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Effects induced by fungal endophytes in *Catharanthus roseus* tissues infected by phytoplasmas

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Abstract

Endophytes are microorganisms (fungi, bacteria, actinomycetes) that live inside host plants without causing disease symptoms or apparent injury. *Aureobasidium pullulans* and *Epicoccum nigrum* are two endophytic fungi of particular interest because they are reported as antibiotic producers and antagonists against different phytopatogenic microorganisms. In this work, the cytological effects caused by endophyte-inoculation in phytoplasma-infected *Catharanthus roseus* tissues were investigated. Ultrastructural observations performed on leaves of endophyte-inoculated cuttings evidenced phytoplasma structural modifications associated with the increasing of host defence response, such as formations of phloem protein plugs, callose occlusions and presence of vacuolar phenolic depositions in the lumen of sieve elements. These modifications lead to the enhancement of physical barriers preventing the phytoplasma movements in the host.

Key words: *Aureobasidium pullulans, Catharanthus roseus*, endophytes, *Epicoccum nigrum*, phytoplasmas.

Introduction

Plants frequently host endophytic fungi and/or bacteria which, for all or part of their lifecycle colonize the tissues of living plants without causing disease symptoms (Wilson, 1995). About fungal endophytes, they can result extremely diverse in the different plants, colonizing all the parts of the host. It is recognized that endophytes are of great importance for the plants, protecting them against pathogenic fungi, bacteria, insects and nematodes (Gimenez *et al*., 2007). They also induce physiological modifications in their hosts, making them more resistant against abiotic or environmental stresses. Many endophytic microorganisms are able to produce compounds of biotechnological value as antibiotics and antitumoral drugs (Schulz *et al*., 2002).

Aureobasidium pullulans (de Bary) Arnaud and *Epicoccum nigrum* Link are two ubiquitous microfungi, frequently reported as endophytes of different crops (Musetti *et al*., 2005; Rodolfi *et al*., 2006; Prasongsuk *et al*., 2005). Both fungi are antibiotic producers, they have antagonistic activity against phytopathogenic fungi (Madrigal *et al*., 1991) and are considered as possible biocontrol agents of pre- or post-harvest diseases (Elmer *et al*., 2001; Nigro *et al*., 2003).

Aim of this work was to investigate the interactions between the two endophytes and phytoplasmas isolated on *Catharanthus roseus* L. plants, verifying in particular, if the fungal treatment could induce ultrastructural modifications also in the host.

Materials and methods

E. nigrum and *A. pullulans* endophytic strains were isolated from grapevine shoots as previously reported (Musetti *et al*., 2005). For *C. roseus* inoculation, both endophytes were grown on malt extract broth (MEB, Pronadisa, CONDA laboratories, Madrid, Spain), for 8 days, at 20 °C in the dark. Two *C. roseus* plants, infected by grafting with '*Candidatus* Phytoplasma mali' (the pathogen associated with apple proliferation-AP), exhibiting symptoms characteristic of the disease since six months from inoculation, and two healthy plants, were used. Twenty cuttings (five for each plant), 8 cm long, were obtained and inoculated by immersion in 20 ml (for each plant) of a suspension containing MEB with actively growing mycelium of *E. nigrum* or *A. pullulans* and sterilized distilled water 1:1 (vol/vol). In parallel, comparable AP-infected or healthy cuttings were incubated in 20 ml of sterile H_2O as controls. All the cuttings were maintained at 20 °C, under 16:8 light/dark photoperiod for 7 days. After this period, vein samples were removed from three leaves of each cutting and submitted to fixation and embedding for transmission electron microscope (TEM) observations, according to the method described by Musetti *et al*., (1999).

Results

Seven days after inoculation with *A. pullulans* or *E. nigrum*, *C. roseus* cuttings did not show visible symptoms or lesions leading to endophyte treatments.

In AP-infected phloem tissues, both untreated and treated with endophytes, TEM observations revealed the presence of numerous phytoplasmas. However, in endophyte-treated cuttings, several phytoplasmas showed modified ultrastructure, such as irregular, distorted shape, presence of cytoplasm confined at the periphery of the cell, not well distinguishable cell membrane (figure 1). Moreover, plant cytological changes, suggesting the activation of defence response, were observed. Abundant callose depositions were found in sieve tubes, particularly occluding sieve plates; P-protein aggregations accumulated in the mature phloem elements, filling the cell lumen and, in some cases, wrapping up the phytoplasma cells; phenolic depositions were also present both in sieve tubes and in the vacuoles of companion cells.

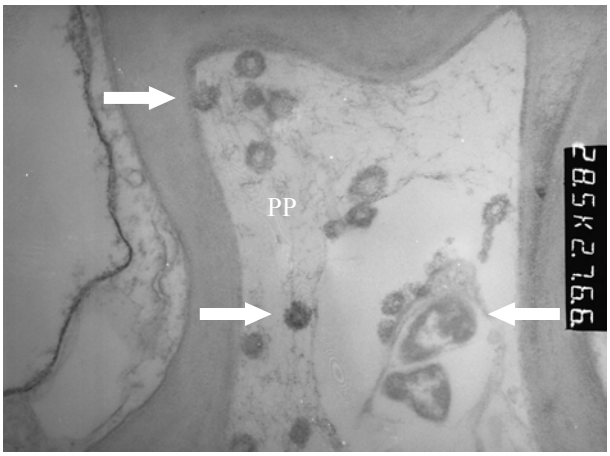

Figure 1. Leaf tissue micrograph of phytoplasma-infected *C. roseus* cutting treated with *E. nigrum*. Phytoplasmas (arrows), immersed in P-protein (PP), appear degenerated.

Similar cytological modifications were observed in endophyte-treated healthy leaf tissues. On the contrary, scattered phloem proteins and slight callose depositions were observed in sieve plates of untreated AP-infected cuttings. In untreated-healthy control cuttings, tissues appeared well preserved and ultrastructural modifications were not observed.

Discussion

During the last ten years the interest for endophyte microorganisms was increased because their presence benefits the host plants, promoting both the development (producing growth substances) and the activation of defence mechanisms (producing antibiotics or elicitors) (Gimenez *et al*., 2007). The results of the current study, even if preliminary, demonstrated that the fungal endophytes *A. pullulans* or *E. nigrum* induced ultrastructural changes both in phytoplasma-infected *C. roseus* tissues and in the pathogen. The described ultrastructural modifications indicated an enhancing of defence response in the host. The presence of P-protein in its aggregate status, callose occlusions and vacuolar phenolic depositions in the lumen of sieve tubes are able to prevent the phytoplasma spread inside the host plant. In particular, the aggregation of P-protein in sieve tubes is one of the first response of phloem cells to pH modification; this phenomenon can be correlated to different causes, among which the pathogen attack. In endophyte-treated cuttings, the described modifications appeared more important than those observed in untreated ones. Similar ultrastructural changes were reported in phytoplasma-infected tomato tissues treated with elicitors (Leherminier *et al*., 2003). Phytopasmas also appeared modified by endophyte treatment. Again, agglutinations and degeneration of phytopasma cells have been also observed in tomato plants treated with arbuscular-mycorrhizal fungi, and correlated with mycorrhizal hormone activity (Lingua *et al*., 2002). Further investigations are in progress to study the relationship among phytoplasmas and endophytes and the possibility to use the latter in the phytoplasma disease control.

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