Application of scanning electron microscopy for diagnosing phytoplasmas in single and mixed (virus-phytoplasma) infection in Papaya

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Abstract

Phytoplasma and some viruses, *papaya ring spot* (PRSV) and *papaya mosaic* (PapMV) have been reported in papaya, from different Mexican states. Some symptoms of yellow type diseases, such as mosaics, stunting, bunchy top and leaf chlorosis, necrosis and malformations are somewhat similar in appearance, but caused by distinct pathogens. Using a scanning electron microscopy (SEM) technique phytoplasmas were detected in the phloem tissues of field and greenhouse-indexed papaya plants from Baja California Sur (BCS). Samples from 32 local varieties, as well as cv. Maradol, showing numerous symptoms of dieback, mosaics, bunchy top, and yellow crinkle were analyzed. The pathogen was detected in stems, leafstalks, roots, axillary leaflets, leaf veins and flowers. Phytoplasma was also detected in dry and in germinated seeds within the fruit, suggesting seed transmission of the pathogen. Some ultrastructural peculiarities of phytoplasma in infected tissues were also observed. No viral infection with PRSV and PapMV was revealed neither in test-plants nor by molecular techniques. Application of SEM technique for analysis of papaya samples from Veracruz and Irapuato, both from field-grown and mechanically inoculated plants with PRSV and PapMV in various combinations also revealed phytoplasmas in the phloem of most of tested samples. In some cases, along with phytoplasmas, rod-shaped bacteria were distinguished.

Keywords: Papaya, phytoplasma, papaya ringspot virus, papaya mosaic virus, scanning electron microscopy, Mexico.

Introduction

Papaya (*Carica papaya* L.) is an important perennial fruit crop in the tropics and subtropics, and is very susceptible to numerous diseases, probably as a result of extensive monoculture and a narrow gene pool ("The Biology of *Carica papaya* L.", 2008). Phytoplasma and virus-associated papaya maladies are among the more destructive, and there is no strategy for controlling these diseases on a commercial scale. A number of viral diseases have been associated with papaya. Papaya ringspot, caused by *Papaya ringspot virus* (PRSV) significantly reduced crop productivity in Hawaii, the Caribbean, Brazil, Southeast Asia and Australia (Yeh and Gonzalves, 1984; Purciful et al., 1984; Gonzalves, 1998; Golnaraghi and Shnhraeen, 2003; Mowlick et al., 2007). *Papaya mosaic virus* (PMV) is also a serious problem in some countries, such as the USA, Venezuela and Bolivia (Purciful and Heibert, 1971; Rajapakse and Herath, 1981).

Phytoplasma associated diseases of papaya were reported from different papaya producing countries. Papaya dieback (PDB), yellow crinkle (PYC) and mosaic (PM) were recognized in Australia (Gibb et al, 1996, 1998; Elder et al, 2002). Papaya disease, Nivun Haamir (NH), similar to PDB, was reported in Israel (Lju et al., 1996), and attributed to the same taxon as PDB, *Ca. Phytoplasma australiense* (Gera et al., 2005). Phytoplasma associated with bunchy top-like disease (BTS), known in Cuba (Arocha et al, 2006), was recently reported in mixed infection with a potyvirus (Arocha et al., 2009).

Mexico is one of the original centers of papaya cultivation (Nakasone & Paull, 1998), and one of the main papaya producers (Ploetz , 2007). PRSV is considered a very important limiting factor in some regions of Mexico (Treviño, 1980; Teliz et al., 1991; Silva-Rosales et al., 2000). PapMV has been reported in Mexico with low economic impact (Noa-Carrazana and Silva-Rosales, 2001). Some symptoms caused by PRSV and PapMV; stunting, chlorosis, leaf mosaic and distortion and filiform appearance, are common with phytoplasma related papaya symptoms.

In Mexico phytoplasmas associated with papaya infection were first reported in Oaxaca, Central Mexico (Rojas-Martinez et al., 2003). In the Yucatan peninsula Australian Papaya Yellow Crinkle (PYC)-like symptoms were noted in association with detected phytoplasmas (Navarette-Yabur et al., 2003; Moreno-Valenzuela and Navarette-Yabur, 2005). Similar symptoms associated with phytoplasma were recorded in different regions of Baja California Sur (BCS state) (Poghosyan et al., 2004). In 2004, two experimental plots were established in El Centenario and El Comitan, with seeds of 32 local papaya varieties and cv. Maradol. Variability of symptoms was observed in diseased plants, some of which were similar to symptoms reported for different yellow-type diseases. Our objective in this investigation was to analyze the role of phytoplasmas in all types of symptom expression, using disease indexing and an SEM technique.

Additionally, we report the results of phytoplasma detection in papaya samples infected with PRSV and PapMV that were obtained from other regions of Mexico.

Materials and methods

Plant samples from BCS: Samples of apical and axillary leaflets from papaya plants exhibiting different symptoms of presumed phytoplasma infection and asymptomatic samples were taken during field surveys in El Centenario and El Comitan from 2004 through 2007, where experimental fields of papaya were established. Plots were prepared with seeds of 32 local papaya lines and cv. Maradol collected during field surveys in 2002 and 2003. Samples were used for disease indexing under greenhouse conditions and for processing for diagnosis by SEM. For SEM samples other plant organs were also used. Samples of micropropagated papaya were used as a control for SEM. Some weed plants with yellow-type symptoms near papaya plantations and among the trees were also collected and analyzed by SEM. Symptoms were transmitted from the field to greenhouse-grown test plants by grafting. Transmission by dodder (*Cuscuta* spp.) from indexed papaya to Madagascar periwinkle (*Catharanthus roseus*) was conducted in some cases. Samples from some symptomatic plants with leaf distortions of possible viral origin have been sent to CINVESTAV, Irapuato, for PRSV and PapMV- analysis.

Plant samples, Irapuato: Samples of papaya with viral infection were collected from the States of Veracruz (PapMV) and Yucatan (PRSV). The presence of each virus was confirmed by a serological DAS-ELISA test and RT-PCR (Ruiz Castro and Silva Rosales, 1997; Noa-Carrazana and Silva-Rosales, 2001). Then a series of inoculations were performed differently in the greenhouse on papaya test-plants to reproduce symptoms of each virus and to elucidate the symptoms of possible mixed viral infection: single infection with either PapMV or PRSV; mixed simultaneously, PRSV+PapMV, and stepwise, PapMV-PRSV (PapMV first, followed by PRSV after 30 days), and PRSV-PapMV (PRSV first, followed by PapMV after 30 days) (Noa-Carrazana et al., unpublished data). This part of the experiment was conducted at the Virology Laboratory of CINVESTAV in Irapuato. To verify the presence of phytoplasma in samples, including the controls, samples were processed for SEM analysis (up to 70 % ethanol grade) and sent to CIBNOR for further processing and phytoplasma analysis by SEM.

Applied SEM technique: Leaf vines, leafstalks, axillary leaflets, stem, roots, floral parts, and fruits (seeds and plantlets germinated within fruits) from symptomatic, asymptomatic, and control papaya samples were fixed in 2.5 % glutaraldehide dissolved in 0.2 M sodium cacodylate buffer (pH 7.2-7.4) for one day at 4 °C. 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 hexamethyl- disilazane), for 20 min in each of these solutions. After dehydration the samples were dried in carbon dioxide (Critical Point Drier, Samdry-PVT-3B), and then attached to SEM stages by double-sided tape. The samples were coated with paladium in an ion sputter (Denton Vacuum, DESK II) and examined in the scanning electron microscope (S-3000N, Hitachi, Japan) at different accelerating voltages (5-20 kV). Samples of wild plants wereprocessed in the same manner and examined by SEM. In the case of infection of papaya with PRSV and PMV, only apical leaves were processed for SEM analysis.

Results

Disease symptoms, BCS: Diverse symptoms of yellow-type diseases were observed in local lines and cv. Maradol papaya in the field. Some plants had stunted growth, with shortened internodes and proliferation of shoots and internodal leaves, while others had a bunchy top appearance;sometimes, thickening of the petioles and formation of tumors on the trunk was noted (Figure 1, G-H). When old leaves abscised, a hole-like “wound” formed on the trunk. Flow of latex was reduced and was either watery or absent, in some lines. Symptoms on flowers were noted as sepal hypertrophy and petal reduction in female flowers and dried or underdeveloped inflorescences of male and hermaphrodite flowers. Fruit formation depended on the growing stage. If infection was late, fruit formation was initially normal, but then ceased. In early infections, the plants formed only 2 or 3 fruits that were deformed and small.

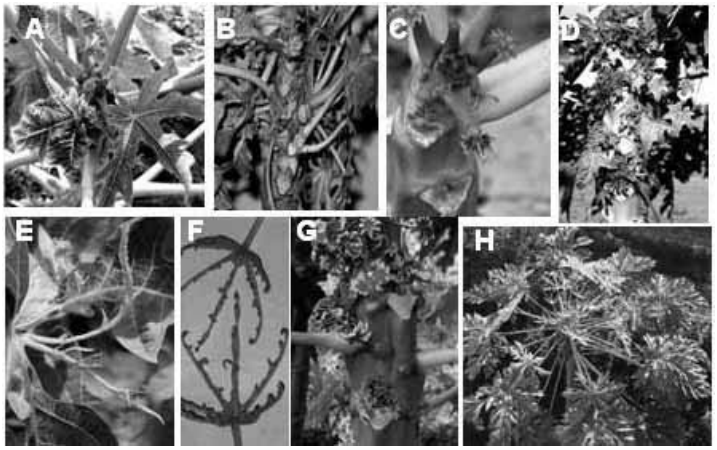

Fig. 1 Symptoms of yellow-type diseases in papaya from BCS: (A) leaf wrinkling; (B) shortening of internodes and proliferation of internodal leaves; (C) button-like leaf reduction; (D) shortening of leafstalks and leaf distortions; (E) filiform leaf structure; (F) claw-like leaf deformation; (G) tumors on trunk; (H) bunchy top.

Many kinds of leaf malformations was observed on plants in different stages of growth, including filiform and claw-like leaf distortion of old leaves, yellowing and wrinkling, severe leaf crinkle and reduction. Small "clawed" or "button-like" bunched leaflets appeared on stem tips, apex, and internodes. When old leaves were falling, very small bunches of distorted leaves with very short leafstalks appeared in the internodes or on the lower parts of the trunk (Fig.1, A-F). Tumors on the trunk turned brown, then necrotic and finally desiccated. When phytoplasma infection was transmitted to papaya test plants by grafting, similar symptoms occurred in the test plants. Disease indexing of symptoms from two papaya local lines having tumors on the trunk did not show this specific symptom on test papaya plants. All diseased test plants died within one year.

Transmission to periwinkle by dodder led to strong interveinal chlorosis, and finally, total necrosis and abscission of leaves and proliferation and necrosis of branches. In some branches only very small leaves survived on the tips, other branches died within one to two years. Formation of flowers was reduced, with malformed and pale rose-colored petals.

Virus symptoms: Symptoms of viral infection in field-grown papaya plants from 15 states of Mexico (not BCS) included yellowing, vein clearing, mosaic, and leaf distortion (Noa –Carranaza et al, unpublished data). Different symptoms were observed in inoculated papaya test-plants, from mild mosaic from infection with only PapMV or PapMV-PRSV, to necrotic lesions and leaf distortion in the case of single infection with PRSV, or mixed infections of PRSV-PapMV or PRSV+PapMV.

SEM analysis: samples from BCS: Phytoplasma cells were detected in the phloem tissue of samples analysed from local papaya lines and the commercial Maradol variety, raised in the field or in the greenhouse (Figure 2).

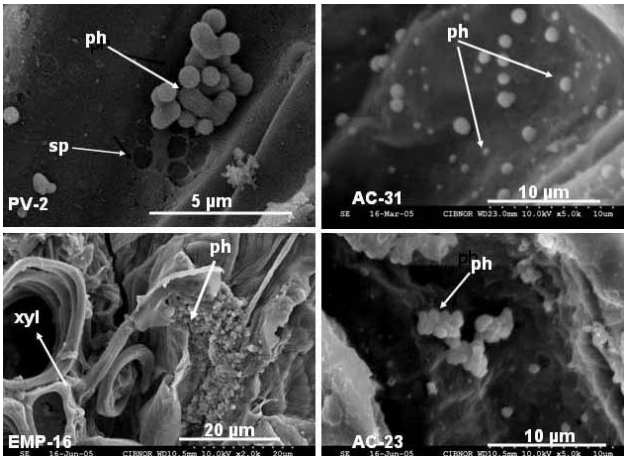

Fig. 2 Scanning electron micrographs of phytoplasmas in phloem tissue of some local papaya lines. (PV-2) and (AC-31), field samples; (EMP-16) and (AC-23), indexed plants. Arrows indicate: ph-phytoplasma cells; sp-sieve pores; xyl- xylem tissue.

The pathogen was detected in different parts of diseased plants: leaves, stems, leafstalks, roots, flowers, fruit and seeds (Figure 3-4). Phytoplasma was not found in symptom-free micropropagated plants, but was however detected in some symptomless field-grown papayas. The concentration of the pathogen in phloem cells depended from the season, plant condition, disease stage and severity. Phytoplasmas were observed as spherical bodies, ranging in size from 500 to 1800 nm. They appear as separate cells or clustered particles in phloem tissue, sometimes observed near or within sieve pores. Some phytoplasma were in the process of binary fusion, or with buds. The fibril structure of thecytoplasm and nucleus with surrounded plastids was distinguishable in host cells in some cases. Uneven distribution of phytoplasmas in sieve tubes was noted.

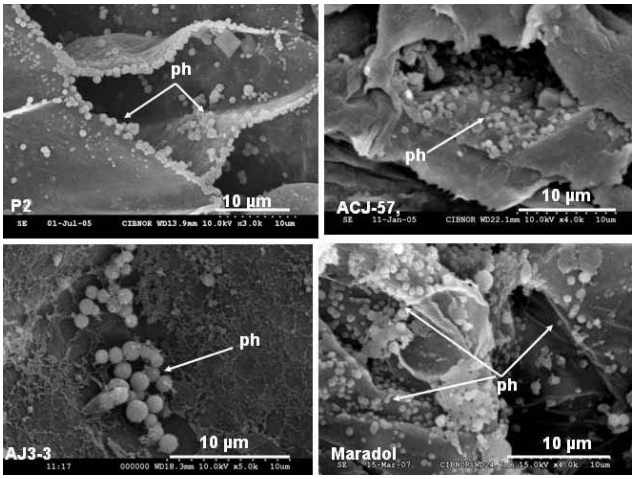

Fig. 3 Phytoplasmas in different organs of diseased local lines and cv. Maradol. (P2), floral bud; (ACJ-57), leaf vine; (AJ3-3), root; (Maradol), leafstalk. Arrows indicate phytoplasma (ph).

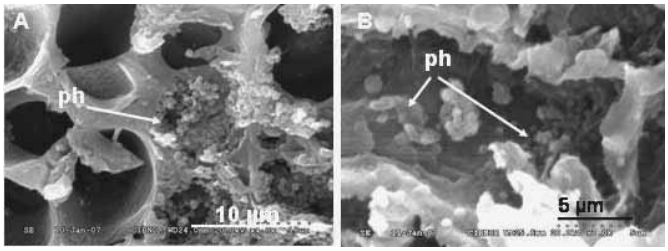

Fig. 4 Phytoplasmas (ph) detected in dry mature seed (A), and in germinated seedling (B).

In some cases the “infected zone” was observed at low magnification in the phloem tissue of a diseased plant, including about 20 neighboring phloem cells in one section plane (Figure 5).

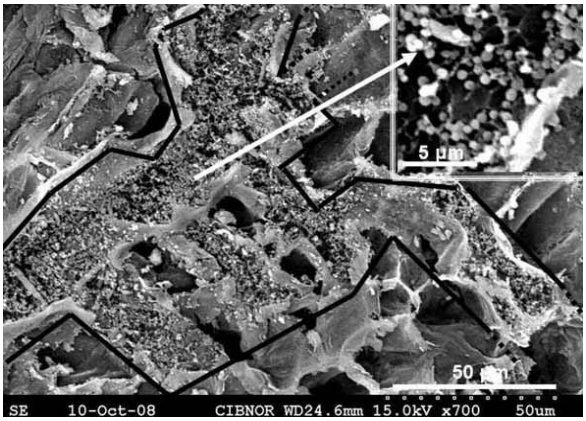

Fig. 5 Distribution of phytoplasmas in phloem of diseased papaya, low Magnification (X700). The “zone” of infection is marked in black. White arrow indicates part of the same image, high magnification (X4.000).

In phloem tissue of papaya with tumor-like structures on the trunkmany rod-shape bacteria were also detected alongside the phytoplasma, (Figure 6). Some ultrastructural features in the diseased host plant phloem tissues were observed: starch granules in phloem parenchyma, many inorganic crystals and some paracrystals.

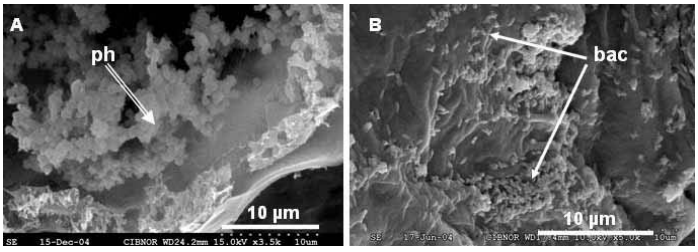

Fig. 6 Trunk section of papaya with tumor. (A). Phytoplasmas (ph), and (B) rod-shaped bacteria (bac).

When symptoms were transmitted by dodder, phytoplasmas were observed in periwinkle and in dodder haustoria (Figure 7).

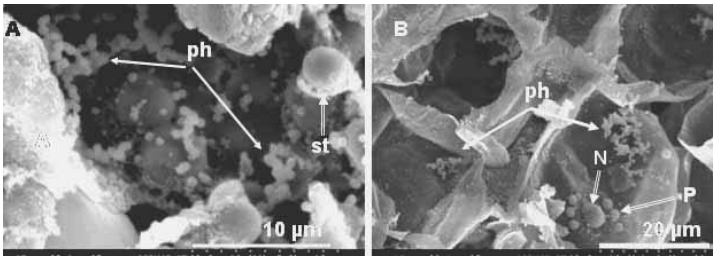

Fig. 7 (A). SEM- image of phytoplasmas transmitted by dodder to periwinkle.(B). Phytoplasmas in dodder haustoria. Indications: ph-phytoplasma; N-nucleus, P-plastids, St-starch granule.

In samples from morning glory (*Convolvulus* spp.) and other symptomatic and asymptomatic weeds, abundant phytoplasma particles were observed in phloem tissue with an average size of 1000nm (Figure 8).

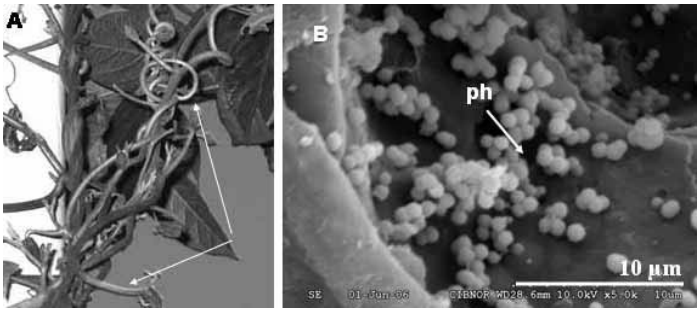

Fig. 8 (A). Morning glory (*Convolvulus* spp.) with symptoms of leaf reduction and distortion (arrows). (B). Phytoplasmas (ph) in phloem of morning glory.

SEM analysis, samples with viral infection: Phytoplasmas were detected in all samples with single (PRSV and PapMV) and mixed (PRSV+PapMV, PRSV-PapMV) viral infection. (Figure 9). Phytoplasmas were more abundant in the samples, inoculated with PRSV only, or simultaneously with PapMV.

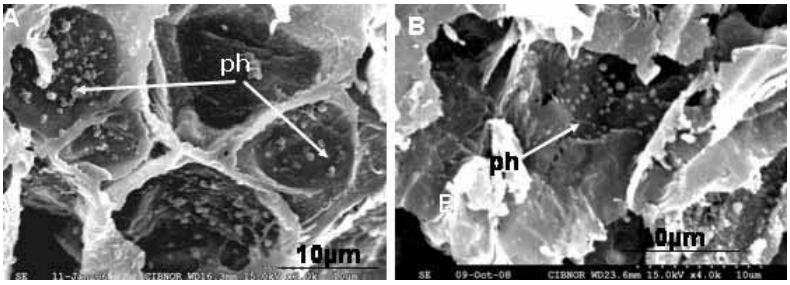

Fig. 9 SEM micrograph of phytoplasmas(ph) in phloem tissue of papaya with PRSV. (A) Field-sample from Veracruz. (B) Test- plant inoculated with PRSV.

A low phytoplasma concentration occurred in samples that contained PapMV only. In the case of mixed PapMV-PRSV (first-PapMV), rod-shaped bacteria but not phytoplasma were detected. Samples without viruses also contained phytoplasmas in phloem tissue.

Discussion

The character of symptom development and plant death observed in distinct papaya local lines was somewhat similar to Australian papaya dieback (PDB), and yellow crinkle (PYC) diseases (Gibb et al., 1996). SEM analysis of the 32 local papaya lines and cv. Maradol revealed phytoplasma in phloem tissue of distinct organs in different growth stages. Among the symptoms in damaged papayas was bunchy top, one of the most frequently reported symptoms among related to yellow-type diseases. This "bunchy" symptom was reported also in Australian PDB disease as an intermediate symptom ("Biology of Papaya", 2008). Bunchy symptoms appeared in our indexed papaya plants two months after grafting from diseased papaya, that did not express this symptom, and phytoplasma cells were observed in phloem tissue of bunched leaves. Button-like and claw-like leaf symptoms at internodes and tips also developed in grafted papaya test-plants, and phytoplasmas were found in their phloem tissues.

Previous to our study, tumors on the trunk of plants had not been reported for any phytoplasma related papaya malady. Phytoplasma cells were observed by SEM both in tumors and tissues of trunk section. Similar tumor-like growths were described within trunks of papaya with an unknown disease reported in the Republic of Congo, but PCR analysis showed the plants to be infected with a potyvirus rather than a phytoplasma (Arocha et al., 2008). On the indexed plants tumors did not develop, but when infection was transmitted from a tumor-bearing papaya to test plants, phytoplasmas were observed in indexed papaya and later, in periwinkle, connected by dodder to this phytoplasma positive papaya plant.

Not all samples collected from symptomatic plants (about 10 % of over 500 analyzed samples) revealed the presence of phytoplasmas in their phloem tissue. This may be related with the mechanisms of phytoplasma movement ("migration") within the plant. The appearance of symptoms, especially in leaves, does not always correlate with phytoplasma presence, and the pathogen in some cases could not be observed (Siddique et al., 1998; Wei et al., 2004). In our experiments with indexed papaya plants, when the temperature in greenhouse was extremely high (>35 °C) or low (<15 °C), phytoplasma was more easily detected in the lower leaves and roots than in the upper parts of symptomatic test plants. These data correlate with phytoplasma distribution in other woody plants (Jiang et al., 2004).

Detection of phytoplasma in mature and germinated seeds within fruit from diseased papaya is of special interest. Vertical transmission of phytoplasma was not recognized earlier, but is now strongly disputed. Phytoplasma DNA was found in embryos from coconut palm with lethal yellowing (LY) disease and maize kernels (Cordova et al. 2003; Jones et al, 2007). Seed transmission of some phytoplasmas into seedlings of alfalfa, tomatoes and oilseed rape, and plantlets of lime was reported (Khan et al., 2002; Botti and Beratccini, 2006). These findings are further supported by a recent report of the possible transmission of ESFY phytoplasma through apricot flowers and seeds (Nečas et al., 2008). No data about possible seed transmission of phytoplasma associated with papaya diseases have been recorded. Detection by SEM of phytoplasma in different papaya organs, including flower parts, plantlets and mature and germinated seeds within the fruit demonstrates that phytoplasma could move from the flower parts to seeds and seedling. Nipah et al. (2007) discussed the possible mechanism of phytoplasma movement to seeds, noting the importance of electron microscopy in the identification of phytoplasmas in seeds. Though further validation with PCR analysis is required in our study of seed transmission, and application of SEM, in some other cases, these findings present new perspectives for studying the epidemiology of diseases associated with phytoplasmas.

The application of both molecular techniques and electron microscopy may be needed to diagnose some complex yellow-type symptoms when disease origin is not clear. With these methods, mixed infection with phytoplasma and viruses was shown in malformed clovers (Franova et al., 2004) and recently demonstrated in papaya with bunchy top symptoms (BTS) in Cuba (Arocha et al., 2009). The presence of rod shaped bacteria along with phytoplasma in phloem tissue of papaya with a tumor on the trunk, suggests a possible mixed infection with two distinct prokaryotic pathogens. The reliability of phytoplasma diagnosis in phloem tissue by SEM technique, was reported and discussed earlier (Poghosyan et.al., 2004; Al-Awadhi et al., 2002).

Diagnosis of phytoplasmas in 32 local papaya lines and cv. Maradol in one state, BCS, and the detection of this pathogen in papaya samples from other Mexican states (Veracruz and Yucatan) with viral infection, was an additional reason for using SEM technique for the diagnosis of phytoplasma.

Future investigations by PCR and other molecular tests could help to characterize phytoplasmas found in diseased papaya plants in BCS. Moreover, studies about the phylogenetic relations between phytoplasmas in papaya from different Mexican states and beyond, could provide more information to better understand mixed infections in papaya.

Literature

Al-Awadhi, H.A.; Hanif, A.; Suleman, P.; Montasser, M.S.; 2002: Molecular and microscopical detection of phytoplasma associated with yellowing disease in date palms *Phoenix datilifera* L. in Kuwait. Kuwait J. Sci. Eng. 29(2), 87-108.

Arocha, Y.; Piñol, B.; Picornell, B.; Almeida, R.; Jones, P.; 2006: Firts report of the 16SrII (*Candidatus Phytoplasma aurantifolia*) group associated with a bunchy-top disease of papaya in Cuba. Plant Pathol. 55, 821.

Arocha, Y., Almeida, R.; Vigheri, N.; Nikoy-Florent; Betts, P., Monger, W.A., Harju, V., Mumford.R.A.; Bekele, B., Dereje Tadesse and Jones, P.; 2008: Unveiling the aetiology of papaya diseases in Cuba, Democratic Republic of Congo (DRC) and Ethiopia. Rev. Protección Veg. 23(1), 21-25.

Arocha Y.; Piñol, B.; Acosta, K; Almeida, R.; Devonshire, J.; Van de Meene, A.; Boa, E.; Lucas, J.; 2009: Detection of phytoplasma and potyvirus pathogens in papaya (*Carica papaya* L.) affected with "Bunchy Top Symptom"(BTS) in eastern Cuba. Crop Protection, 28(8), 640-646.

Botti, S., Bertaccini, A.; 2006: Phytoplasma infection through seed transmission: further observations, pp.76. In: R.D. Ayling; C. Citti; R.A.J. Nicholas (Eds.): Proc.16th Intern. Organ. Mycoplasmasmol.Congr., 76. Cambridge, UK, Veterinary Laboratories Agency.

Cordova, I.; Jones, P; Harrison, N.A.; Oropeza, C.; 2003: In situ PCR detection of phytoplasma DNA in embryos from coconut palms with lethal yellowing diseases Molecular plant pathology 4(2), 99-108.

Elder, R.J.; Mine, J.R.; Reid, D.J.; Guthrie, J.N.; Persley, D.M.; 2002: Temporal incidence of three phytoplasma associated diseases of *Carica papaya* and their potential hemipteran vectors in central and southeast Queensland. Aust. Plant Pathol.31(2),165- 176.

Fitch, M.M M.; 2005: *Carica papaya* Papaya.Chapter 6.1. In: R.E. Litz (Ed.): Biotechnology of fruit and nut crops. CABI Publishing, 174-207.

Fránova J.; Paltrinieri, S.; Botti, S.; Šimkova, M.; Bertaccini, A.; 2004: Association of phytoplasmas and viruses with malformed clovers. Folia Microbiol. 49(5), 617-624.

Gera, A.; Mawassi, M.; Zeidan, A.; Spiegel, S.; Bar-Joseph, M.; 2005: An isolate of „*Candidatus Phytoplasma australiense*" group associated with Nivun Haamir dieback disease of papaya in Israel. Plant Pathol. 54, 560.

Gibb, K.; Persley, D.; Schneider, B.; Thomas, J.; 1996: Phytoplasmas associated with papaya diseases in Australia. Plant Dis. 80, 174-178.

Gibb, K.S; Shneider, B; Padovan, A.C.; 1998: Differential detection and genetic relatedness of phytoplasmas in papaya. Plant Pathol. 47, 325-332.

Gonsalves, D.; 1998: Papaya ringspot. In: Compendium of Tropical Fruit Diseases; Ploetz, R.C., Zentmyer, G.A.; Nishijima, W.T.; Rohrbach, K.G.; Ohr, H.D.; Eds.; APS Press: Minnesota, U.S.A., 66–68.

Jiang, H.; Wei, W.; Saiki, T.; Kawakita, H.; Watanabe K.; Sato, M.; 2004: Distribution patterns of mulberry dwarf phytoplasma in reproductive organs, winter buds and roots of mulberry trees. J. General Plant Pathol. 70(3), 168-173.

Khan, A.J.; Botti, S.; Paltnieri, S.; Al-Subdhi, A.M.; Bertaccini, A.F.; 2002: Phytoplasma in alfalfa seedlings: infected or contaminated seeds? In: Abstracts, 14-th Intern. Org. of Mycoplsmol.Conf., 148. Vienna, Austria.

Kostova, D.; Tsorlianis, S.;Yordanova, A.; Lisa, V.;Vaira, A.M.; 2006: New cucumovirus on beans in Bulgaria - an attempt for characterization. Journal of Culture Collections, 5(1), 94-101.

Liu, B.; White D.T.; Walsh K.B.; Scott, P.T.; 1996: Detection of phytoplasmas in dieback, yellow crinkle and mosaic diseases of papaya using polymerase chain reaction techniques. Austral. J. of Agric. Res. 47(3), 387-394.

Moreno-Valenzuela, O.; Navarette-Yabur, A.; 2005: Detección e identificación de fitoplasmas presentes en papayo (*Carica papaya L.*) en Yucatán. Articulo técnico del órgano informativo de la Fundacion Produce Yucatán, 13-16.

Mowlick, S.; Akanda, A.M.; Rahman, A.H.; 2007: Development of mild strains of Papaya Ringspot Virus-Papaya Strain. J. Agric. Rural. Dev. 5(1-2), 94-97.

Nakasone, H.J., Paull, R.E.; 1998: Tropical Fruits. CAB International, Wallingford.

Nečas, T.; Maškova,V.; Krška, B.; 2008: The possibility of ESFY phytoplasma transmission through flowers and seeds. ISHS Acta Horticultura 781: XX International Symposium on virus and virus-like diseases of template fruit crops-fruit trees diseases.

Nipah, J.O.; Jones, P.; Hodgetts, J.; Dickinson, M.; 2007: Detection of phytoplasma DNA in embryos from coconut palms in Ghana, and kernels from maize in Peru. Bulletin of Insectology 60 (2), 385-386.

Noa-Carrazana, J. C.; Silva-Rosales, L.; 2001: First report of a Mexican isolate of Papaya mosaic virus in Papaya (*Carica papaya*) and pumpkin (*Cucurbita pepo*). Plant Dis. 85, 558.

Padovan, A.C.; Gibb, K.S.; 2001: Epidemiology of phytoplasma diseases in papaya in northern Australia. J. Phytopathol. 149, 649-658.

Ploetz, R.Z.; 2007: Diseases of tropical perennial crops: challenging problems in diverse environments. Plant Dis. 91(6), 644-663.

Pourrahim, R; Farzadfar, A.; Golnaraghi, A.R.; Shnhraeen, N.; 2003: First Report of Papaya ringspot virus on Papaya in Iran. Plant Dis. 87, 1148.

Purcifull, D.E.; Heibert, E.; 1971: Papaya mosaic virus. AAB Description of Plant Viruses. http://www.dpweb.net.

Purcifull, D.; Edwardson, J.; Heibert, E.; Gonzalves, D.; 1984: Papaya ringspot virus. In:Descriptions of Plant Viruses, 292. Commonwealth Mycological Institute and Association of Applied Biologists: Kew, England.

Rajapakse, R.H.; Herath, H.M.; 1981: Host susceptibility of the papaya mosaic virus in Sri Lanka. Beitr Trop Landwirtsch Veterinarmed 19, 429-32.

Ruiz-Castro, S.; Silva-Rosales, L.; 1997: Use of RT-PCR for papaya ringspot virus detection in papaya (*Carica papaya*) plants from Veracruz, Tabasco and Chiapas. Rev. Mex. Fitopatol. 15, 83-87.

Siddique, A.B.M.; Guthrie, J.N.; Walsh, K.B.; White, D.T.; Scott, P.T.; 1998: Hystopathology and within-plant distribution of the phytoplasma associated with Australian papaya dieback. Plant Dis. 82, 112-1120.

Silva-Rosales, L.; Becerra-Leor, N.; Ruiz-Castro, S.; Teliz-Ortiz, D.; Noa-Carrazana, J. C.; 2000: Coat protein sequence comparisons of three Mexican isolates of papaya ringspot virus with other geographical isolates reveal a close relationship to American and Australian isolates. Arch.Virol. 145, 835-43.

Teixeira da Silva, J., Rashid, Z.; Tan Nhut, D.; Sivakumar, D; Gera, A; Teixeira Souza Jr., Tennant P. F.; 2007: Papaya (*Carica papaya* L.) Biology and Biotechnology. Tree and Forestry Science and Biotechnology, © 2007 Global Science Books.

Teliz, D.; Mora, G.; Nieto, D.; Gonsalves, D.; Garcia, E.; Matheis, L.; Avila, C.; 1991: La mancha anular del papayo en México. Rev. Mex. Fitopatol. 9, 64-68.

"The Biology of *Carica papaya* L"; 2008: Australian government, Department of Health and Ageing Office on the gene technology regulator.Overview, version 2.

Treviño, M. H.;1980: Incidencia de Virosis en Papayo: CAECOT-INIA. Mexico.

Wei, W.; Kakisawa, S; Suzuki, S., Jung, H.-Y.; Nishigawa, H.; Miyata, S.-I.; Oshima, K.; Ugaki, M.; Hibi, T.; Namba S.; 2004: *In planta* dynamic analysis of onion yellows phytoplasma using localized inoculation by insect transmission. Phytopathology 94, 244-250.

Yeh, S.-D.; Gonsalves, D.; 1984: Evaluation of induced mutants of papaya ringspot virus for control by cross protection. Phytopathology 74, 1086-1091.