Available online at www.sciencedirect.com
SCIENCE DIRECT®
Scientia Horticulturae 102 (2004) 1–13
SCIENTIA HORTICULTURAE
www.elsevier.com/locate/scihorti

Oxidative metabolism and phenolic compounds in *Capsicum annuum* L. var. *annuum* infected by *Phytophthora capsici* Leon

C. Gayoso, F. Pomar, F. Merino, M.A. Bernal*

Department of Animal Biology, Plant Biology and Ecology, Science Faculty, La Coruña University, La Zapateira s/n, 15071 La Coruña, Spain

Accepted 14 November 2003

Abstract

Changes in peroxide levels, the activity of antioxidant enzymes and phenolic compounds were studied in *Capsicum annuum* L. var. *annuum* plants inoculated with *Phytophthora capsici* P_{1UDC} (Galician field isolate). The extent of oxidative stress during the interaction *Capsicum–Phytophthora* was evidenced, at the final period of interaction, by the at least 87% increase in peroxide levels. Catalase activity showed the biggest changes in the stems by 114% increase, ascorbate peroxidase fell during the interaction, peroxidase levels remained constant from the first hours to the fifth day, and only showed an increment by 54 and 90% in stems and leaves, respectively, during the final period of interaction as phenolic compounds (free and bound fraction). Collectively, these results provide evidence that the interaction *Capsicum–Phytophthora* is accompanied by a substantial increase in oxidative stress, probably as a direct consequence of a progressive decline in the enzymatic systems responsible for catabolism of active oxygen species.

Keywords: *Capsicum annuum*; *Phytophthora capsici*; Active oxygen species; Antioxidant enzymes; Phenolic compounds

1. Introduction

The pepper is an important crop in Galicia (NW Spain), the annual production of pepper fruits being 20 000 Tm in a total area of 950 ha and the most relevant cultivar cultivated being the Padrón pepper. The fruits are harvested and commercially available when immature,

* Corresponding author. Tel.: +34-981-167000; fax: +34-981-167065.
E-mail address: bernal@udc.es (M.A. Bernal).

0304-4238/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2003.11.015

and their flavor is moderately hot, as they contain only small levels of capsaicin (Estrada et al., 2002). The capsaicinoid content in the Padrón pepper plant is genetically controlled, but also subjected to environmental variables such as water supply and fertilization level (Estrada et al., 1998, 1999).

As with other crops, the pepper is susceptible to the attack of several pathogens, hence resulting in losses in production. In a study conducted in 1998, Pomar et al. (2001) found that *Phytophthora capsici* Leon and *Verticillium dahliae* Kleb. are the most prevalent fungal pathogens to attack pepper plants in Galicia. *Phytophthora* blight has a wide geographic distribution and can occur at any stage of pepper growth and all parts of the plant may be affected. The collar rot and wilt phase is most common and is characterized by a dark brown stem discoloration extending upward from the soil line accompanied by a sudden wilt of the entire plant without foliar yellowing.

The production of various active oxygen species (AOS) is a common event in all organisms living under aerobic conditions. AOS are toxic intermediates that result from successive one-electron steps in the reduction of molecular O_2 and are routinely generated at low levels by plant cells in chloroplasts, mitochondria, and in other cell compartments involved in reduction–oxidation processes.

When plants are attacked by pathogens they respond by activating a variety of defense mechanisms, including the rapid production and accumulation of AOS, primarily the superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) (Medhy, 1994; Low and Merida, 1996). Multiple roles for active oxygen in plant defense have been suggested, including acting as second messengers, as substrates in synthesis of structural defense components, and as the toxic agents that cause plant cell and pathogen death (Baker and Orlandi, 1995; Lamb and Dixon, 1997; Medhy et al., 1996). These studies have documented a strong correlation between production of AOS and the occurrence of the hypersensitive reaction (HR), the most common and dramatic defense reaction in plants, as well as of systemic acquired resistance (SAR), a non-specific induced resistance response.

Plants possess a complex battery of enzymatic and non-enzymatic antioxidants that can protect cells from oxidative damage by scavenging AOS. H_2O_2 is destroyed predominantly by ascorbate peroxidases (APX) and catalases (CAT) (Asada, 1997; Willekens et al., 1997). Although CATs are restricted to the peroxisomes, and perhaps mitochondria, APXs have been found in every compartment of the plant cell in which they have sought (Asada, 1997; Jimenez et al., 1997).

Evidence is presented showing that peroxidases catalyze the polymerization of phenolic compounds to produce a variety of products (Kobayashi et al., 1994) which may take part in the defense system of plants against pathogens. Moreover, the oxidation of phenolic compounds generally leads to the production of quinones (Thipyapong et al., 1995), which are highly toxic compounds responsible for the generation of reactive oxygen species.

It has been recently suggested that reduction in the activity/expression of enzymes responsible for AOS catabolism is also relevant for AOS increase. Repression of the antioxidative defense could be another mechanism for accumulation of AOS. Both catalase and ascorbate peroxidase, enzymes responsible for hydrogen peroxide scavenging in plant cells, seem to be locally down-regulated during the HR (Dorey et al., 1998; Mittler et al., 1998, 1999). In the interaction between tobacco and TMV, the expression of a cytosolic form of the

antioxidative enzyme APX is repressed post-transcriptionally (Mittler et al., 1999). Furthermore, the number of antioxidative compounds and enzymes changes in the apoplast of barley leaves inoculated with powdery mildew (Vanacker et al., 1998).

Phytophthora wilt of pepper is not well controlled by the use of fungicides or resistant cultivars. This fact encouraged us to do research on the metabolism of the active species since their participation in this type of interaction has gained great importance in recent years, in what refers to detoxification mechanisms as well as in their involvement as a defense signal. Here we present results on the process of oxidative metabolism and the level of phenolic compounds associated with the infection of *Capsicum annuum* L. var. *annuum* by *P. capsici*.

2. Materials and methods

2.1. Chemicals

PD-10 Sephadex G-25 column was purchased from Pharmacia Biotech. All other chemicals used in this work were obtained from Sigma Chemical Co. (Madrid, Spain) and were of the highest purity available.

2.2. Plant and fungus material

Seeds of *C. annuum* L. var. *annuum* were soaked overnight in tap water before being sown in vermiculite. The seedlings emerged 8 days later and were grown thereafter at 25 °C in a 16 h photoperiod. The fungus used was *P. capsici* Leon, isolated P_{1UDC} (obtained from a field in Galicia and isolated by our group in a study conducted in 1998), which was kept in the dark on a potato dextrose agar (PDA) medium at 25 °C.

2.3. Inoculation procedure

Zoospores were produced by growing an isolate of *P. capsici* (P_{1UDC}) on water-agar at 25 °C for 8 days. Twenty agar blocks were removed from the actively growing cultures and placed into Erlenmeyer flasks containing 20 ml of 10% sterile soil extract. Numerous sporangia were induced to release zoospores by chilling at 4 °C for 30 min. After rewarming at 25 °C for 30 min, contents of each Erlenmeyer were decanted into a 100 ml beaker through cheesecloth to remove agar blocks and attached mycelia. The zoospore suspension was quantified using a hemocytometer.

Forty plants, grown for 15 days and having two to four fully expanded leaves, were used for inoculation and disease assessment. The inoculation was carried out with a 5 ml water suspension of 300 zoospores ml^{-1}. The control plants were inoculated with sterile water not containing zoospores. Samples of leaves, stems and roots were taken 12 h, 1–3, 5 and 7 days after inoculation. Each assay involved eight plants per treatment and was performed at least twice. Infection by *P. capsici* was confirmed by reisolating the pathogen from infected stems and roots. All the results, of control plants as well as of inoculated plants, are means of three independent experiments.

2.4. Plant enzyme extraction

All steps were performed at 4 °C. In the case of peroxidase extraction, the samples were homogenized in a buffer containing 50 mM Tris, 1 mM EDTA, 1 M KCl, pH 7.50 (2 ml buffer g^{-1} fresh tissue) with the addition of 0.05 g PVPP per g of fresh weight and 1 mg sodium bisulphite per ml of buffer. To study ascorbate peroxidase and catalase activity, samples were homogenized in a buffer containing 50 mM potassium phosphate, 1 mM EDTA, 1 mM ascorbic acid, pH 7.00.

Homogenates were filtered through four layers of nylon gauze and centrifuged at 14 000 × *g* for 30 min. The supernatants for peroxidase and ascorbate peroxidase activity were desalted using a PD-10 Sephadex G-25 column from Pharmacia Biotech and stored at −20 °C until use.

2.5. Enzymatic assays

Peroxidase activity was assayed at 25 °C using 4-methoxy-α-naphthol (4MN) as a substrate according to Ferrer et al. (1990). The incubation mixture was composed of 50 mM Tris–HCl buffer (pH 7.5), 1 mM 4MN, 0.33 mM H_2O_2 and 50 μl of sample. Ascorbate peroxidase activity was studied according to the method of Amako et al. (1994). The incubation mixture consisted of 50 mM potassium phosphate buffer (pH 7.00), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 50 μl of sample. Catalase activity was measured according to the method of Aebi (1984) with minor modifications. The incubation mixture was composed of 50 mM potassium phosphate buffer (pH 7.00), 10.6 mM H_2O_2 and 100 μl of sample.

Enzymatic activities, expressed as katal, were defined as the amount of enzyme that converts 1 mol s^{-1} of substrate into product.

2.6. Extraction and determination of total phenolics

Each sample (500 mg fresh weight) was homogenized in 2.5 ml 80% MeOH. The homogenized sample was incubated for 15 min at 80 °C and then centrifuged at 500 × *g* for 10 min. The supernatant was dried at 80 °C and resuspended in 0.5 ml 80% MeOH, after which was filtered through a 0.45 μm filter. This fraction was regarded as the free phenolics fraction.

The pellet was resuspended in 4 ml NaOH and incubated for 4 h at 37 °C. After adjusting the pH to 1–2 with HCl, the phenolics were extracted by diethyl ether. The ether was then evaporated to dryness and the residue was resuspended in MeOH, after which was filtered through a 0.45 μm filter. This fraction was regarded as the bound phenolics fraction.

Phenol content was determined using the Folin–Ciocalteu reagent, according to the method of Singleton and Rossi (1965), and calculated from a standard curve obtained using different concentrations of ferulic acid.

2.7. Peroxide extraction and measurement

To determine the peroxide concentration, the samples were extracted as they were being collected. The samples were homogenized in 0.1 M potassium phosphate buffer (pH 6.90), at a 1:2 ratio (w/v), and then centrifuged at 14 000 × *g* for 30 min, extracting the supernatant.

Peroxide was measured according to the method of Frew et al. (1983). The incubation mixture was composed of 0.1 M potassium phosphate buffer (pH 6.90), 5 mM 4-aminoantipirine, 2.5 mM phenol, 2.0 nkat horseradish peroxidase and H_2O_2. One kat of peroxidase was defined as the amount of protein that oxidized 1 mol s^{-1} of 4-methoxy-α-naphthol, assayed according to Ferrer et al. (1990). A calibration curve of different peroxide concentrations prepared with Milli-Q water was used.

2.8. Statistical analyses

Data were analyzed by application of the Duncan test. Significant differences between the control and treated plants were those for $P \leq 0.05$.

3. Results

3.1. Changes in H_2O_2 levels

The control plants showed a similar pattern of peroxide accumulation in the different organs, as their levels were lower than 50 nmol g^{-1} FW during the period studied. In inoculated plants, peroxide accumulation showed an increase in all the organs during the interaction. This increment was observed from the fifth day after inoculation, with the roots exhibiting the highest levels after inoculation (40.6 nmol g^{-1} FW).

3.2. Changes in antioxidant enzymes

Catalase activity in stems (Fig. 1B) and leaves (Fig. 1C) of inoculated plants increased as compared to the control plants. The values of catalase activity in those organs of inoculated plants were 2.1- and 2.0-fold greater than the control values, respectively, 7 days after inoculation and the stem showed the highest value of activity. In roots (Fig. 1A) a different pattern was observed, characterized by first a decrease in roots of both control and inoculated plants but then an increase at the end.

Ascorbate peroxidase activity in control and inoculated plants was detected in all the organs studied. As indicated in Fig. 2A, ascorbate peroxidase levels in roots decreased as a result of the inoculation, whereas in the control plant, an increase was found. Ascorbate peroxidase levels in stems and leaves (Fig. 2B and C) showed no clear pattern of changes when the control and inoculated plants were compared, but a lower level was always detected in inoculated plants.

The peroxidase activity (Fig. 3) in the *C. annuum–P. capsici* interaction showed no differences between control and inoculated plants, except for a possible transient increase in leaves (90%) and stems (54%) (Fig. 3B and C, respectively) 7 days after inoculation.

3.3. Changes in phenolic contents

In control plants, free phenolics showed their lowest value at around 3 days in all the organs studied (Fig. 4A–C). It then began increasing and remained high for 7 days (Fig. 4A)

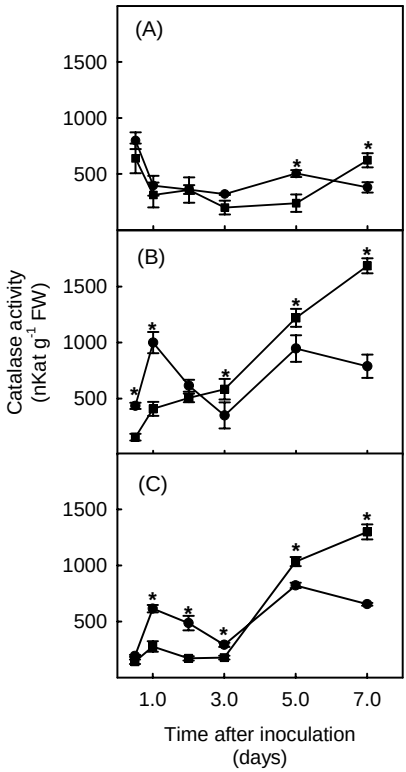

Fig. 1. Changes in catalase activity (CAT) in roots (A), stems (B) and leaves (C) of control (●) and inoculated (■) plants at different times after inoculation. Means values (±S.E.), within each day for each organ (*) indicates significant differences at $P \leq 0.05$.

in roots (330 μg ferulic acid g^{-1} FW) and for 5 days in stems (304 μg ferulic acid g^{-1} FW) and leaves (649 μg ferulic acid g^{-1} FW) (Fig. 4B and C). In inoculated plants, a change in this pattern of accumulation was found because the highest levels were detected in stems and leaves at the end of period studied (Fig. 4B and C) accompanied by a significant increase in response to inoculation, in both cases. The roots, however, showed lower levels than control plants roots.

In the case of bound phenolic contents (Fig. 4D and E), the level in control plants decreased over time, showing an increase of at least 15% on the seventh day. During the initial study period, inoculated plants also presented a decrease, with the minimum levels being observed 3 days after inoculation, but they later underwent a major increase in the stems and leaves.

When considering total phenolic contents (Fig. 5), the overall behavior is similar to that observed for the soluble contents, showing a decrease only in roots (Fig. 5A), but increasing in stems and leaves after the third day of inoculation (Fig. 5B and C).

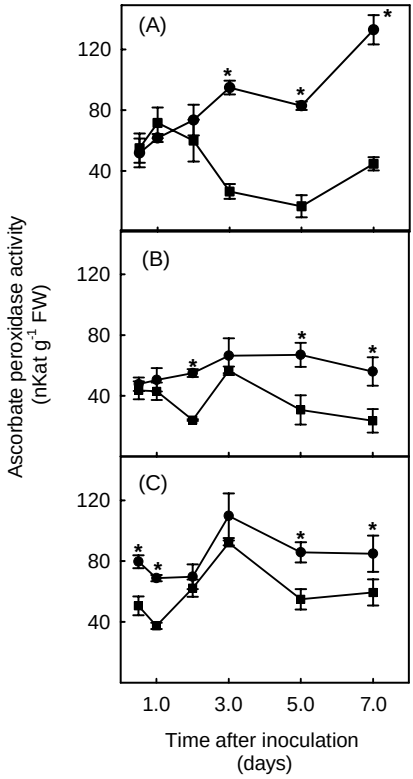

Fig. 2. Changes in ascorbate peroxidase activity (APX) in roots (A), stems (B) and leaves (C) of control (●) and inoculated (■) plants at different times after inoculation. Means values (±S.E.), within each day for each organ (*) indicates significant differences at $P \leq 0.05$.

4. Discussion

Oxidative stress can be activated in response to a variety of stimuli, such as high and low temperatures, exposure to UV rays, nutrient deficiency, drought, herbicides and pathogen attack (Inze and Van Montagu, 1995). An increase in AOS often occurs following pathogen infection (Sandermann, 2000).

Oxidative processes during the interaction of *C. annuum–P. capsici* Leon were determined by measuring changes in H_2O_2 content. The results show that *C. annuum* seedlings infected by *P. capsici* Leon lead to an increase in the peroxide level, in all the organs studied, as compared to the organs of control plants (Fig. 6).

The susceptibility to oxidative stress is a function of the overall balance between the factors that increase oxidant generation and those substances that exhibit antioxidant capability (Foyer et al., 1994). To determine the possible association of the oxidative processes accompanying the interaction with the ability to regulate AOS, the activities of catalase, ascorbate peroxidase and peroxidase in the different organs were monitored and changes in

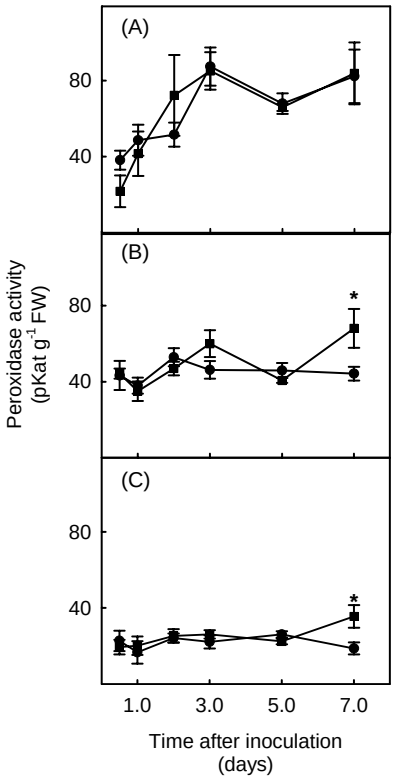

Fig. 3. Changes in peroxidase activity (PRX) in roots (A), stems (B) and leaves (C) of control (●) and inoculated (■) plants at different times after inoculation. Means values (±S.E.), within each day for each organ (*) indicates significant differences at $P \leq 0.05$.

these enzyme activities may be regarded as a consequence of pathogen-induced oxidative stress.

An increment in the catalase activity in stems and leaves of inoculated plants was detected (Fig. 1B and C). As a precursor of the highly reactive hydroxyl radical ($OH^{\bullet}$), H_2O_2-caused destruction in cells was quite significant (Heiser et al., 1998). So CAT induction appeared to be a common response to stress that could strengthen plants against reactive oxygen species overproduction. The interaction *C. annuum–P. capsici* was accompanied by the accumulation of H_2O_2 in the different tissues. It is likely that increases in CAT activities might contribute to lowered H_2O_2 content, but the antioxidant potential in the tissues of *Capsicum* plants might not be enough to block the degeneration process.

Levels of ascorbate peroxidase were found to decrease in inoculated plants as compared to control plants (Fig. 2) in all the organs studied. This enzyme is located in the chloroplasts (Asada, 1999) and is responsible for the scavenging of peroxide in these organelles. During the time that the pathogen is introduced into the plant, the activation of lipooxygenases and phospholipases takes place and this occurrence produces the de-compartmentalisation

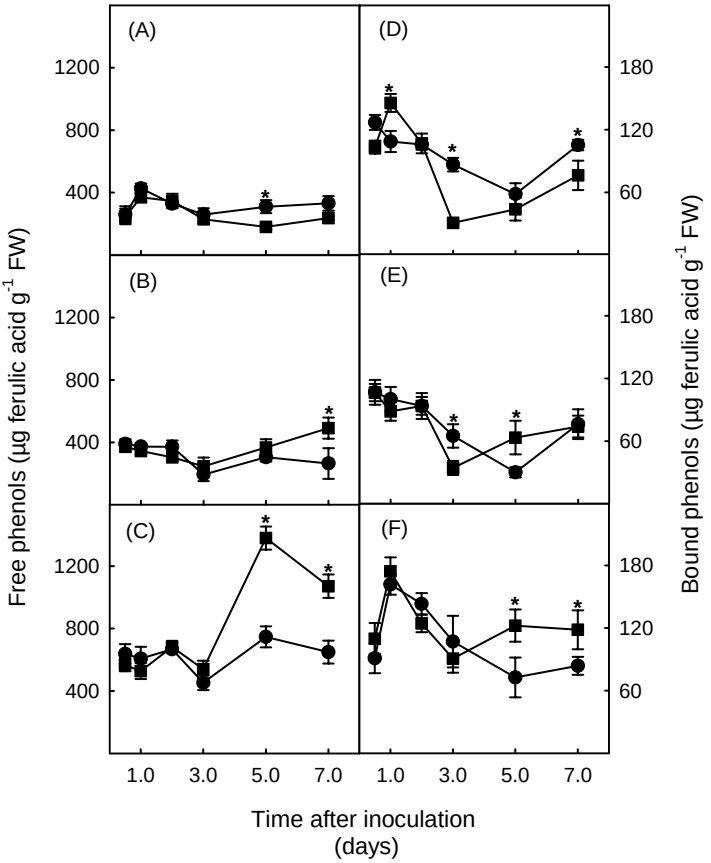

Fig. 4. Changes in free (A–C) and bound (D–F) phenolic compounds in roots (A and D), stems (B and E) and leaves (C and F) of control (□) and inoculated (■) plants at different times after inoculation. Means values (±S.E.), within each day for each organ (*) indicates significant differences at $P \leq 0.05$.

of the chloroplasts. Ascorbate peroxidase is very sensitive at high levels of peroxide and requires ascorbic acid in the medium where it is found, otherwise it will diminish (Asada, 1999).

Mittler et al. (1998) found that during viral-induced programmed cell dead (PCD) in tobacco the expression of cytosolic ascorbate peroxidase (cAPX) is post-transcriptionally suppressed. This suppression is likely to contribute to a reduction in the capability of cells to scavenge H_2O_2, which in turn enables the accumulation of H_2O_2 and the acceleration of PCD. Suppression of cAPX activity may have an even more dramatic effect on the induction of PCD compared with catalase, because cAPX has a higher affinity for H_2O_2 than does catalase (Amako et al., 1994). Recently de Pinto et al. (2002) reported a similar suppression of ascorbate peroxidase activity when they studied the relationship between cell death induced by nitric oxide and/or AOS and the cellular antioxidant capabilities in tobacco BY-2 cell suspensions. It is conceivable that a decrease in enzymatic scavengers

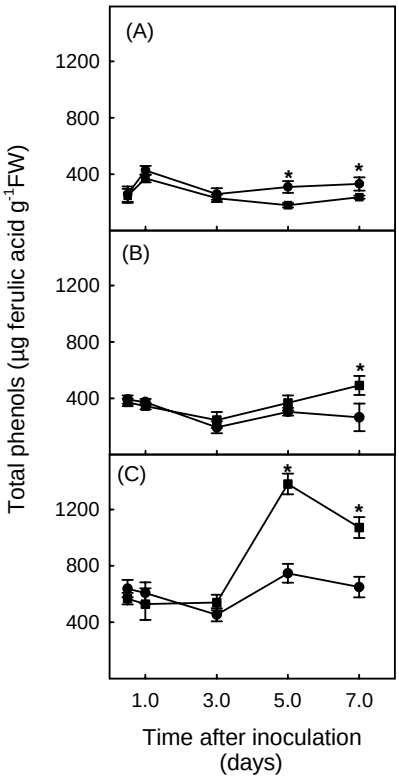

Fig. 5. Changes in total phenolic compounds in roots (A), stems (B) and leaves (C) of control (●) and inoculated (■) plants at different times after inoculation. Means values (±S.E.), within each day for each organ (*) indicates significant differences at $P \leq 0.05$.

caused by the interaction plant-pathogen may also contribute to the shift in the balance of free radical metabolism towards peroxide accumulation.

A correlation between peroxidase activity and phenol levels has been proposed for various crops including *C. annuum* (Candela et al., 1995). Pepper peroxidase is capable of oxidizing different phenols (Bernal et al., 1995) and it would seem plausible that this enzyme may be involved in the insolubilisation of phenylpropanoids in muro. The observation that peroxidase activity increases in stems and leaves at day 7 after inoculation (Fig. 3B and C) at the same time that phenols reach maximum levels (Fig. 4B, C, E and F) would suggest that in the pepper, this enzyme may be involved in the defense response. However, the role played by peroxidase enhancement in the resistance to plant pathogens has not been established unequivocally and it is still not clear whether it is a cause or a consequence of this phenomenon (Esnault and Chibar, 1997). Auh and Murphy (1995) showed that DPI and several other inhibitors of the oxidative burst did not affect extracellular peroxidase activity in rose cells and their data suggest that increased peroxidase activity is not a major source of AOS during the HR.

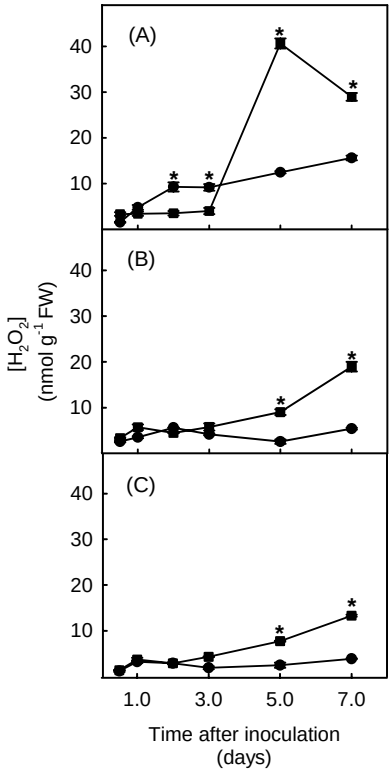

Fig. 6. Changes in peroxide content in roots (A), stems (B) and leaves (C) of control (●) and inoculated (■) plants at different times after inoculation. Means values (±S.E.), within each day for each organ (*) indicates significant differences at $P \leq 0.05$.

Phenolic compounds are among the most widely distributed secondary products in the plant kingdom. Since they are known to accumulate in response to infection in some species, it has also been suggested that they play a potential role in disease resistance (Hahlbrock and Scheel, 1989). The results presented here for the pepper bear a number of similarities to those reported previously by Candela et al. (1995). In a stem of *C. annuum* infected by *P. capsici*, these authors found an increase in the total phenols as a result of the increase in the free forms.

Collectively, these results provide evidence that the interaction *Capsicum–Phytophthora* is accompanied by a substantial increase in oxidative stress, probably as a direct consequence of a progressive decline in the enzymatic systems responsible for catabolism of active oxygen species. On the basis of quantitative data, among the three-enzymes tested, changes in APX and CAT activity seem to be related with the interaction *C. annuum–P. capsici*. Since all of the measurements reported here have been made in total extracts, the possibility that there could be differential changes in antioxidant enzymes between subcellular compartments cannot be excluded. Further studies are needed to address the changes that occur at

the subcellular level during the interaction, which would lead to a better understanding of this complex but highly regulated process.

Acknowledgements

This research was funded by a Grant from La Coruña University (UDC1999-00) and Xunta de Galicia (XUGA10303A97). F. Pomar was awarded a scholarship from La Coruña University.

References

Aebi, H., 1984. Catalase in vitro. Meth. Enzymol. 105, 121–126.

Amako, K., Gong-Xiang, Ch., Asada, K., 1994. Separate assays specific for ascorbate peroxidase and guaiacol peroxidase and for the chloroplastic and cytosolic isozymes of ascorbate peroxidase in plants. Plant Cell Physiol. 35 (3), 497–504.

Asada, K., 1997. The role of ascorbate peroxidase and monodehydroascorbate reductase in H_2O_2 scavenging in plants. In: Scandalios, J.G. (Ed.), Oxidative Stress and the Molecular Biology of Antioxidant Defenses, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 715–735.

Asada, K., 1999. The water–water cycle in chloroplasts scavenging of active oxigens and dissipation of excess fotons. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50, 601–639.

Auh, C.K., Murphy, T.M., 1995. Plasma membrane redox enzyme is involve in the synthesis of O_2^- and H_2O_2 by *Phytophthora* elicitor-stimulated rose cells. Plant Physiol. 107, 1241–1247.

Baker, C.J., Orlandi, E.W., 1995. Active oxygen in plant/pathogen interactions. Annu. Rev. Phytopathol. 33, 299–321.

Bernal, M.A., Calderon, A.A., Ferrer, M.A., Merino, F., Ros Barcelo, A., 1995. Oxidation of capsaicin and capsaicin phenolic precursors by the basic peroxidase isoenzyme B_6 from hot pepper. J. Agric. Food Chem. 43, 352–355.

Candela, M.E., Alcazar, M.D., Espín, A., Egea, C., Almela, L., 1995. Soluble phenolics acids in *Capsicum annuum* stems infected with *Phytophthora capsici*. Plant Pathol. 44, 116–123.

de Pinto, C., Tommasi, F., De Gara, L., 2002. Changes in the antioxidant systems as part of the signaling pathway responsible for the programmed cell death activated by nitric oxide and reactive oxygen species in tobacco BY-2 cells. Plant Physiol. 130, 698–708.

Dorey, S., Baillieul, F., Saindrenan, P., Fritig, B., Kauffmann, S., 1998. Tobacco class I and II catalases are differentially expressed during elicitor-induced hypersensitive cell death and localized acquired resistance. Mol. Plant Microbe. Interact. 11, 1102–1109.

Esnault, R., Chibar, R.N., 1997. Peroxidases and plant defense. Plant Perox. Newslett. 10, 34–41.

Estrada, B., Pomar, F., Díaz, J., Merino, F., Bernal, A., 1998. Effects of mineral fertilizer supplementation on fruit development and pungency in Padrón peppers. J. Hort. Sci. Biotech. 73 (4), 493–497.

Estrada, B., Pomar, F., Díaz, J., Merino, F., Bernal, A., 1999. Pungency level in fruits of the Padrón pepper with different water supply. Scientia Hort. 81, 385–396.

Estrada, B., Pomar, F., Díaz, J., Merino, F., Bernal, A., 2002. Capsaicinoids in vegetative organs of *Capsicum annuum* L. in relation to fruiting. J. Agric. Food Chem. 50 (5), 1188–1191.

Ferrer, M.A., Pedreño, M.A., Muñoz, R., Ros Barceló, A., 1990. Oxidation of coniferyl alcohol by cell wall peroxidases at the expense of indole-3-acetic acid and O_2. FEBBS Lett. 276, 127–130.

Foyer, C.H., Lelandais, M., Kunert, K.J., 1994. Photooxidative stress in plants. Physiol. Plant. 92, 696–717.

Frew, J.E., Jones, P., Scholes, G., 1983. Spectrophotometric determination of hydrogen peroxide and organic hydroperoxides at low concentrations in aqueous solution. Anal. Chim. Acta 155, 139–150.

Hahlbrock, K., Scheel, D., 1989. Physiology and molecular biology of phenylpropanoi metabolism. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40, 347–369.

Heiser, I., Ebwald, W., Elstner, E.F., 1998. The formation of reactive oxygen species by fungal and bacterial phytotoxins. Plant Physiol. Biochem. 36, 703–713.

Inze, D., Van Montagu, M., 1995. Oxidative stress in plants. Curr. Opin. Biotechnol. 6, 153–158.

Jimenez, A., Hernandez, J.A., Del Rio, L.A., Sevilla, F., 1997. Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. Plant Physiol. 114, 275–284.

Kobayashi, A., Koguchi, Y., Kanzaki, H., Kajiyama, S., Kawazu, K., 1994. A new type of antimicrobial phenolics produced by plant peroxidase and its possible role in the chemical defense system against plant pathogens. Z. Naturforsch 49c, 411–414.

Lamb, C., Dixon, R.A., 1997. The oxidative burst in plant disease resistance. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48, 251–275.

Low, P.S., Merida, J.R., 1996. The oxidative burts in plant defense: function and signal transduction. Physiol. Plant. 96, 533–542.

Medhy, M.C., 1994. Active oxygen species in plant defense against pathogens. Plant Physiol. 195, 467–472.

Medhy, M.C., Sharma, Y.K., Sathasivan, K., Bays, N.W., 1996. The role of activated oxygen species in plant disease resistance. Physiol. Plant. 98, 365–374.

Mittler, R., Feng, X., Cohen, M., 1998. Post-transcriptional suppression of cytosolic ascorbate peroxidase expression during pathogen-induced programmed cell death in tobacco. Plant Cell 10, 461–473.

Mittler, R., Herr, E.H., Orvar, B.L., Van Camp, W., Willekens, H., Inze, D., Ellis, B.E., 1999. Transgenic tobacco plants with reduced capability to detoxify reactive oxygen intermediates are hyperresponsive to pathogen infection. Proc. Natl. Acad. Sci. U.S.A. 96, 14165–14170.

Pomar, F., Bernal, M.A., Collar, J., Díaz, J., Caramelo, C., Gayoso, C., Novo, M., Prego, C., Saavedra, A., Silvar, C.Y., Merino, F., 2001. A survey of “tristeza” of pepper in Galicia and the fungal pathogens causing the disease. *Capsicum Eggplant* Newsp. 20, 90–93.

Sandermann, H., 2000. Active oxygen species as mediators of plant immunity: three case studies. Biol. Chem. 381, 649–653.

Singleton, V.L., Rossi, J.A., 1965. Colorimetric of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vit. 16, 144–158.

Thipyapong, P., Hunt, M.D., Steffens, J.C., 1995. Systemic wound induction of potato (*Solanum tuberosum*) polyphenol oxidase. Phytochemistry 40, 673–676.

Vanacker, H., Carver, T.L.W., Foyer, C.H., 1998. Pathogen-induced changes in the antioxidant status of the apoplast in barley leaves. Plant Physiol. 117, 1103–1114.

Willekens, H., Chamonongpol, S., Davey, M., Schraudner, M., Langebartels, C., Van Montagu, M., Inze, D., Van Camp, W., 1997. Catalase is a sink for H_2O_2 and is indispensable for stress defense in C3 plants. EMBO J. 16, 4806–4816.