PEROXIDASE ACTIVITY IN COTTON CELL CULTURE INFECTED WITH *VERTICILLIUM DAHLIAE*

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Abstract

In our studies with cotton, we have shown that the plant's induced anionic peroxidases bind to chitin, which is a component of the cell wall of the plant pathogenic fungus *Verticillium dahliae*. In binding to the cell wall surface, they disrupt the integrity of the pathogen's cell wall. Thus, these chitin-specific peroxidase isoforms constitute an integral part of the plant's defense mechanism. In the present study, we investigated the response of the resistant cotton cultivar An-Baunt-2 and the susceptible cultivar C-4727 to infection with the plant pathogen *V. dahliae* as determined by measurements of the endocellular and exocellular peroxidase activity. We found that in all samples the endocellular peroxidase was lower in comparison with extracellular isozyme activity when the tissue was challenged with *V. dahliae*. In a dynamic study, we found that peroxidase activity after infection with *V. dahliae* resulted in a sharp increase in activity, in both endocellular, and extracellular, chitin-specific peroxidases in the resistance cultivar An-Bayaut-2 within 1 hour after infection. In An-Bauut-2, maximal activity was reached in 12 hours for endocellular enzymes and 18 hours for exocellular enzymes. Enzyme activity remained at these elevated levels for 30 hours. In the case of susceptible C-4727, the response was slower and did not reach maximal levels until 18 hours after infection with *V. dahliae* for the extracellular enzymes and 24 hours for the endocellular enzymes; the total levels were 35% less than that of An-Bayaut-2.

Introduction

Cotton is the major agricultural crop produced in Uzbekistan, and as such it is an important component in the economy of the Republic of Uzbekistan. Thus, yield and fiber quality loses can have a significant effect on the country's agricultural income. Because cotton must be grown in Uzbekistan under unusually cool conditions compared to other cotton growing regions, Verticillium wilt is a major concern to the government of Uzbekistan. Therefore, breeding for resistance to Verticillium wilt is of fundamental importance in developing new cotton cultivars. An important response to pathogen infection is the release of active oxygen species (AOS) by the plant. This rapid release of oxidative species as part of the defense response is referred to as the oxidative burst and the enzymes responsible for this release are largely located on the cell plasmolemma. Among these are peroxidases.

Peroxidases are classically considered as the antioxidants protecting cells from destructive action of hydrogen peroxide. However peroxidases can show oxidase activity with oxidation of heme iron from +2 valence to +3 valence and by transfer of electron from reducers, such as NADH to oxygen. It has been hypothesized that cell wall peroxidase is responsible for producing hydrogen peroxide during the oxidative burst in response to elicitor action from pathogenic fungi in string bean cells (Allan and Fluhr, 1997). Bestwick et al. (1997) have experimentally confirmed the presence of peroxidase secretion and hydrogen peroxides in pathogenic infection sites of tobacco leaves. Similar peroxidase activity can lead to formation of superoxide and hydrogen peroxides. Some plant peroxidases are localized in the apoplast space of cells where they are bound *via* ionic or covalent bonds with cell wall polymers. These cell wall peroxidases participate in lignin biosynthesis and cross-section linking. However, we have shown that these peroxidase enzymes have a wider spectrum of the physiological functions associated with their AOS-producing activity.

The induction of protective activity by extracellular peroxidase in plants occurs in response to many biotic and abiotic stimuli. For example, Cipollini (1998) has shown that wind and mechanical friction on string bean sprouts stimulate soluble peroxidase activity in primary leaves. Anionic peroxidase isoform activity was found to be higher in the hydathode of cabbage infected with *Xanthomonas campestris* compared to a control (Gay, 1995). The liquid from the hydathode possesses antibacterial properties. In addition, sprouting seeds of garden radish secret AOS and peroxidase, and the process depends on light, abscisic acid and gibberellin (Schopfer, et al., 2001). The abscisic acid in rice sprout roots induced production of hydrogen peroxide and activated NADH-dependent peroxidase of cell wall (Lin and Kao, 2001). Furthermore, the cultivated cells of tobacco secret more than 100 proteins; of these three are peroxidase isozymes (Allan, 1997).

Research Goals

In order to discover detailed information on the mechanism of resistance of cotton to *V. dahliae* we set as our research goals to:

1. Investigate the effect of pathogens on peroxidase formation in callous and cell culture of resistant and susceptible cotton species.
2. Study the dynamics of chitin-specific peroxidase activity during plant-phytopathogen interaction.

Results and Discussion

We challenged callus and cell suspensions of resistant An-Bayaut-2 and susceptible C-4727 cotton cultivars with *V. dahliae*. We found that isozymes produced from callus cells have a higher peroxidase activity compared to isozymes from suspension cell culture.

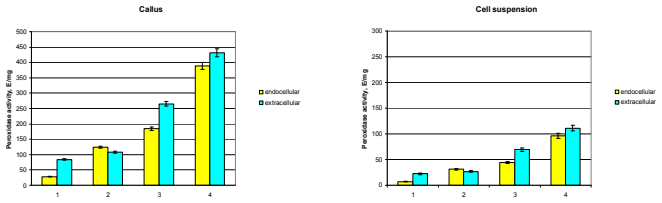

Figure 1. Change of cotton chitin-specific isoforms peroxidase activity of An-Bayaut-2 and C-4727 species at infection with pathogenic fungi *Verticillium dahliae* as measured after 24 hours.

1. C-4727, control
2. An-Bayaut-2, control
3. C-4727, treated
4. An-Bayat-2, treated

Interestingly, significant changes in peroxidase activity were noted between chitin-specific isoforms of calluses and of those from cell suspension. The activity of peroxidase chitin-specific isoforms from callus tissue was approximately 4 times greater than that from suspension cell culture. Endocellular isozymes in all samples had lower peroxidase activity in comparison with extracellular isozymes. In the case of the extracellular peroxidases, since they are stress proteins and adhere to the surface of the cell membrane, they may be programmed to react more quickly, in particular to *V. dahliae*. The increase peroxidase activity after infection with *V. dahliae* in all samples confirms activation of these enzymes. This ability to respond more quickly to stress may be a major contributor to phyto-immunity. Recognition of this response may help breeders in developing plants with enhanced resistance to pathogens.

Figure 2. Peroxidase spectrum of chitin-specific isoforms of An-Bayaut-2 and C-4727 cotton species at infection with pathogenic fungi *Verticillium dahliae*: A - calluses; B – cell suspension

1.	C-4727	endocellular	control
2.	C-4727	extracellular	control
3.	An-Bayaut-2	endocellular	control
4.	An-Bayaut-2	extracellular	control
5.	C-4727	endocellular	experiment
6.	C-4727	extracellular	experiment
7.	An-Bayaut-2	endocellular	experiment
8.	An-Bayaut-2	extracellular	experiment

Experiments to determine the range of endocellular and extracellular peroxidase chitin-specific isoforms activity have not revealed distinctions in isozyme composition among samples. However, differences in activity are easily observed based on the intensity of color on the gels.

We conducted experiments with plant cell suspensions to determine the dynamics of peroxidase activity after infection with *V. dahliae* (Figure 3). We observed a sharp increase in activity in both endocellular, and extracellular, chitin-specific peroxidases in the resistance cultivar An-Bayaut-2 within 1 hour after infection. The maximal activity was reached in 12 hours for endocellular enzymes and 18 hours for exocellular enzymes, and remained at these levels after 30 hours. The susceptible variety C-4727 responded more slowly and did not reach and it maximal levels until 18 hours after infection with *V. dahliae* for the extracellular enzymes and 24 hours for the endocellular enzymes; the total levels were 35% less compared to An-Bayaut-2. We believe that the difference in activation speed of the protective reactions in resistance cottons verses the susceptible cotton can be used to differentiate resistant and susceptible progeny generated in a cotton breeding.

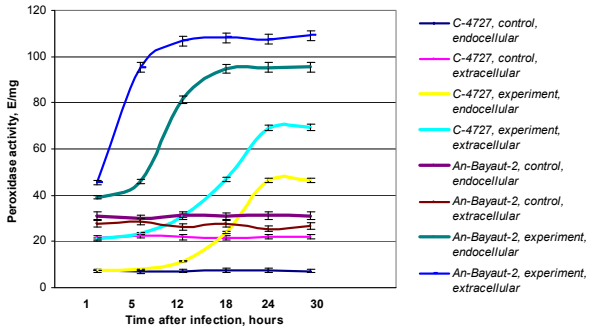

Figure 3. Dynamic change in endocellular and exocellular peroxidase activity of chitin-specific isoforms of resistant An-Bayaut-2 and susceptible C-4727 cotton species in dynamics in control and after infection with *Verticillium dahliae*.

References

Allan A., Fluhr R. Two distinct sources of elicited reactive oxygen in tobacco epidermal cells. Plant Cell. 1997. 9:1559-1577.

Bestwick C.S., Brown I.R., Bennett M.H.R., Mansfield J.V. Localization of hydrogen peroxide accumulation during the hypersensitive reaction of lettuce cells to *Pseudomonas syringae pv.* phaseolicola. Plant Cell. 1997. 9:209-221.

Cipollini D.F., Jr. The Induction of soluble peroxidase activity in bean leaves by wind-induced mechanical perturbation. Am. J. Bot. 1998. 85:1586-1591.

Gay P.A., Trezun S. Analisys of Anionic Peroxidase Isozymes in Hydatode Fluid of Resistant and Susceptible Cabbage Varieties during Pathogenesis with *Xanthomonas campestris* pv. *campestris* (XCC) // Abstr. APS Annu. Neet., Pittsburg PA, 12-16, 1995. Phytopathology. 1995. V. 85. P. 1164.

Lin C.C., Kao C.H. Abscisic acid induced changes in cell wall peroxidase activity and hydrogen peroxide level in roots of rice seedings. Plant Sci. 2001. 160:323-329.

Schopfer P., Plachy C., Frahry G. Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. Plant Physiol. 2001. 125:1591-1602.