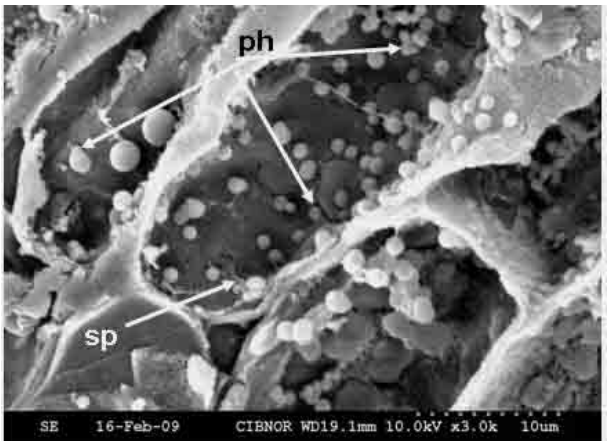

Figure 1. Phytoplasmas (ph) in sieve tubes of tomato. Sp: sieve pore.

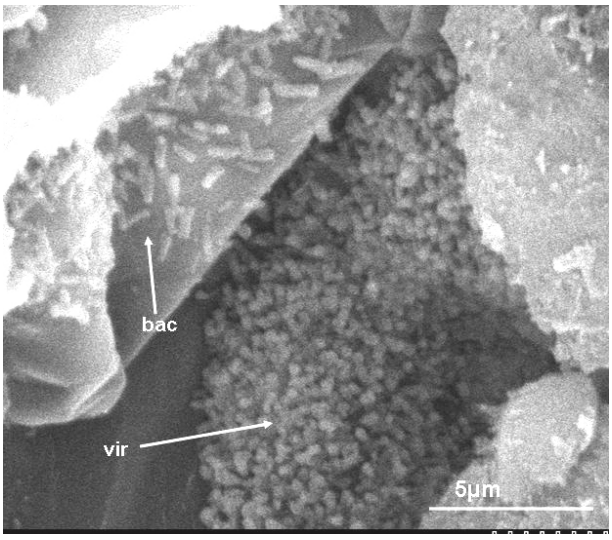

Figure 2. Groups of geminate virus particles (vir) in phloem parenchyma of the same tomato. Bac: rod shaped bacteria.

Nested PCR analysis of both pepper and tomato samples from the plants tested by SEM, revealed a 1,200 bp amplified product, thus supporting the presence of phytoplasmas in plants with yellows symptoms.

Phytoplasma were not detected in micropropagated plants. Sequence analysis of amplified products proved that the phytoplasma detected in pepper belonged to 16SrIII X-disease group. Sequence analysis of amplified DNA products of tomato demonstrated only 80% of similarity with known phytoplasmas. The taxonomic grouping of this phytoplasma needs further confirmation.

The results of PCR assays indicated association of two different begomoviruses in pepper: *Tomato yellow leaf curl virus* (TYLCV), reported first time in Baja California Peninsula, and *Tomato chino La Paz virus* (first report of ToChLPV in pepper, Cardenas-Conejo *et al*., 2010). Virus analysis of tomato symptomatic samples by nested PCR and subsequent procedures also revealed a co-infection with two begomoviruses: TYLCV and *Pepper golden mosaic virus* (PepGMV).

Discussion

The presence of phytoplasmas and two different begomoviruses in tomato and pepper samples in Mexico was verified by SEM and PCR assays. This si the first evidence of mixed phytoplasma-begomovirus infection in tomato and pepper plants. In the last decade the complex phytoplasma-virus associations with plant diseases are discussed more extensively (Arocha *et al*., 2009). Such mixed infections need to be analysed from both epidemiological and pathogens interaction point of view. The work is in progress to define the phytoplasma identity in tomato and the phylogenetic position of phytoplasmas detected in tomato and pepper plants showing yellows symptoms in Mexico.

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