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In vitro system for studying the interaction between *Erwinia amylovora* and genotypes of pear

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Abstract

A new *in vitro* system is described for studying an interaction between *Erwinia amylovora* and *Pyrus communis* (L.). The system uses single shoots placed onto the solid medium, and it enables to detect changes in pH of the medium and differential appearance of shoot necrosis. Shoots of susceptible cultivar (Williams) and tolerant cultivar (Harrow Sweet) were compared measuring the necrosis rate along the *in vitro* shoots and the pH variation following proton extrusion of both plant and pathogen. Shoots acidified differentially the culture medium depending on the presence of the pathogen, cultivar susceptibility and shoot inoculation methods. Differences in the tolerance level against pathogen among the cultivars were distinguishable only when the shoots were inoculated at the basal end. In susceptible cultivar, the necrosis appeared after 48 h of inoculation, while in tolerant cultivars after 72 h. This system is repeatable and more reliable than already known methods, such as *in vitro* leaf explants or *in vivo* plants; it can be used all around the year to test the gene expression and products essential to characterize the genes involved in the pathogenesis. This system showed the effects of *E. amylovora* on the photosystem dependent system of host cells, confirmed by the effects of pathogen attack on the variation of chlorophyll *a* and chlorophyll *b* ratios and positive effects of light on the appearance of the first disease symptoms.

Introduction

Erwinia amylovora is the causal agent of fire blight, which in the most devastating disease of apple, pear and some other members of the family *Rosaceae*. *E. amylovora* produces harpins under both situations of its compatibility and incompatibility interactions with plant cells: harpins are acidic, heat-stable proteins with an apparent molecular weight 44 kDa. The gene encoding harpin (*hrpN*) was located in the 40 kb *hrp* gene cluster of *E. amylovora* (Bauer and Beer, 1991). Recent reports elucidated the genetic and biochemical basis of the pathogenicity of *E. amylovora* (Wei et al., 1992, 2000; Wei and Beer, 1995; Bogdanove et al., 1996; Gaudriault et al., 1997; Jin et al., 2001). The molecular events leading to interaction between pathogen and host plants, which cause disease (compatible interaction), or non-host plants develop hypersensitivity (incompatible interaction), are less known.

All plant cells extrude protons out of the membranes and consequently a positive charge outside the cells is established. This gradient of electrical charge constitutes a driving force for cations or solutes to enter the cells (Heldt, 1997). The activity of P-type H^+-ATPase of plasma membrane produces this electrical charge gradient at the expense of ATP. Cations, anions and neutral solutes are able to enter the cell through various specific carrier proteins, that are energized by the concomitant uptake of protons (Palmgren, 2001). In most plants, the activity of H^+-ATPase pump of membrane regulates the pH of cytoplasm to