# A combined microscopy approach to study plant-phytoplasma interaction using Arabidopsis thaliana.

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Phytoplasmas, obligate parasites of plants and phloem-feeding insects, belong to Mollicutes (Lee et al., 2004) and are associated with several hundreds of diseases affecting over one thousand plant species, including many economically important crops (Marcone, 2014). There is no effective curative strategy available so far, so the sole ways to limit the infection outbreaks are the use of insecticides and the removal of symptomatic plants (Bertaccini et al., 2014).

Even if not all infections are necessarily deleterious, symptoms in infected plants suggest heavy disorders of phloem functions and growth-regulator balancing (Lee et al., 2000). Upon their discovery (Doi et al., 1967), the study of phytoplasmas has been hindered by the extreme difficulty to culture them in vitro, due to their lack of fundamental metabolic pathways (Bai et al., 2006). Moreover, the study in natural plant hosts is often limited by environmental conditions, long plant life cycle and poor knowledge of host-plant biology. Therefore, in the last decade some authors suggested to use *Arabidopsis thaliana* as model plant for studying phytoplasma-plant interactions. This choice was supported by the correspondence between the macroscopic symptoms developed in infected *A. thaliana* and those observed in natural host plants (Bressan and Purcell, 2005; Hoshi et al., 2009; Cettul and Firrao, 2011; MacLean et al., 2011). Nevertheless, morphological and ultrastructural modifications occurring in infected *A. thaliana* tissues have never been described in detail.

In this work, we adopted a combined microscopy approach to verify if this plant is a reliable model for the study of phytoplasma-plant interactions at microscopical level.

Using DAPI and fluorescence microscopy (FM), phytoplasma presence and localization were demonstrated in every infected plant. Transmission electron microscopy (TEM) observations confirmed phytoplasma massive presence into the sieve elements (SEs) (Figure 1). Phytoplasma

appeared well preserved, with typical pleomorphic shape, free-floating and dividing in the lumen or adhered to SE membrane, probably connecting to the host (Marcone et al., 2014; Buxa et al., 2015). Phytoplasmas also established relationships with sieve element reticulum (SER). Pathogen presence, probably linked to nutrient uptake (Celli et al., 2015; Musetti et al., 2016), caused SER hyperproliferation, as observed in many other plant-phytoplasma interaction (Rudzinska-Langwald and Kaminska, 2001; Buxa et al., 2015) (Figure 1). Pathogen spread was documented by the passage through sieve pores.

As remarked above, phytoplasma presence affected host plant development (Lee et al., 2000). In infected *A. thaliana* plants, light microscopy (LM) evidenced a profound disturbance in phloem morphology at histological level, mainly consisting in collapse, necrosis and hyperplasia of the phloem components. The relationship between necrosis and hyperplasia could be explained as a plant response to the impaired phloem functionality (Oshima et al., 2001) or due to pathogen effectors (Bai et al., 2009; Sugio et al., 2011).

At ultrastructural level, as previously observed in other phytoplasma hosts (Musetti et al., 2000; 2013; Kaminska et al., 2001; Santi et al., 2013), phloem components showed plasmolysis or were collapsed or necrotized. Even in vital SEs, abnormalities of cell membrane profile and cell wall thickness were visible. TEM observations showed two typical plant responses to phytoplasma infection: phloem-protein agglutination and callose deposition at the sieve plates, which limited sieve-pore diameter (Figure 1). These phenomena have been interpreted as a plant reaction to physically limit pathogen spread (Lherminier et al., 2003; Gamalero et al., 2010; Luna et al., 2011; Musetti et al., 2010; 2013).

Phloem functionality experiments using CFDA and confocal laser scanner microscopy (CLSM) suggested that sieve-pore obstruction leads to phloem impairment (Figure 2 A, C). This phenomenon is also associated to the accumulation of photo-assimilates, visible as chloroplast starch deposits under LM and TEM (Figure 2 B, D), as previously reported in other host plants (Maust et al., 2003; Junqueira et al., 2004; Musetti et al., 2013).

This study proved that phloem tissue of infected *A. thaliana* presented the main morphological and ultrastructural response to phytoplasma infection as reported in natural hosts. Moreover, analyses carried on *A. thaliana* were not affected by troubles linked to low phytoplasma titre and uneven distribution, typical of woody plants. Therefore, we can state that *A. thaliana* revealed a reliable model plant for phytoplasma-plant interactions, concerning both macroscopic symptoms and morphological and ultrastructural changes.

**Figures:**

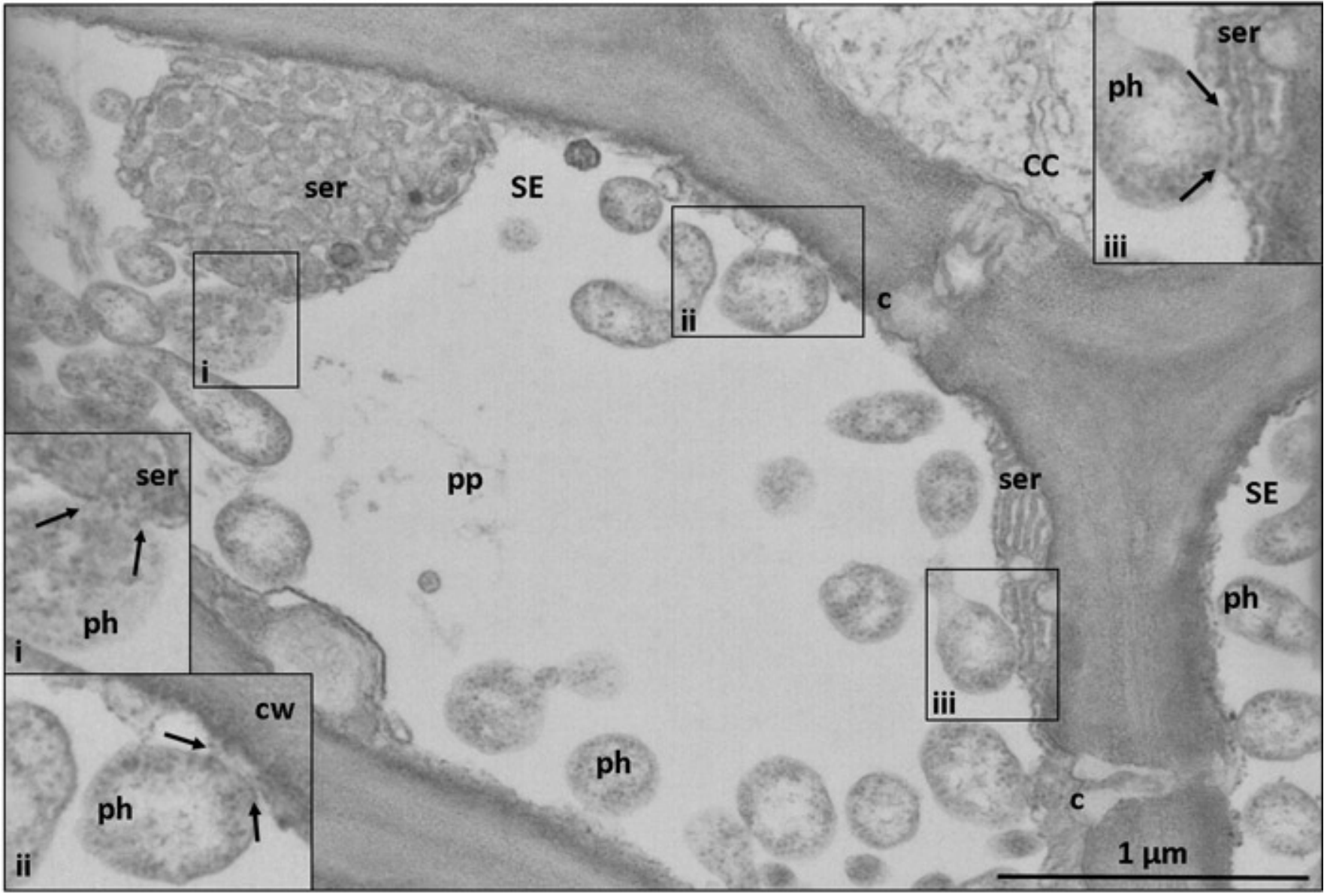


Figure 1. TEM micrograph of phytoplasmas in infected SE. They are floating and dividing in lumen or adhered to SE plasma membrane. Phytoplasmas establish relationships with hyperproliferated SER. Insets show the phytoplasma adhesion to different SE components (black arrows). c: callose, CC: companion cell; cw: cell wall; ph: phytoplasma; pp: phloem protein; SE: sieve element; ser: sieve endoplasmic reticulum

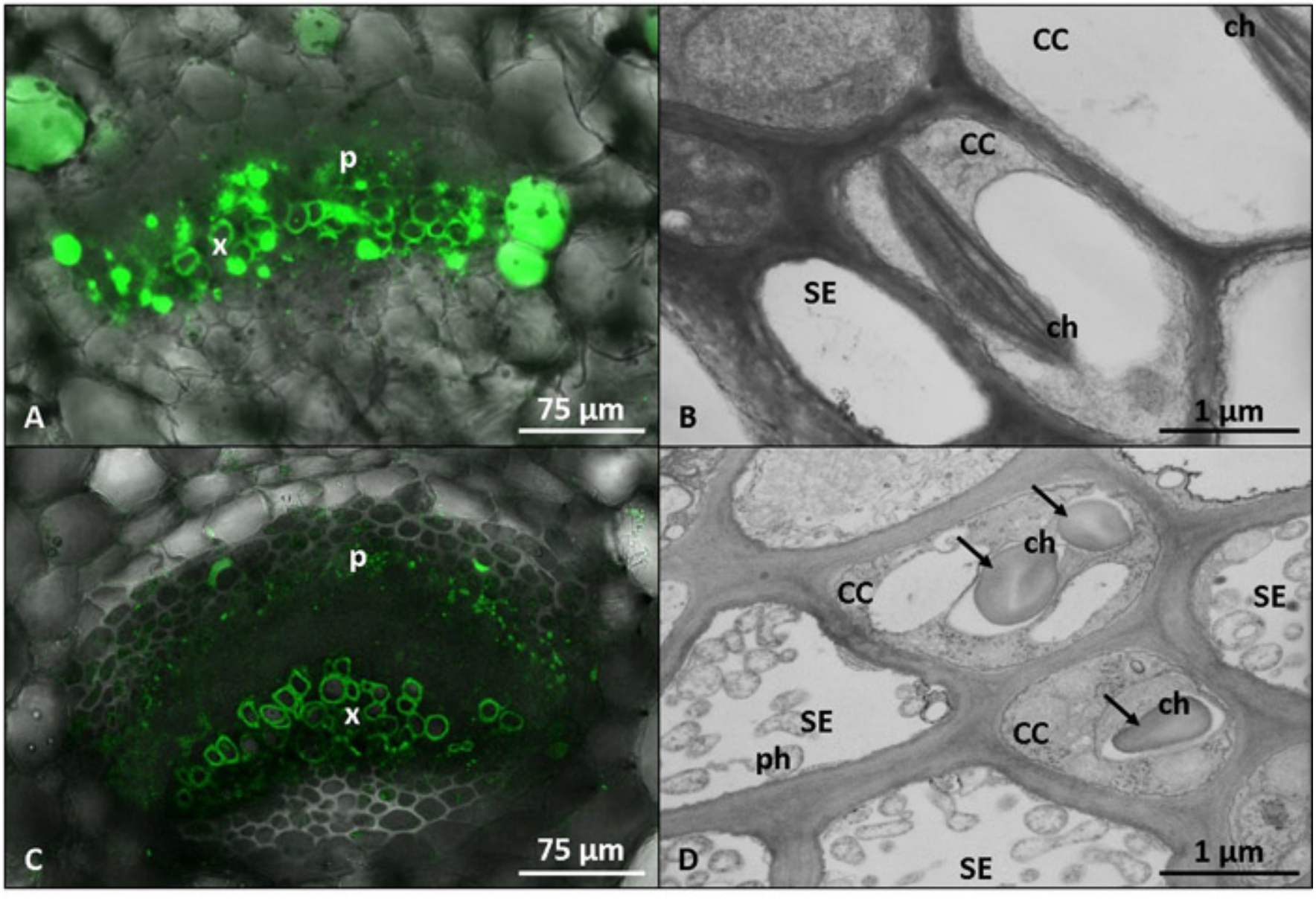


Figure 2. CSLM images and TEM micrographs of healthy (A, C) and infected (B, D) plants. CFDA experiments demonstrated that SE mass flow is significantly reduced in infected plants (A, B). In infected plants massive starch deposits (black arrows) are present in chloroplasts, provoking a distortion of the thylakoid membrane system. CC: companion cell; ch: chloroplast; p: phloem; ph: phytoplasma; SE: sieve element; x: xylem.

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