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REACTIVE OXYGEN SPECIES AND ANTIOXIDANT ENZYMES IN TOMATO CULTIVARS WITH DIFFERENT SUSCEPTIBILITY TO FUNGAL INFECTION

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The leaves of two tomato cultivars: susceptible cv. Torena and relatively resistant cv. Perkoz were infected with fungus *Botrytis cinerea*. The levels of hydrogen peroxide and hydroxyl radical as well as the activities of ascorbate peroxidase, catalase, peroxidase assayed with guaiacol, syringaldazine and ferulic acid were determined. Tomato cultivars used in this research responded to infection with *B. cinerea* in different ways. Main difference concerned the postinfectional changes in H_2O_2 content and the level of peroxidase activity assayed with guaiacol, syringaldazine and ferulic acid detected in leaves before infection.

INTRODUCTION

Generation of reactive oxygen species (ROS) is one of the earliest plant responses to pathogen attack. ROS may cause damage to plant cells and thus facilitate the infection spread. The most harmful is the hydroxyl radical which can cause lipid peroxidation, destruction of DNA and proteins. On the other hand, some ROS, especially H_2O_2, are considered to play an important role in the protection of plants against invading pathogens. H_2O_2 has been reported to protect plant tissues by direct killing pathogenic microorganisms. It has also been suggested to act as a signal for the activation of genes encoding cellular protectants such as phytoalexins, phenylalanine ammonia lyase, glutathione transferase and glutathione peroxidase (Willekens, Inzé, van Montagu & van Camp, 1995). In addition, H_2O_2 might contribute to the inhibition of infection spread in the host hypersensitive response (Tenhaken, Levine, Brisson, Dixon & Lamb, 1995). There is also some evidence that H_2O_2 participates in the induction of systemic acquired resistance (Chen, Silva & Klessig, 1993). The level of ROS is determined by the intesity of their generation as well as the activity of the antioxidant system. In order to protect themselves against the toxic effect of too high H_2O_2 concentrations plants possess antioxidant enzymes. The main enzymes that scavenge H_2O_2 are ascorbate peroxidase and catalase. Some peroxidases oxidizing phenolic compounds use H_2O_2 as a substrate for cell wall reinforcement processes, e.g. lignification, suberization, cross-linking of hydroxyproline-rich proteins and polysaccharides.

In our previous studies we investigated defence reactions of tomato cv. Perkoz that was relatively resistant to *Botrytis cinerea*. The roles of ROS and the antioxidant system in plant-pathogen interactions have been widely investigated in recent years but they still remain not sufficiently understood. In order to demonstrate whether they are crucial for resistance of tomato plants to fungal infection we compared the changes in hydrogen peroxide and hydroxyl radical levels as well as antioxidant enzyme activities during the process of pathogenesis in two tomato cultivars with different susceptibility to *B. cinerea*.

MATERIAL AND METHODS

Two tomato (*Lycopersicon esculentum* Mill.) cultivars showing different susceptibility to pathogenic fungus *Botrytis cinerea* were used in this study: susceptible cv. Torena and relatively resistant cv. Perkoz. The plants were grown in a growth chamber under a 16 h light/8 h dark photoperiod, at 22°C, with 350 $\mu E\ m^{-2}\ s^{-1}$ light intensity. At the age of 6 weeks the leaves were infected with conidial suspension of *B. cinerea* (10^6 conidia/ml). The levels of H_2O_2 (Capaldi & Taylor, 1983), $OH^{\cdot}$ (Tiedemann, 1997) and the activities of ascorbate peroxidase (APX) (Nakano & Asada, 1981), catalase (CAT) (Dhindsa, Plumb-Dhindsa & Thorpe, 1981) and peroxidase assayed with guaiacol

(PODg) (Maehly & Chance, 1954), syringaldazine (PODs) (Imberty, Goldberg & Catesson, 1985), ferulic acid (PODf) (Takahama, 1995) were determined. The results presented are the means of 4 independent experiments (n = 4–8). The significance of differences between mean values (non-infected plants – infected plants of a particular tomato cultivar) was determined by a non-parametric Mann-Whitney Rank Sum Test. Values that differ significantly from the control (non-infected plants) were indicated in figures as follows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

RESULTS AND DISCUSSION

H_2O_2 content started to increase as early as 12 h after infection of the leaves of cv. Perkoz and it reached the maximum level 24 h after inoculation. In infected leaves of the more susceptible cv. Torena the concentration of H_2O_2 did not change significantly during the time course of the experiment. (Fig. 1A). It has also been found in our laboratory that 5 mM H_2O_2 completely inhibited germination of *B. cinerea* spores *in vitro* (data not presented). Infection caused only slight changes in $OH^{\cdot}$ level in the leaves of both cultivars (Fig. 1B). At the early stages of pathogenesis in the leaves of cv. Perkoz the activities of APX and CAT remained unchanged or decreased below the control level (Fig. 2). Significant increase in APX and CAT activities was observed in the leaves of cv. Perkoz following the second day after inoculation, whereas in case of cv. Torena the activities of these enzymes did not change significantly in response to infection. The activity of POD assayed with guaiacol (PODg) (Fig. 3A), syringaldazine (PODs) (Fig. 3B) and ferulic acid (PODf) (Fig. 3C) increased markedly after treatment with the fungus in the leaves of both cv. Torena and cv. Perkoz. The activity of POS oxidizing syringaldazine increased more intensively and earlier in comparison with those measured with guaiacol and ferulic acid. Although treatment with the fungus caused similar induction of PODg, PODs and PODf in both cultivars, the activities of these enzymes before infection were 2–3 times higher in more resistant cultivar (Table 1).

Differences in defence reactions between susceptible cv. Torena and relatively resistant cv. Perkoz were observed. After infection the level of H_2O_2 increased significantly in the leaves of cv. Perkoz, whereas in those of cv. Torena it remained almost unchanged. The increased level of H_2O_2 shortly after inoculation of tomato plants cv. Perkoz may directly affect spore germination and thus delay the pathogen growth. Lu and Higgins (1999) reported that germination and germ tube growth of conidia of *Cladosporium fulvum* were significantly retarded by milimolar concentrations of H_2O_2. The spore germination of *Peronospora tabacina* and *Colletotrichum lagenarium* was totally inhibited by H_2O_2 at 2.61×10^{-5} M (Peng & Kuć, 1992). Moreover, H_2O_2 is used by some peroxidases in the processes that are aimed at giving structural protection against the invading pathogen.

The activties of peroxidases oxidizing phenolic compounds increased similarly after infection of both cultivars, however the levels of these enzymes activities detected before inoculation were much lower in the leaves of more susceptible cv.

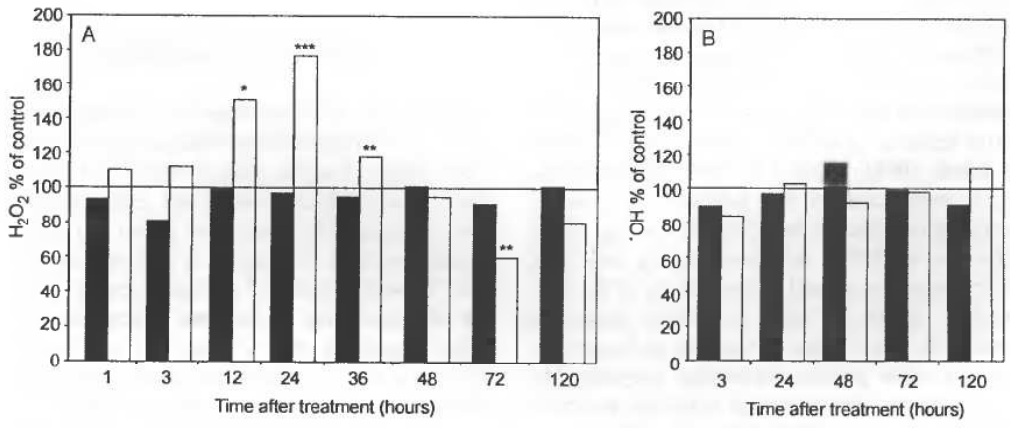

Fig. 1. Changes in H_2O_2 (A) and $OH^{\cdot}$ (B) levels after infection of tomato leaves cv. Torena (black bars) and cv. Perkoz (white bars) with *Botrytis cinerea*

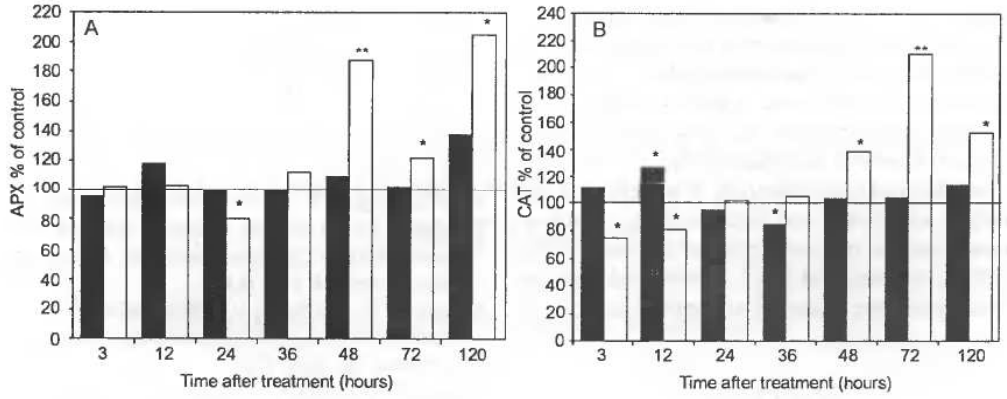

Fig. 2. Changes in APX (A) and CAT (B) activities after infection of tomato leaves cv. Torena (black bars) and cv. Perkoz (white bars) with *Botrytis cinerea*

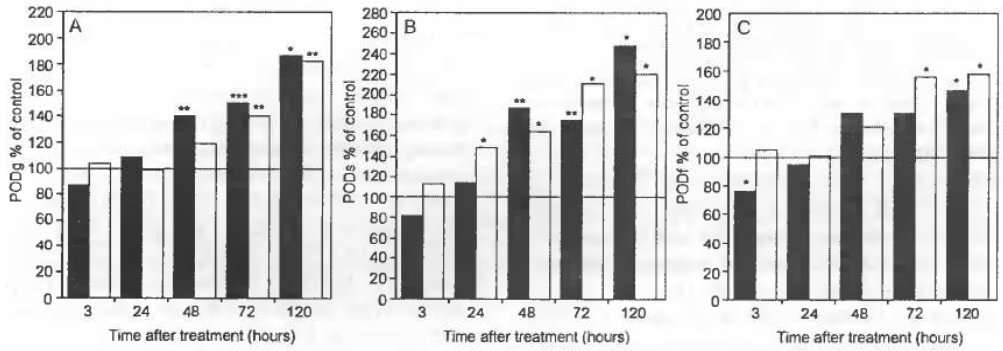

Fig. 3. Changes in POD activity assayed with guaiacol (PODg) (A), syringaldazine (PODs) (B) and ferulic acid (PODf) (C) after infection of tomato leaves cv. Torena (black bars) and cv. Perkoz (white bars) with *Botrytis cinerea*

Table 1. The levels of reactive oxygen species and the activities of antioxidant enzymes in control leaves of cv. Torena and Perkoz

Parameter	Torena	Perkoz
H_2O_2 (nmol/g f. w.)	81.42	117.28
$OH^{\cdot}$ (A_{540}/g f. w.)	0.460	0.677
APX (U/mg protein)	0.069	0.127
CAT (U/mg protein)	34.25	31.96
PODg (U/mg protein)	0.093	0.244
PODs (U/mg protein)	3.97	10.58
PODf (U/mg protein)	3.89	8.98

Torena and even after infection they did not reach the level of those measured in cv. Perkoz. According to some authors induction of POD oxidizing syringaldazine is related to the intensification of lignin biosynthesis (Goldberg, Catesson & Czaninski, 1983). Hammerschmidt, Lamport and Muldoon (1984) reported that infection of cucumber with *Cladosporium cucumerinum* resulted in lignin deposition. Intensive lignification occurred in resistant but not in susceptible cultivars of cucumber. POD oxidizing ferulic acid is thought to be involved in the cross-linking of cell wall polysaccharides (Zimmerlin, Wojtaszek & Bolwell, 1994). After infection of oat leaves with *Puccinia coronata* f. sp. *avenae* Ikegawa, Mayama, Nakayashiki and Kato (1996) observed the cross-linking of wall matrix polysaccharides by diferulic acid formation. Ikegawa *et al.* (1996)

suggested that this cross-linking was caused by a coupling reaction initiated by ionically-bound cell wall peroxidase. Cross-linking makes the polysaccharides of cell wall matrix more resistant to cell wall degrading enzymes and thus provides a mechanical barrier to pathogen ingress.

In conclusion, increase in H_2O_2 content observed only in cv. Perkoz and higher activity of peroxidases involved in the stiffening of cell walls in this cultivar as compared to cv. Torena, indicate that these factors are important to plant resistance.

REFERENCES

Capaldi D. J. & Taylor K. E. (1983). A new peroxidase colour reaction: Oxidative coupling of 3-methyl-2-benzothiazolinone hydrazone (MBTH) with its formaldehyde azine. Application to glucose and choline oxidases. *Anal. Biochem.*, **129**, 329–336.

Chen Z., Silva H. & Klessig D. F. (1993). Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. *Science*, **262**, 1883–1886.

Dhindsa R.S., Plumb-Dhindsa P. & Thorpe T. A. (1981). Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.*, **32**, 93–101.

Goldberg R., Catesson A.-M. & Czaninski Y. (1983). Some properties of syringaldazine oxidase, a peroxidase specifically involved in the lignification process. *Z. Pflanzenphysiol.*, **110**, 267–279.

Hammerschmidt R., Lamport D. T. A. & Muldoon E. P. (1984). Cell wall hydroxyproline enhancement and lignin deposition as an early event in the resistance of cucumber to *Cladosporium cucumerinum*. *Physiol. Plant Pathol.*, **24**, 43–47.

Ikegawa T., Mayama S., Nakayashiki H. & Kato H. (1996). Accumulation of diferulic acid during the hypersensitive response of oat leaves to *Puccinia coronata* f. sp. *avenae* and its role in the resistance of oat tissues to cell wall degrading enzymes. *Physiol. Mol. Plant Pathol.*, **48**, 245–255.

Imberty A., Goldberg R. & Catesson A. M. (1985). Isolation and characterization of *Populus* isoperoxidases involved in the last step of lignin formation. *Planta*, **164**, 221–226.

Lu H. & Higgins V. J. (1999). The effect of hydrogen peroxide on the viability of tomato cells and of the fungal pathogen *Cladosporium fulvum*. *Physiol. Mol. Plant Pathol.*, **54**, 131–143.

Maehly A. C. & Chance B. (1954). *Methods of Biochemical Analysis*, Vol. 1, pp. 357–424. Interscience Publishers Inc., New York.

Nakano Y. & Asada K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.*, **22**, 867–880.

Peng M. & Kuć J. (1992). Peroxidase-generated hydrogen peroxide as a source of antifungal activity *in vitro* and on tobacco leaf disks. *Phytopathol.*, **82**, 696–699.

Takahama U. (1995). Oxidation of hydroxycinnamic acid and hydroxycinnamyl alcohol derivatives by laccase and peroxidase. Interactions among p-hydroxyphenyl, guaiacyl and syringyl groups during the oxidation reactions. *Physiol. Plant.*, **93**, 61–68.

Tenhaken R., Levine A., Brisson L. F., Dixon R. A & Lamb C. (1995). Function of the oxidative burst in the hypersensitive disease resistance. *Proc. Natl. Acad. Sci. USA*, **92**, 4158–4163.

Tiedemann A. N. (1997). Evidence for a primary role of active oxygen species in induction of host cell death during infection of bean leaves with *Botrytis cinerea*. *Physiol. Mol. Plant Pathol.*, **50**, 151–166.

Willekens H., Inzé D., van Montagu M. & van Camp W. (1995). Catalases in plants. *Molec.Breeding*, **1**, 207–228.

Zimmerlin A., Wojtaszek P. & Bolwell G. P. (1994). Synthesis of dehydrogenation polymers of ferulic acid with high specificity by a purified cell-wall peroxidase from French bean (*Phaseolus vulgaris* L.). *Biochem J.*, **299**, 747–753.