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Some biochemical changes in pear fruit tissue induced by *Erwinia aymlovora*

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ABSTRACT Enzyme activities (peroxidase and polyphenol oxidase) were measured in unripe resistant – Vicar of Winkfield – and susceptible – Max Red Bartlett – pear fruits during disease process caused by *Erwinia amylovora* infection. Samples were taken from the inoculation point and neighboring tissues during three days intervals. Host responses showed significant differences. Increases in enzymatic activities started to occur on the second day after inoculation (DAI) in the susceptible cultivar, while these changes were detectable only 3 DAI in resistant fruits. In the resistant cultivar, activities of both enzymes increased. However, in the susceptible cultivar peroxidase activity started to decrease after symptom development. Both enzyme activities were suitable markers of the susceptible and resistant host responses.
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KEY WORDS

resistant
susceptible pear fruit
peroxidase
polyphenol oxidase
bacterial infection

Plants are exposed to different stresses, either biotic, or abiotic, which results in a shift of metabolism towards the oxidative direction (Baker and Orlandi 1995; Staskawicz et al. 1995; El Zahabi et al. 2004). Plants mobilize antioxidative defense mechanisms, in order to eliminate the effect of free radicals, the causal agents of most stresses. The components of these defense mechanisms are several stress-enzymes (superoxid dismutase, catalase, peroxidase and polyphenol oxidase) and other components like phenols. It is well documented that secondary metabolites (e.g. phenols) accumulate during stress responses in plants. *Erwinia amylovora* is responsible for fire blight; since 1999, we have been evaluating susceptibility of pear cultivars with the aim of searching gene sources of resistance. Fruit susceptibility of 25 pear cultivars was determined earlier, which have given different host responses to infection. In the present study we have compared biochemical changes in unripe pear fruits in susceptible and resistant cultivars on the basis of peroxidase (POD), polyphenol oxidase (PPO) enzyme activities at the place of inoculation and in neighboring tissues as well. Experiments were carried out in greenhouse conditions in 2003 and 2004.

Materials and Methods

Erwinia amylovora pear isolates (Ea 21, 23) collected from various growing regions of Hungary were used in a mixture at a density of $5x10^8$ cells/ml for inoculation.

Unripe pear fruits were inoculated with a needle dipped into the bacterial suspension, and evaluated after 4 days of incubation on a 0-5 grade scale (disease category) based on the diameter of infected tissue. On the basis of different host responses, a susceptible (water soaked, diffused infected area of cv. Max Red Bartlett)- and a resistant (dry, dark and hypersensitive type necrotic area of cv. Vicar of Winkfield) cultivar was chosen for analysis. Six fruits per cultivar (3-4 cm in diameter) were used for inoculation by six prickings per fruit.

Samples – flesh cutouts (1 cm ø) – were taken by a cork borer around the inoculation point (a) and from the neighboring tissue 1 cm far (b), and 2 cm far from (c) immediately, and following 2, 48 and 72 hours of incubation.

250 mg fruit samples were homogenized in 1 ml ice-cold Tris (pH=7,5) buffer containing glycerol (10%), Triton x 100 (10%), PEG 4000 (5%), NaCl (5%).

POD activity was determined by spectrophotometry with H_2O_2 as a substrate and ortho-dianidizine as a choromegenic reagent ($\varepsilon = 11.3$), at $\lambda = 460$ nm (Shannon et al. 1966). PPO activity were also followed by spectrophotometry using catechol at λ=420 nm (Jen and Kahler 1974). Results are expressed in terms of U/ml.

Results

Cv. 'Vicar of Winkfield' was the most resistant, cv. 'Max Red Bartlett' the most susceptible one (disease rating: 0.7 and 4.22, respectively). Biochemical responses to infection by *E. amylovora* were investigated on these two cultivars. Both cultivars responded with changes in enzyme activity (POD, PPO) following symptom development. The time between samplings, as seen on the x-axis of the figures, was not uniform, in order to monitor early (2 hours AI) stress responses.

POD activity decreased in the infection point (a) 2 hours AI (HAI) both in resistant and susceptible combinations. It has significantly increased in susceptible fruits in the infection point (a) up to 48 HAI, between 48-72 HAI enzymatic

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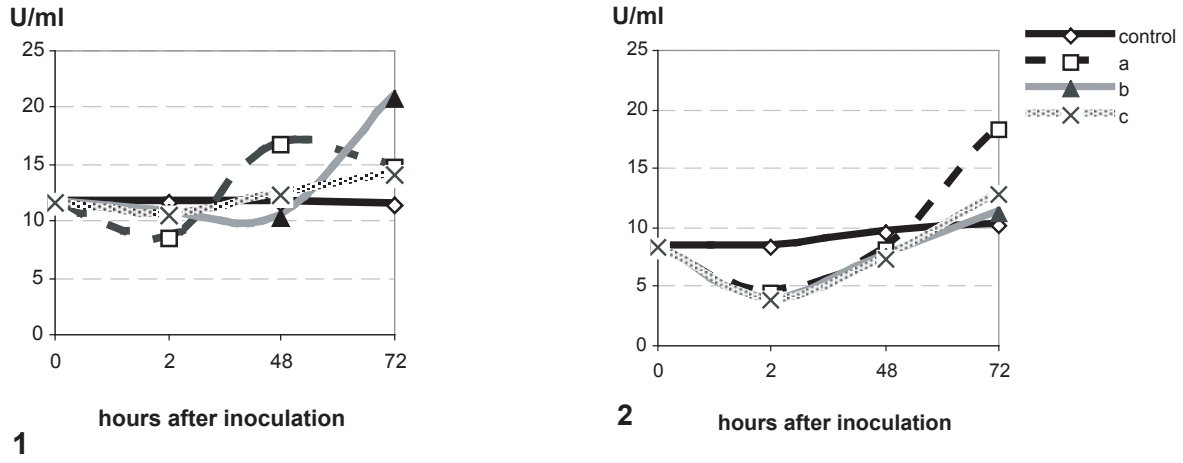
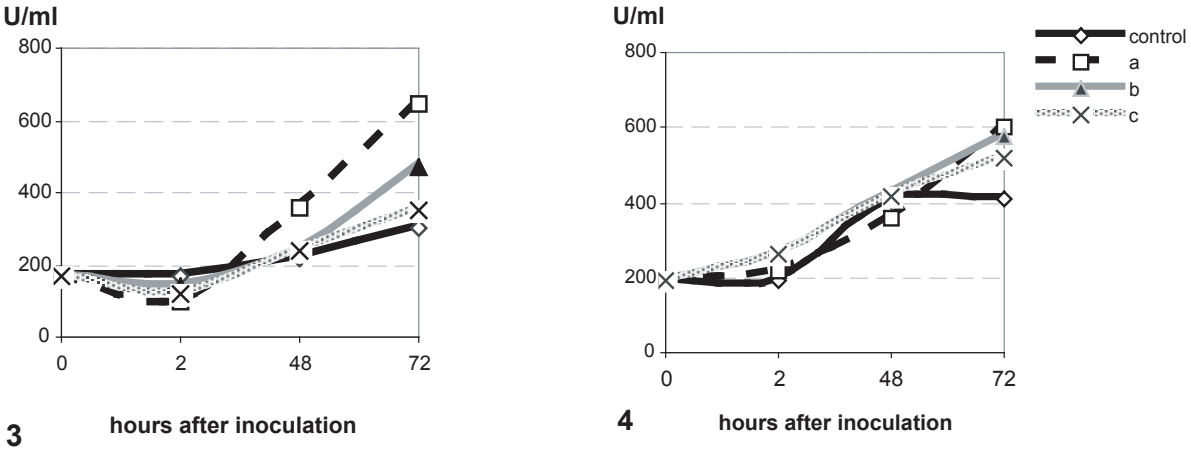


a – samples from the inoculation point; b - samples 1 cm far from the inoculation point; c - samples 2 cm far from the inoculation point

Figures 1-2. POD activity in susceptible (Max Red Bartlett) and resistant (Vicar of Winkield) pear fruit inoculated with *E. amylovora* (2003-2004).

activity in the infection point (a) decreased relative to previous levels (Fig. 1). In tissues neighboring infection (b), a significant increase in enzymatic activity was detected. In case of the resistant cultivar we have not found any increases in enzymatic activity up to 48 HAI neither in the infection point (a), nor in tissues neighboring infection (b, c). Between 48-72 HAI enzymatic activity has significantly increased in the infection point (a), while in distal tissues (b) activity values were lower (Fig. 2).

PPO activity values steadily increased in all uninfected control fruits too. Similar to POD activity, PPO enzymatic activity has significantly increased in susceptible fruits in the infection point up to 48 HAI (a), and between 48-72 HAI it has further increased in the infection point (a), while in distal tissues (b, c) the rate of increase was smaller (Fig. 3). In the resistant cultivar we have not found any increases in enzymatic activity up to 48 HAI neither in the infection point (a), nor in tissues neighboring infection (b, c). On the other hand, between 48-72 HAI enzymatic activity has significantly



a – samples from the inoculation point; b - samples 1 cm far from the inoculation point; c - samples 2 cm far from the inoculation point

Figures 3-4. PPO activity in susceptible (Max Red Bartlett) and resistant (Vicar of Winkfield) pear fruit inoculated with *E. amylovora* (2003-2004).

increased both in the infection point (a) and in distal tissues (b) but these increases were much less as those assayed in the susceptible cultivar (Fig. 4).

Discussion

Increases in POD and PPO enzymatic activities in response to biotic stress followed the development of bacterial infection, as described in earlier works by (Havlickova et al. 1998; Sárdi et al. 2000; Keck et al. 2002). In susceptible fruits, symptoms of *E. amylovora* infection in the inoculation point (a) have developed 48 HAI, with a likely consequence of a gradual decrease in POD synthesis. During this time period POD activity continuously increased in tissues neighboring infection (b), which could indicate mobilization of plant defense processes in healthy neighboring tissues, in response to disease symptoms at the inoculation site. In all controls a relatively high PPO activity was continuously detected, probably due to mechanical stress during sampling. Increases in enzymatic activities were more significant in the resistant cultivar which could indicate operation of a more effective defense system. Besides the well-known phenomenon of increases in POD (peroxidase) activity in response to infection PPO (polyphenol oxidase) is likely related to synthesis of secondary metabolites (e.g. phenols).

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