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Changes in the antioxidant status in leaves of *Solanum* species in response to elicitor from *Phytophthora infestans*

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Summary

Three *Solanum* genotypes with various polygenic resistance levels to the oomycete pathogen *Phytophthora infestans* (Mont.) De Bary were studied for their antioxidant response to the pathogen culture filtrate (CF). Detached plant leaves were treated with CF for 6, 18 and 30 h, and assayed for changes in hydrogen peroxide content, total ascorbate and glutathione pools and redox ratios (reduced form to total pool), as well as for changes in activities of ascorbate peroxidase, glutathione reductase and glutathione-*S*-transferase. In CF treated leaves of non-host resistant *S. nigrum* var. *gigantea* and field resistant *S. tuberosum* cv Bzura, the H_2O_2 content did not change in comparison to water treated control leaves, whereas in the susceptible *S. tuberosum* clone H-8105 it decreased below the control level. In CF treated leaves of all genotypes, the total ascorbate pools were relatively unaltered and their redox ratio changed only transiently. In Bzura leaves the total glutathione content increased earlier than in the two other genotypes. The glutathione redox ratio remained rather stable, except for the susceptible clone H-8105, where it decreased transiently by about 42%. The relative increases in activity of all the studied enzymes were the highest in the susceptible clone H-8105. The results are discussed in the light of oxidative processes occurring in CF treated leaves. We conclude that stringent control of pro- and anti-oxidant reactions bringing the H_2O_2 and/or cellular redox state to the threshold level is decisive for deployment of an effective defense strategy.

Abbreviations: Asc, ascorbate; Asc–GSH cycle, ascorbate-glutathione cycle; CDNB, 1-chloro-2, 4-dinitrobenzene; CF, culture filtrate; DHAsc, dehydroascorbate; DMAB, 3-dimethylaminobenzoic acid; DTT, dithiothreitol; HR, hypersensitive response; LOX, lipoxygenase; MB, monobromobimane; MBTH, 3-methyl-2-benzothiazoline hydrazone; NBT, nitroblue tetrazolium (2, 2′-di-*p*-nitrophenyl-5, 5′-diphenyl-3, 3, -[3, 3′-dimethoxy-4, 4′-diphenylene]-ditetrazolium chloride); ROS, reactive oxygen species.

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Introduction

The oxidative burst, a rapid production of reactive oxygen species (ROS), is a well documented early plant response to biotic and abiotic stress (e.g., Apel and Hirt, 2004). ROS comprise radicals and other non-radical but reactive species derived from oxygen. Among them, much attention has been focused on the superoxide anion ($O_2^{\bullet -}$) and on hydrogen peroxide (H_2O_2), which, directly or in cooperation with other molecules, exert various effects on cells. In excess, ROS pose a threat to important bio-molecules and cell membranes. On the other hand, numerous studies indicate an essential role of ROS in plant defense responses to biotic stress. In addition to direct antimicrobial activity and contribution to the strengthening of barriers against pathogens, recent reports point to H_2O_2 and $O_2^{\bullet -}$ as signal transduction agents activating defense pathways and as key mediators in cell death during hypersensitive response (HR) (Grant and Loake, 2000). To maintain a balance between negative and beneficial functions of ROS, their levels are strictly controlled by a complex and flexible network of antioxidant systems (Mittler et al., 2004). The major enzymatic ROS-scavenging components of this network are superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). Superoxide dismutases dismute $O_2^{\bullet -}$ to H_2O_2, an excess of which may be subsequently detoxified by CATs and/or APX. Ascorbate peroxidases, in contrast to CATs localized in peroxisomes, are present in almost all cellular compartments; they exhibit a high affinity to H_2O_2 and are considered to be responsible for the fine modulation of ROS level (Mittler, 2002). Moreover, APX, in cooperation with two main low molecular antioxidants, ascorbate (Asc) and glutathione (GSH), and enzymes for their regeneration, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase (GR), constitute the ascorbate–glutathione (Asc–GSH) cycle, believed to be the central part of the antioxidant network (Noctor and Foyer, 1998). Ascorbate, present at high concentrations in all cellular compartments and capable of direct scavenging of $O_2^{\bullet -}$ and hydroxyl radicals, is considered to be the most powerful cell antioxidant (Noctor and Foyer, 1998). Ascorbate and glutathione are the major cellular redox buffers, which together with their oxidized forms, dehydroascorbate (DHAsc) and glutathione disulfide (GSSG), enable cells to maintain a redox balance. Changes in the levels or redox state of ascorbate and glutathione pools as well as in H_2O_2 homeostasis, and thereby in cellular/compartment redox state, are considered to be pivotal signaling events influencing gene expression and modulating plant defense response (Pastori and Foyer, 2002; Foyer and Noctor, 2005). This hypothesis is in line with the findings that accumulation of ROS resulted in enhanced HR cell death in transgenic tobacco with reduced ability to scavenge ROS (i.e., antisense APX or CAT) (Mittler et al., 1999).

One of the consequences of ROS activity is oxidative damage of membrane integrity due to lipid peroxidation processes, which may result in generation of highly cytotoxic compounds. Glutathione *S*-transferases (GSTs), induced upon pathogen attack, may detoxify lipid peroxides by conjugating them with GSH. These enzymes can also catalyze the GSH-dependent reduction/inactivation of H_2O_2, forming GSSG and increasing GSH synthesis by feedback induction (Marrs, 1996).

There are numerous discrepancies in the literature with respect to changes in antioxidant metabolites and in activity of antioxidant enzymes induced in plant/pathogen interactions (e.g., El-Zahaby et al., 1995; Vanacker et al., 1998; De Gara et al., 2003). These discrepancies may result from the diversity of plant/pathogen systems used in these studies, from analyses of only some of the antioxidant parameters and the lack of non-invasive, sensitive and accurate assays for ROS. Therefore, questions regarding the common plant defense strategy and its relationship to phyto-pathogen resistance remain to be addressed.

Our studies concern defense mechanisms in *Solanum* species in response to the oomycete pathogen *Phytophthora infestans* that causes one of the most devastating potato diseases called the late blight. These plant/pathosystems form unique interactions, in which different wild *Solanum* species and highly resistant and fully susceptible clones, as well as representative non-host species display HR upon infection (Vleeshouwers et al., 2000). The three *Solanum* genotypes, *S. nigrum* var. *gigantea*, *S. tuberosum* cv Bzura and *S. tuberosum* clone H-8105 were used. In interaction with *P. infestans* or its elicitor they exhibit, respectively, non-host resistance, polygenic field (partial) resistance or susceptibility. Previously, we demonstrated differences in kinetics and intensity of ROS production, lipid peroxidation and lipoxygenase activity induced by the elicitor in plant leaves (Polkowska-Kowalczyk et al., 2004). In view of the complexity of pro- and anti-oxidant processes in plant/pathogen interaction, we have now examined changes in leaf hydrogen peroxide content, in the total pool and redox state of ascorbate and glutathione, as well as in activities of APX, GR and GST in leaves of *Solanum* genotypes treated with an elicitor, the culture filtrate (CF) from *P. infestans*.