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Phytoplasma associated diseases in tomato and pepper in the state of BCS, Mexico: a brief overview

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Abstract

Multiple symptoms of yellows-type diseases were observed during field surveys of chili pepper and tomato farms in the agricultural areas in the state of Baja California Sur (BCS) from 2004 to 2007. Symptoms were successfully transmitted by grafting to test plants of tomato and pepper. Using scanning electron microscopy (SEM) techniques, phytoplasma cells ranging from 400 to 2000 nm were detected in phloem tissue in field samples and indexed plants, as well as in wild and weedy areas among the crops. Starch granules and salt crystals of unknown etiology were also observed.

Key words: yellows type diseases, phytoplasma, spiroplasma, indexing, SEM, tomato, chili peppers, BCS.

Introduction

Mexican state of BCS at the southern end of Baja California Peninsula is one of the lesser-studied states of Mexico from a phytopathological view. Phytoplasmas were first reported in this state in 2004 in association with papaya maladies (Poghosyan *et al*., 2004). Until then, nothing was known in this region about the incidence of phytoplasma caused diseases in any crop.

During 2006-2007, regular phytosanitary surveys were made in tomato and pepper plantings in Valle de Santo Domingo, El Carrizal, and other agricultural areas in the state in open fields and greenhouses. Symptoms of a yellows-type disease of suspected phytoplasma origin were observed in field and greenhouse growing bell peppers (*Capsicum annuum* tipus Morron), and cherry tomatoes *(Lycopersicon esculentum* Mill. var. Cherry).

Similar symptoms were observed earlier in field grown chiltepin chili peppers (*Capsicum frutescens*) (Poghosyan *et al*., 2005), and poblano chili peppers (*Capsicum annuum* L. var. Anchor poblano*)* (Laski *et al*., 2006).

With the aim to determine the occurrence of phytoplasmas in yellows-type diseases in peppers and tomatoes in Baja California Sur, we used disease indexing in test-plants and detection of phytoplasmas in phloem tissue of suspected diseased plants using SEM techniques.

Materials and methods

Samples of symptomatic and asymptomatic plants were collected in various stages of growth during field surveys. These were grafted onto tomato and pepper test-plants in insect-proof greenhouse conditions. In some cases, young diseased plants were transferred to pots in the greenhouse to observe disease development. For SEM analysis, the same collected samples were used, as well as wild or volunteer plants growing in tomato and pepper fields, including cilantro (*Coriandrum sativum*). Micropropagated phytoplasma-free plants were used as controls.

Apical leaf veins, leafstalks, axillary leaflets and floral parts were cut and fixed with 2.5% glutaraldehyde in 0.2 M sodium cacodilate buffer (pH 7.2) for at least 12 h at 4ºC. After rinsing the samples in the same buffer, they were dehydrated in increasing grades of ethanol (30%, 50%, 70%, 95%, 100%), followed by absolute acetone or hexamethyldisilazane, for 20 min in each solution. After dehydration, the samples were dried in carbon dioxide in a critical point drier, (Samdry-PVT-3B), attached to SEM stubs with double-glue tape and coated with palladium in an ion sputter (DESK II, Denton Vacuum), for 40 seconds. Prepared specimens were examined in a SEM (S-3000N Hitachi) at 5-20 kV.

Results

Diseased tomato and pepper plants were stunted, with shortened internodes, chlorotic and deformed old leaves with necrotic margins and crinkled leaf veins. Apical and internodal leaves were reduced, wrinkled and curled. Leaves of diseased tomato were often rounded, with erect leafstalks. Floral parts were usually dried or reduced. Various symptoms of witches' broom were observed on cilantro, including leaf deformation and reduction, shoot proliferation.

When transmitted to test plants, the symptom expression depended on seasonal temperature variations and typical symptoms appeared within one to two months after grafting. Diseased plants gradually lost their foliage from top to bottom and declined and died during one year.

A study of field and greenhouse indexed specimens by SEM technique revealed the presence of phytoplasma cells in the phloem of diseased plants of tomato and pepper ranging from 400 to 2000 nm. Some asymptomatic field samples also had a low concentration of phytoplasmas in their phloem tissue. No pathogen was detected in phloem tissue of healthy micropropagated plants. Phytoplasmas were observed as separate particles or clusters in the phloem tissue. The most abundant

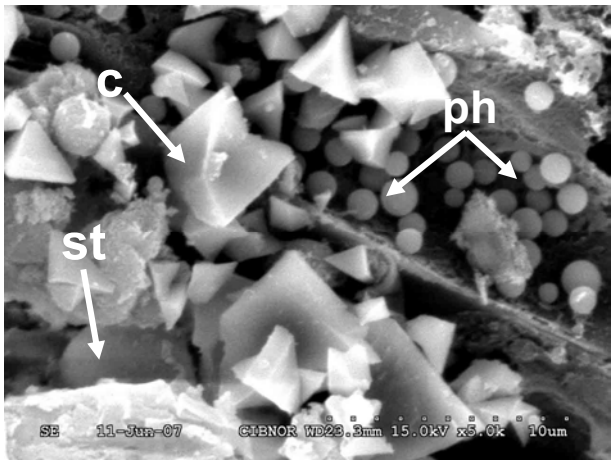

Figure 1. Phytoplasmas (ph), crystals (c), and starch granules (st) in phloem tissue of field sample of bell pepper (chile Morron).

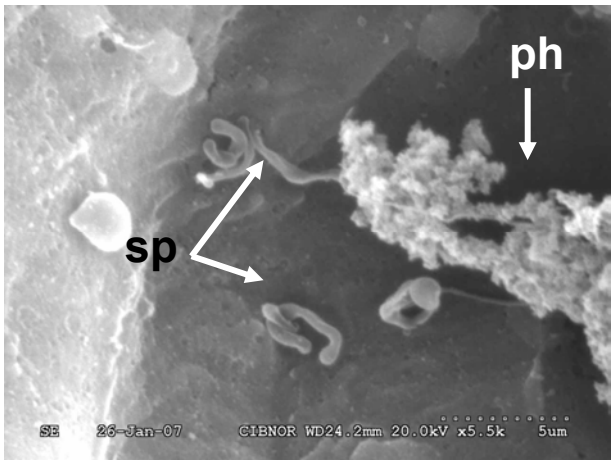

Figure 2. Spiroplasma-like structures (sp) and phytoplasmas (ph) in phloem tissue of diseased cilantro.

concentration of pathogens was noted during the early flowering stage. Some phytoplasma cells were observed near and within sieve pores, demonstrating the mode of their movement within phloem sieve elements and thus transmitting the infection by phloem tissue.

Many starch granules and crystals, particularly of calcium oxalate, were detected in phloem tissue of infected plants (figure 1). The crystals were packed in special cells (idioblasts) and some of them were also seen in xylem tissue.

In phloem tissue of cilantro, both phytoplasma particles and spiroplasma-like structures were observed, with approximate sizes of 6,000 x 500 nm (figure 2).

Discussion

The presence of phytoplasmas in phloem tissue of symptomatic and some asymptomatic tomato and peppers, as well as wild and volunteer plants among the crops, observed by SEM, and the absence of similar structures in healthy micropropagated plants is reliable evidence of phytoplasma pathogen in pepper and tomato diseases in the state of BCS.

The morphology and size of observed structures as well as their localization in sieve elements correspond to phytoplasma cells detected by SEM in association with papaya diseases in the same state (Poghosyan *et al*., 2004), as well as other yellows type diseases elsewhere (Bertaccini *et al*., 1999; Marcone and Ragozzino, 1996).

Viral pathogens from the begomovirus group were also reported in BCS (Holguin *et al*., 2004), with some symptoms very similar to those observed during our surveys and associated with phytoplasma infection. The possibility of a mixed virus and phytoplasma and/or spiroplasma infection and the presence of distinct phytoplasma/spiroplasma groups in BCS will be the subject of future research, incorporating both molecular and ultrastructural techniques.

Acknowledgements

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