Molecular Defense Responses of Apple Genotypes in Compatible and Incompatible Interactions with *Erwinia amylovora*

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Abstract

Erwinia amylovora is the causal agent of fire blight, a bacterial disease of apple and pear. Pathogenicity determinants of the bacteria are identified (*hrp-dsp* cluster, capsule, siderophore) but molecular mechanisms leading to susceptibility or resistance of the plant are not yet understood. To address this question, we challenged two genotypes of apple, known for their contrasting susceptibility to fire blight, with a wild-type strain of *E. amylovora* (*Ea wt)*, an avirulent *hrp* mutant of this bacteria (*Ea hrp*) or a wild-type strain of the incompatible pathogen *Pseudomonas syringae* pv. *tabaci* (*Pst wt)*. Mechanisms usually related to resistance responses were investigated i.e. oxidative stress, accumulation of PR-proteins and induction of genes encoding various enzymes of the phenylpropanoid pathway. Results showed two kinds of responses (i) some mechanisms were elicited in both susceptible and resistant genotypes by *Ea wt* and *Pst wt* with similar kinetics and not induced by *Ea hrp*, (ii) others were specifically repressed by *Ea wt* in its susceptible host, when induced by *Pst wt* and *Ea hrp*. These results suggest several hypothesis about the cross-talk between *E. amylovora* and its host plants.

Keywords: *Erwinia amylovora*; defense; compatibility; incompatibility; *hrp* mutant

INTRODUCTION

Pathogenicity factors of *E. amylovora* have been intensively studied and mostly identified (*hrp-dsp* cluster, capsule, siderophore), while molecular host responses to infection by this bacteria as well as the precise role of the various pathogenicity factors during the interaction remain open questions (for a review see VANNESTE 2000). To address this question, we investigated as a first approach a wide panel of defense-related mechanisms including various events related to oxidative stress, accumulation of PR-proteins and modulation of the phenylpropanoid pathway after infection of a susceptible and a resistant genotype of *Malus* by a virulent wild-type strain of *E. amylovora*. Plant responses were compared to those obtained from the same genotypes of *Malus* challenged with an avirulent *hrp* secretory mutant of *E. amylovora* and with the tobacco pathogen *Pseudomonas syringae* pv. *tabaci*, both used as controls.

MATERIALS AND METHODS

Intact leaves of apple grafted scions of *Malus domestica* cv. Evereste (resistant) and cv. MM106 (susceptible) were infiltrated with a wild-type strain of *E. amylovora* (CFBP1430), an *hrp* secretion mutant of CFBP1430 (PMV6023) and a wild-type strain of *P. s.* pv. *tabaci* (CFBP2106). Infiltrated leaves were sampled within 48 h following infiltration and submitted to analysis.

Targetted plant responses known as being elicited in various incompatible plant/pathogen interactions were chosen for this study. They include the occurrence of

an oxidative stress (production of superoxide anion $O_2^{\circ -}$, activation of ascorbate peroxidase, glutathione reductase, glutathione-S-transferase), the accumulation of PR-proteins (β-1,3-glucanases, chitinases, PR-5) and the induction of the phenylpropanoid pathway [through the study of gene transcripts of PAL (l-phenylalanine ammonia-lyase), CHS (chalcone synthase), CHI (chalcone isomerase), DFR (dihydroflavonol reductase), FLS (flavonol synthase)]. Assessment of $O_2^{\circ -}$ – production and analysis of enzymatic activities or defense gene expression were performed as already described (BRISSET *et al*. 2000; VENISSE *et al*. 2001, 2002).

RESULTS AND DISCUSSION

Results showed that an oxidative stress and accumulation of PR-proteins were activated in each genotype (either susceptible or resistant) by both wild-type strains of *E. amylovora* and *P. s.* pv. *tabaci* at similar levels and according to similar kinetics. These plant responses were elicited by *Hrp* effectors of *E. amylovora* since the *hrp* secretion mutant was ineffective. These results suggest that oxidative stress and PR-proteins are not related to resistance in the case of fire blight. On the contrary, *E. amylovora* could use the production of active oxygen species as a tool to kill the plant cells and to colonize its host plant.

Amongst the genes encoding enzymes of the phenylpropanoid pathway, the expression of CHS, DFR, FLS were specifically not induced by the wild-type strain of *E. amylovora* in leaves of the susceptible cultivar MM106, while these genes were consistently expressed in all other plant/bacteria combinations tested. As expressions of these genes were also found after infiltration of the *hrp* secretion mutant of *E. amylovora*, this suggests that i) these events are induced by mechanisms independant of the *Hrp* secretion system and ii) suppressors of these plant responses could be secreted through the Hrp apparatus. On the whole, the analysis of modulation of expression of the five genes of the phenylpropanoid pathway lead to the hypothesis that *E. amylovora* either induces the accumulation of the three main phenolics of apple (catechin, quercetin and phloretin – TREUTTER 2001) in the resistant genotype or prevents their accumulation in the susceptible genotype. As these compounds have potential antibacterial and/or antioxidant properties, their presence or their absence could take part in the resistance or in the susceptibility of plant tissues.

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