PLANT CELLULAR-LEVEL DEFENSE RESPONSES INDUCED BY SOIL BACTERIA

FLORENȚA-ELENA HELEPCIUC[1], ELENA MONICA MITOI[1],
AURELIA BREZEANU[1], CĂLINA PETRUȚA CORNEA[2]

In their interaction with microbiota plants numerous defense reactions have evolved, many of them consisting in structural defense responses. In this study, induction of structural defense mechanisms in cucumber *(Cucumis sativus* L, Wisconsin SMR58 cultivar) treated with bacteria and fungi was investigated. Cucumber plants treated with phytopathogen *Pythium debaryanum* Hesse and bacterial strain *Pseudomonas* sp. P18 were analyzed for structural defense response activation. The results revealed significant plant cellular modifications induced by the treatment applied.

Key words: plant-microbe interaction, defense response, cell wall appositions.

INTRODUCTION

In their natural environment, plants are surrounded by a myriad of pathogenic or beneficial organisms. In order to resist to this continuous challenge, plants are equipped with constitutive defense mechanisms, which can be either structural (primary and secondary cell wall, cuticle) or chemical (phytoanticipins). Besides these preformed defense mechanisms, different induced reactions can be activated as a result of plant interaction with surrounding organisms. During plant-microbe interactions various chemical defense responses are activated: oxidative burst (Wojtaszek, 1997), lytic enzymes synthesis (Mauch *et al*., 1988), phenolic compounds production and deposition (Dai *et al*., 1996). Other induced defense mechanisms can have a structural role, among them cell wall appositions or nonpenetrating papillae conferring resistance to fungal hydrolytic or mechanical force (Hückelhoven, 2014). The aim of this study was to evidence additional structural defense responses induced in plants treated with certain necrotrophic fungi and soil bacteria.

[1] Institute of Biology Bucharest, Romanian Academy, 296 Splaiul Independenței, 060031, Bucharest, Romania, e-mail: monica.carasan@ibiol.ro
[2] Faculty of Biotechnology, University of Agronomical Sciences and Veterinary Medicine, Bd. Mărăşti No. 59, 71331 Bucharest, Romania.

ROM. J. BIOL. – PLANT BIOL., VOLUME 63, Nos. 1–2, P. 45–51, BUCHAREST, 2018

MATERIALS AND METHODS

BIOLOGICAL MATERIALS

The fungal pathogen employed for this study, *Pythium debaryanum*, was provided by the Institute of Plant Protection (Bucharest, Romania). The *Pseudomonas* sp. (P18) strain employed for the experiments belong to the Faculty of Biotechnology collection, USAVM (Bucharest, Romania). Cucumber plants were obtained by germinating cucumber seeds (Wisconsin SMR58 cultivar).

PLANT TREATMENT PROTOCOL

Cucumber seeds were sterilized for one hour in 1% sodium hypochlorite solution and then washed thoroughly with sterile water. Sterile cucumber seeds were treated with bacterial cultures (grown for 4 days on CPM medium at 28°C), and after germination were placed in pots with sterile perlite. The plants were wet with Knop nutritive solution, for two weeks. In parallel, the pathogen *P. debaryanum* was grown for seven days on PDA medium at 28°C. For the infection of plantlets, slices of 5 squared mm PDA medium with grown mycelium were placed in the hypocotyls area. After five days, the hypocotyls of treated plants were processed and afterwards analyzed by light and electron microscopy.

SAMPLE PROCESSING FOR LIGHT AND ELECTRON MICROSCOPY

For electron microscopy specimen processing, the method described by Mascorro and Bozzola (2007) was used. The initial step was sample prefixation in a solution of 3% (v/v) glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.2 at 4°C, overnight, followed by rinsing with the same buffer. The plant fragments were washed for 2-3 hours with 0.05 M sodium cacodylate buffer, then fixed in 1% (w/v) osmium tetraoxide solution in the same buffer, at 4°C, overnight. The samples were dehydrated in a graded series of 10-100% (v/v) ethanol after being washed for 2 h with distilled water. Afterwards, the samples were washed with propylene oxide, embedded in Epon 812 resin and then ultrasectioned. The ultrathin sections were stained according to Reynolds' double coloration (Reynolds, 1963) and afterwards were examined with an EM-125 transmission electron microscope (Selemi, Ukraine) at 50 kV. Semithin sections (1–2 μm thick) were stained with a solution of 1% toluidine blue in 1% borax and then visualized using a MC1 light microscope (Pickett-Heaps, 1966).

RESULTS AND DISCUSSION

The semithin sections of cucumber treated with *Pseudomonas* sp. P18 and *Pythium debaryanum* showed characteristic plant defense responses. One of the

responses, intercellular space obturation, was observed as toluidine blue intensely stained areas (Figure 1a, b). In a few sections the presence of some fungal cells in intercellular spaces was recorded (Figure 1b, white arrow). However, the cells in the immediate proximity of the pathogen ingression did not show a very intense defense response (Figure 1b, white arrow). This is attributed to the fact that the resistance phenomenon manifests at the cell level, meaning that neighboring cells with similar characteristics have different rates of success in blocking fungal penetration in the intact host cell (Hückelhoven, 2014).

Previously analyzed electron microscopy samples highlighted the presence of P18 bacteria in intercellular spaces, but resistance associated defense responses were not observed (Helepciuc *et al*., 2013). In contrast with our previous results, which did not evidence significant structural changes induced by P18 strain, the ultrathin sewctions examined for this study showed essential defense related structural adjustments. An important modification induced in plant tissues was represented by osmiophilic materials accumulation in the intercellular spaces, forming electronodense deposits (Figure 2 a, b). Once the pathogen succeeds to penetrate the extracellular protection barriers like cuticle and epicuticular waxes, the intercellular spaces are the first territories colonized by necrotrophic pathogens, which obtain nutrients for growth and reproduction by promoting the host cell death (Pawlowski and Hartman, 2016). Intercellular space sealing is thus an efficient strategy to delay or even stop the pathogen colonization of the surrounding tissues.

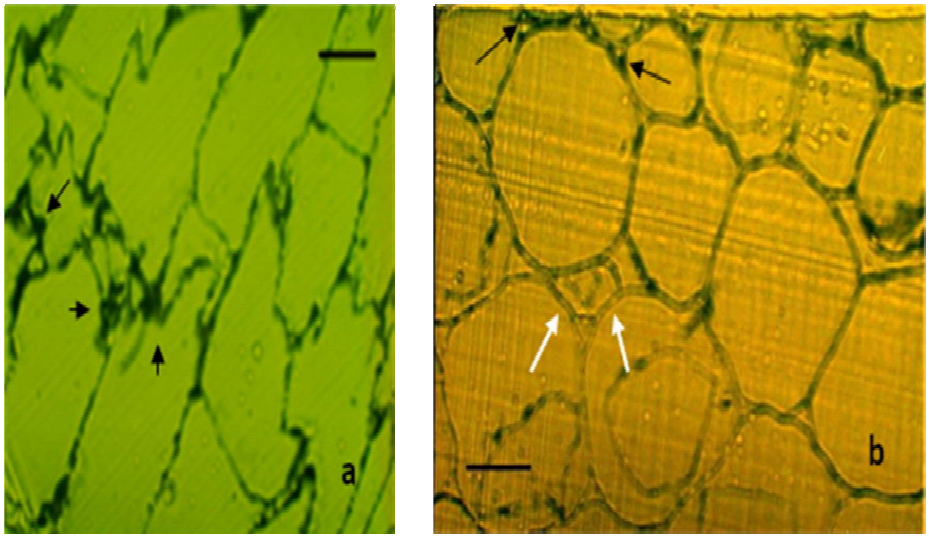

Figure 1. Aspects of cucumber hypocotyls from plants treated with *P. debaryanum* and *Pseudomonas* sp. P18 strain visualized by optic microscopy on semithin sections stained with toluidine blue: intercellular hyphae (b, white arrows), intercellular space filling (a, b, black arrows). Scale bars = 10μm.

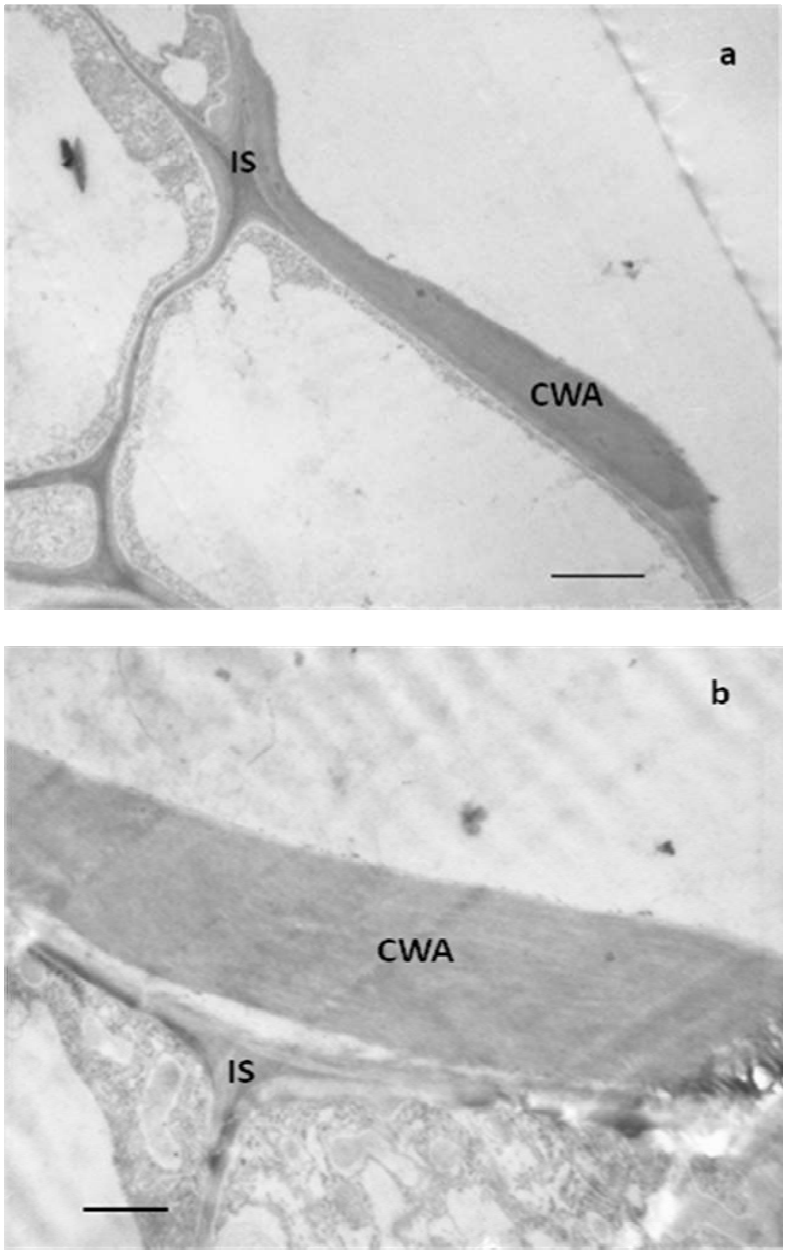

Figure 2. Ultrastructural aspects of cucumber hypocotyl from plants treated with *P. debaryanum* and *Pseudomonas* sp. P18 strain visualized by electron microscopy: osmiophilic materials accumulation in the intercellular spaces (IS) (a, b), cell wall appositions (CWA)(a, b). Scale bars = 1μm.

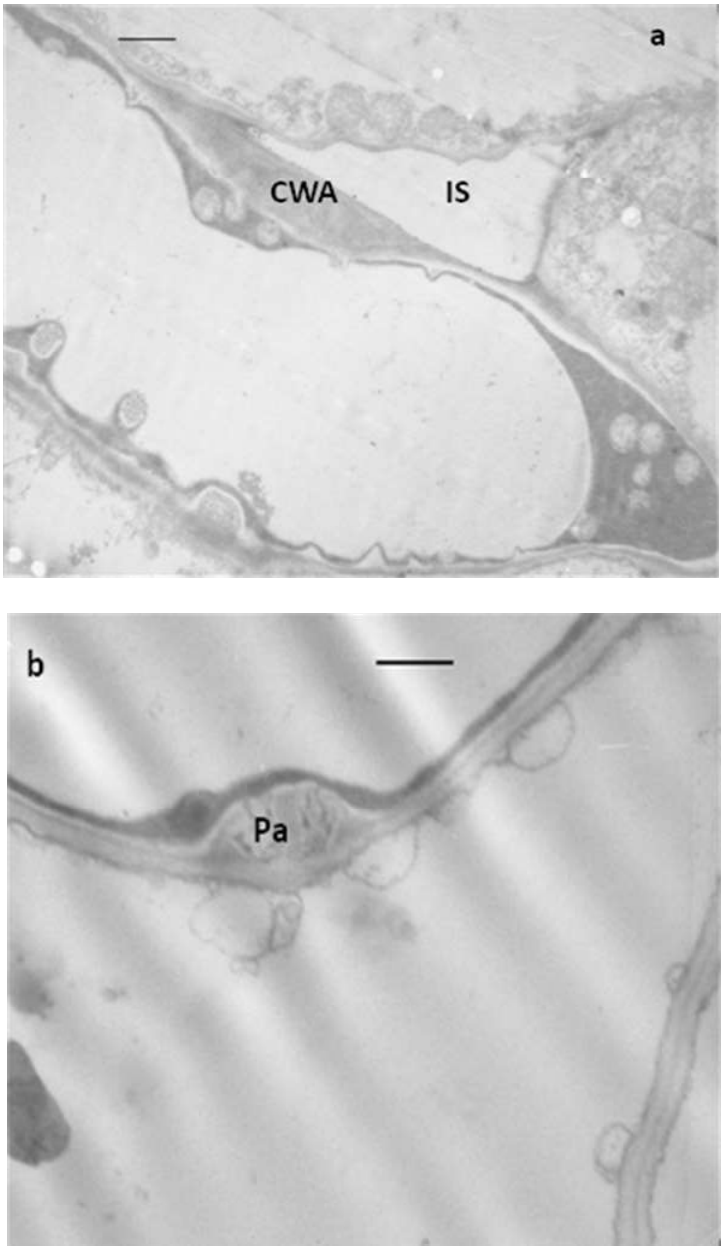

Figure 3. Ultrastructural aspects of cucumber hypocotyl from plants treated with *P. debaryanum* and *Pseudomonas* sp. P18 strain visualized by electron microscopy: cell wall appositions (CWA)(a) and papillae (Pa) formation (b). Scale bars = 1µm.

Other cellular alterations observed within this type of interaction were related to plant cell wall structure. Cell wall appositions were frequently observed in the ultrathin sections studied, the cell walls exhibiting different degrees of thickness (Figure 2 a, b, Figure 3 a). Also, an early defense response, papillae formation, was observed at the cell wall level (Figure 3 a, b). These types of modifications have an important role in plant cell wall fortification, contributing to temporary or permanent pathogen isolation. The effectiveness of some papillae (nonpenetrated papillae) was evidenced in experiments which employed *Blumeria graminis* f. sp. *hordei* and barley (*Hordeum vulgare*), the results showing that the papillae have a layered structure, with the inner core consisting of callose and arabinoxylan and the outer layer containing arabinoxylan and crystalline cellulose (Chowdhury *et al*., 2014). Also, cell wall thickenings can have a great contribution to slowing or limiting the diffusion of cell wall degrading enzymes and phytotoxic compounds secreted by the pathogen to induce plant cell death. Papillae formation is also slowing the pathogen invasion in the attacked tissue and thus offers more time for the activation of more complex defense responses involving gene activation and expression (Voigt, 2014).

CONCLUSIONS

The bacterial treatment applied induced typical structural defenses in the plant cell. Besides intercellular space obstruction, numerous cells with reinforced cell walls were observed. These structural barriers prevented *Pythium debaryanum* pathogen ingress and further colonization of cucumber cells.

Acknowledgements. The study was funded by project no. RO1567-IBB06/2014 from the Institute of Biology Bucharest of the Romanian Academy.

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