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# Effects of Salicylic Acid on Paraquat Tolerance in *Arabidopsis thaliana* Plants

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**Foliar spraying of *Arabidopsis thaliana* (Columbia ecotype) plants with a 1.0-mM salicylic acid (SA) solution significantly improved their tolerance to subsequent paraquat (PQ)-induced oxidative damage. Leaf injuries, including losses of chlorophyll, protein, and fresh weight, were reduced. Our analysis of antioxidant enzymes in the leaves showed that SA pre-treatment effectively retarded rapid decreases in the activities of superoxide dismutase (SOD), catalase, and ascorbate peroxidase that are normally associated with PQ exposure. In addition, guaiacol peroxidase activity was remarkably increased. In a native gel assay of peroxidase (POD) isozymes, staining activity of the POD1 isozyme, which disappeared in plants exposed only to 10 μM PQ, was significantly recovered by the 1.0-mM SA pre-treatment. POD2 isozyme activity was also pronounced in all SA-treated plants compared with the control. A 12-h SA pre-treatment, without subsequent PQ stress, also caused a small increase in the endogenous $H_2O_2$ content that accompanies the symptoms of mild leaf injuries. This enhanced level occurred in parallel with a slight SOD increase and a catalase decrease. From our results, it can be assumed that, due to the small increase in SOD as well as catalase inactivation via SA pre-treatment, a moderate increase in $H_2O_2$ levels may occur. In turn, a large induction of guaiacol peroxidase leads to enhanced PQ tolerance in *A. thaliana* plants.**

*Keywords*: antioxidant enzymes, *Arabidopsis thaliana*, $H_2O_2$, paraquat, salicylic acid (SA)

Salicylic acid (SA) is a natural plant phenolic. Now known as an endogenous signal molecule, it has several physiological and biochemical effects, including those related to plant disease resistance and growth regulation (Raskin, 1992; Pancheva et al., 1996; Dat et al., 1998; Janda et al., 1999). Although SA has received particular attention because of its ability to induce protection against plant pathogens (Raskin, 1992), it might also play an important role in controlling plant sensitivity to different types of abiotic stress (Dat et al., 1998; Janda et al., 1999; Rao and Davis, 1999). For example, Dat et al. (1998) have demonstrated that exogenous SA application to mustard plants significantly improves their heat-shock tolerance. Pre-treatment of maize plants with SA can increase their chilling tolerance through the induction of glutathione reductase and guaiacol peroxidase (Janda et al., 1999). In *Arabidopsis* plants, SA has been suggested as functioning in the induction of antioxidant defense responses (Sharma et al., 1996). Finally, it has also been shown that SA accumulates in the leaves of tobacco and *Arabidopsis thaliana* during oxidative stress, e.g., exposure to ozone or UV light (Yalpani et al., 1994; Sharma et al., 1996).

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All these observations have led to the proposal that SA may be a possible element linked to the induction of abiotic stress tolerance in plants. However, little information is available about a concrete mode of action. Meanwhile, some researchers have reported that, although $H_2O_2$ is toxic at high concentrations, at moderate levels it may serve as a secondary messenger in signal-transfer processes leading to protection against various oxidative stresses (Prasad et al., 1994; Foyer et al., 1997). Moreover, plants treated with SA have been shown to accumulate $H_2O_2$ (Chen et al., 1993; Fauth et al., 1996; Rao et al., 1997).

In plant systems, the herbicidal chemical paraquat (PQ) has been used as a model to study oxidative stresses (Martinez et al., 2001). PQ is a redox-active compound that acts in a chain reaction to produce active oxygen species. It transfers electrons from Photosystem I of the chloroplasts to molecular oxygen, forming a superoxide radical that, in turn, can give rise to deleterious oxygen radicals (Babbs et al., 1989). Such action makes PQ a useful tool for initiating the accumulation of oxidative stress.

The objective in this study was to investigate the physiological role of SA in protecting *A. thaliana* (Columbia ecotype) plants from PQ-induced oxidative damage. Our particular focus was on the interactions

among SA, $H_2O_2$, and antioxidant enzymes.

## MATERIALS AND METHODS

### Plant Materials and Treatments

*A. thaliana* (Columbia ecotype) seeds were sterilized in 10% NaOCl for 10 min and washed completely with sterile water. The seeds were then sown on a mixture of peat moss and perlite, and were watered with a 1/2-strength Hoagland solution. Plants were reared for 21 d in a growth chamber at 23°C, with 70% relative humidity and a 16-h photoperiod provided by 8000-lux light. Under these conditions, the plants achieved the full vegetative state and formed six rosette leaves each. For the pre-treatment, SA (Sigma Co.) was applied to the 21-d-old plants as a foliar spray (1.0 mL/plant) 12 h before beginning the paraquat (PQ) treatment. Stocks of SA had been prepared in a small volume of ethanol, then diluted to the two treatment concentrations (0.5 and 1.0 mM) in a 2-mM sodium phosphate buffer (pH 7.0). The buffer solution alone was sprayed on our control plants. For the induction of PQ stress, 10 μM PQ in a 2-mM sodium phosphate buffer (pH 7.0) was applied as a spray to plants that had been pre-treated with or without SA. After being exposed to the PQ for 24 h, the leaves were harvested for assay.

### Physiological Parameters

To evaluate growth, fresh rosette leaves were harvested at designated intervals and immediately weighed to determine their fresh mass. Chlorophyll content, in 80% acetone extractions, was measured via the technique of Arnon (1949). Leaf protein contents were determined according to the method of Lowry et al. (1951), using BSA as the standard.

### Determination of $H_2O_2$ Content

To assay for $H_2O_2$ content, six rosette leaves each from five plants were homogenized in a mortar on ice with 2 mL of 0.1 M sodium phosphate buffer (pH 6.8). The homogenate was centrifuged at 20,000*g* for 20 min and the supernatant was collected for assay. Determination of $H_2O_2$ content was performed according to the method of Bernt and Bergmeyer (1974), using a peroxidase enzyme. A 0.5-mL aliquot of the supernatant was mixed with 2.5 mL of the peroxide reagent [83 mM sodium phosphate (pH 7.0), 0.005% (w/v) *o*-dianisidine, and 40 μg peroxidase/mL], then incubated at 30°C for 10 min in a water bath. The reaction was stopped by adding 0.5 mL of 1 N perchloric acid, followed by centrifuging at 10,000*g* for 3 min. The resultant clear supernatant was read at 436 nm and its absorbance was compared to the extinction of an $H_2O_2$ standard.

### Enzyme Extraction and Assays

Catalase (CAT) and guaiacol peroxidase (GPX) were extracted from 24 rosette leaves (6 leaves each from 4 plants) in a mortar on ice with 3 mL of a cold-extraction medium containing 0.1 M K-phosphate (pH 7.5), 2 mM $Na_2$-EDTA, and 1% PVPP. The supernatant, recovered at 4°C by centrifugation (20,000*g*, 20 min), was used for the enzyme assays. Ascorbate peroxidase (APX) was extracted using 2 mL of 0.1 M K-phosphate buffer (pH 7.0) that contained 2 mM $Na_2$-EDTA, 5 mM ascorbate, and 1% PVPP. Superoxide dismutase (SOD) was extracted from the rosette leaves of eight plants, using 3 mL of 70 mM K-phosphate buffer (pH 8.0) containing 2 mM $Na_2$-EDTA and 1% PVPP.

CAT activity was determined by monitoring the decrease in absorbance at 240 nm ($E_{240}$ = 39.4 $M^{-1}$ $cm^{-1}$) due to $H_2O_2$ decomposition. The 3-mL reaction mixture contained 50 mM potassium phosphate (pH 7.0), 25 mM $H_2O_2$, and 0.2 mL enzyme extract. The enzyme reaction was then initiated by adding $H_2O_2$ (Rao et al., 1996). GPX activity was determined according to the increase in absorbance at 470 nm ($E_{470}$ = 25.2 $mM^{-1}cm^{-1}$) caused by guaiacol oxidation (Polle et al., 1994). The 3-mL reaction mixture contained 100 mM potassium phosphate (pH 6.5), 16 mM guaiacol, 10 mM $H_2O_2$, and 0.2 mL enzyme extract. The reaction was initiated by adding the enzyme extract. APX activity was measured according to the procedure described by Asada (1984), in which the rate of ascorbate oxidation was monitored at 290 nm ($E_{290}$ = 2.8 $mM^{-1}cm^{-1}$). The 2-mL reaction mixture contained 50 mM potassium phosphate (pH 7.0), 0.5 mM ascorbate, 0.25 mM $H_2O_2$, and 0.2 mL enzyme extract. A correction was done for the low nonenzymatic oxidation of ascorbate by $H_2O_2$. The assay for SOD activity was based on the photochemical method of Giannopolitis and Ries (1977), as modified by Dhindsa et al. (1981). The 3-mL reaction mixture was composed of 50 mM K-phosphate (pH 7.8), 13 mM methionine, 75 μM *p*-nitro blue tetrazolium (NBT), 2 μM riboflavin, 0.1 mM $Na_2$-EDTA, and 0 to 50 μL enzyme extract. Glass tubes containing the 3-mL reaction mixtures were illuminated with two 20-W fluorescent tubes to start the reaction. Turning off the lights terminated the process. One unit of SOD activity was defined as the amount of enzyme resulting in 50% inhibition of

the rate of NBT reduction at 560 nm.

### Peroxidase Isozyme Assay

Proteins from the leaf extracts were subjected to native PAGE using the modified discontinuous buffer system of Laemmli (1970), from which SDS was omitted. Peroxidase isozymes were separated on a 10% polyacrylamide gel, with a 4% stacking gel, at 100 V for 5 h at 4°C. The loading samples contained 125 mM Tris-HCl (pH 6.8), 5% glycerol, 0.002% BPB, and 190 µg leaf extract. The peroxidase isozymes were stained by incubating the gels for 10 min in 50 mM sodium acetate buffer (pH 4.5) containing 2 mM benzidine. The reaction was initiated by adding 3 mM $H_2O_2$, and was allowed to continue for 20 min. Afterward, the reaction was stopped by a brief wash in deionized water, followed by its photographing (Rao et al., 1996).

## RESULTS

### Effect of SA Pre-Treatment on PQ-Induced Leaf Damage Symptoms

We examined whether SA pre-treatment could protect *A. thaliana* seedlings against PQ-induced oxidative stress. After reaching the full vegetative state in a growth chamber, the plants were pre-treated with or without a foliar spray of SA (0.5 mM or 1.0 mM), 12 h before being subjected to 1 d under a 10-µM PQ-induced stress. Severe leaf-damage symptoms (necrosis and wilting) were observed in plants exposed to PQ alone, while those that had been pre-treated with SAs were less affected (Fig. 1). Those plants sprayed with 1.0 mM SA were better able to overcome PQ toxicity. In addition, the incidence of leaf injuries, e.g., losses of chlorophyll, protein, and fresh weight, were reduced in the pre-treated plants, particularly at the 1.0-mM level (Fig. 2).

### Effect of SA Pre-Treatment on Developmental Changes in Antioxidant Enzyme Contents

The effect of SA pre-treatment on changes in the levels of antioxidant enzymes after PQ stress was also investigated. Activities of SOD, catalase, guaiacol peroxidase, and ascorbate peroxidase were markedly decreased in response to 1 d of exposure to PQ alone, compared with our untreated control plants. However, the pre-application of SA effectively retarded this rapid decline in enzymatic activities that resulted from PQ stress,


**Figure 1.** Protection of 21-d-old *A. thaliana* plants from paraquat (PQ) toxicity by exogenous application of salicylic acid (SA). Plants were pre-treated for 12 h with either 0.5 mM or 1.0 mM SA in a 2-mM Na-phosphate (pH 7.0) buffer as foliar sprays (1 mL SA solution/plant) before being exposed for 1 d to 10 µM PQ. **Control,** plants treated with 2 mM Na-phosphate (pH 7.0) alone; **PQ alone,** plants treated with 10 µM PQ alone; **0.5 mM SA + PQ,** plants pre-treated with 0.5 mM SA before 10 µM PQ application; **1.0 mM SA + PQ,** plants pre-treated with 1.0 mM SA before 10 µM PQ application.

with the effect being more pronounced in the 1.0-mM treatment (Fig. 3). In addition, activity of guaiacol peroxidase in plants pre-treated with 1.0 mM SA was higher than in the controls.

Gels that had been stained for guaiacol peroxidase revealed two isozyme bands (Fig. 4). Activity of the POD1 isozyme was absent in plants exposed to PQ alone, but was significantly recovered in 1.0-mM SA-treated plants. We propose that the reappearance of this band could have been responsible for the increase in guaiacol peroxidase activity. In contrast, expression of the POD2 isozyme was more enhanced for all SA+PQ combinations as well as the PQ-alone treatment compared with the untreated control plants.

### In-Vivo $H_2O_2$ Content and Activities of Catalase and SOD after 12-h SA Treatment in the Absence of PQ Stress

Although $H_2O_2$ is reportedly toxic at high concentrations, moderate levels of this compound may play a role in the signal-transfer processes that lead to protection from various oxidative stresses in both plant

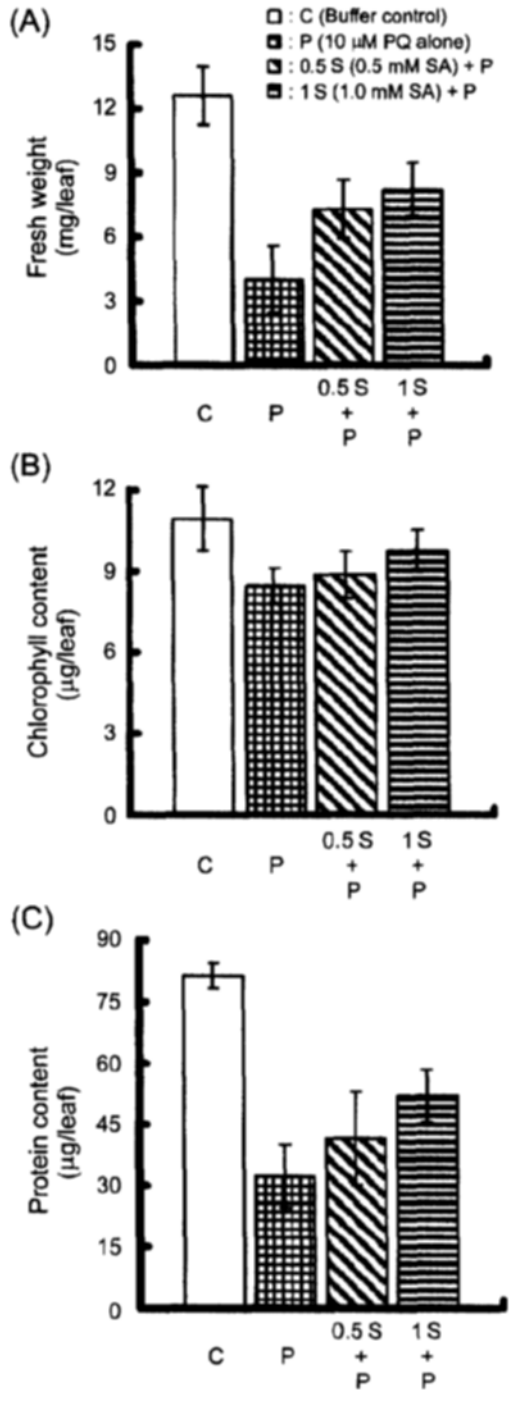


**Figure 2.** Effects of SA pre-treatment on PQ-induced leaf-damage symptoms ([A]: fresh weight, [B]: chlorophyll content, [C]: protein content). The 21-d-old *A. thaliana* plants were pre-treated with SA in a foliar spray 12 h before PQ application. Data shown represent mean ± SE of three independent experiments. Symbols are as follows: **C,** leaves treated with 2 mM Na-phosphate (pH 7.0) buffer alone; **P,** leaves treated with 10 μM PQ alone; **0.5 S + P,** leaves pre-treated with 0.5 mM SA before 10 μM PQ application; **1 S + P,** leaves pre-treated with 1.0 mM SA before 10 μM PQ application.

and animal systems (Prasad et al., 1994; Foyer et al., 1997). Plants treated with SA have previously been shown to accumulate in-vivo $H_2O_2$ (Chen et al., 1993; Rao et al., 1997). Therefore, we examined the parallel changes in $H_2O_2$ levels and catalase and SOD activities in *A. thaliana* leaves that were sprayed only with SA, but without the subsequent PQ application (Table 1). Treatment with 1 mM SA enhanced $H_2O_2$ levels by ~23% compared with the untreated control. At the same time, catalase activity was reduced by ~35%, while the $H_2O_2$-generating SOD activity showed a small increase (~13%). Incubating the plants only with 1.0 mM SA caused no severe leaf injuries, although symptoms of minor damage did appear.

## DISCUSSION

Whereas most previous research has focused on how SA contributes to plant defense responses during attack by various pathogens, its protective role and mode of action against abiotic stresses has only recently been investigated. SA has been shown to induce heat tolerance in the potato (Lopez-Delgado et al., 1998) and to decrease the inhibitory effect of salt stress in wheat (Al-Hakimi and Hamada, 2001). In *Arabidopsis* plants, SA may aid in the induction of antioxidant defenses and maintenance of the glutathione pool (Sharma et al., 1996), both possible indications of its involvement in plant protection against oxidative stress (Rao and Davis, 1999). Likewise, the addition of SA to the hydroponic solution for maize plants decreases the effects of low-temperature stress (Janda et al., 1999).

In the current study, pre-treatment with 1.0 mM SA effectively retarded the rapid, stress-associated decreases in SOD, catalase, and ascorbate peroxidase activities, while that of guaiacol peroxidase showed a pronounced increase (Fig. 3). Furthermore, in native gels of the peroxidase (POD) isozymes, staining activity for the POD1 band disappeared completely after the PQ-only treatment, but was again recovered in plants that had been pre-treated with 1.0 mM SA. This reappearance of the POD1 band leads us to think that this isozyme could be responsible for increased guaiacol peroxidase activity. Therefore, we assume that, among the antioxidant enzymes studied here, guaiacol peroxidase is the most important for producing PQ-stress tolerance in *Arabidopsis* plants.

The generation of $H_2O_2$ during abiotic stresses has been suggested as a part of the signaling reactions leading to protection from those stresses (Foyer et al., 1997). Prasad et al. (1994) have demonstrated that the

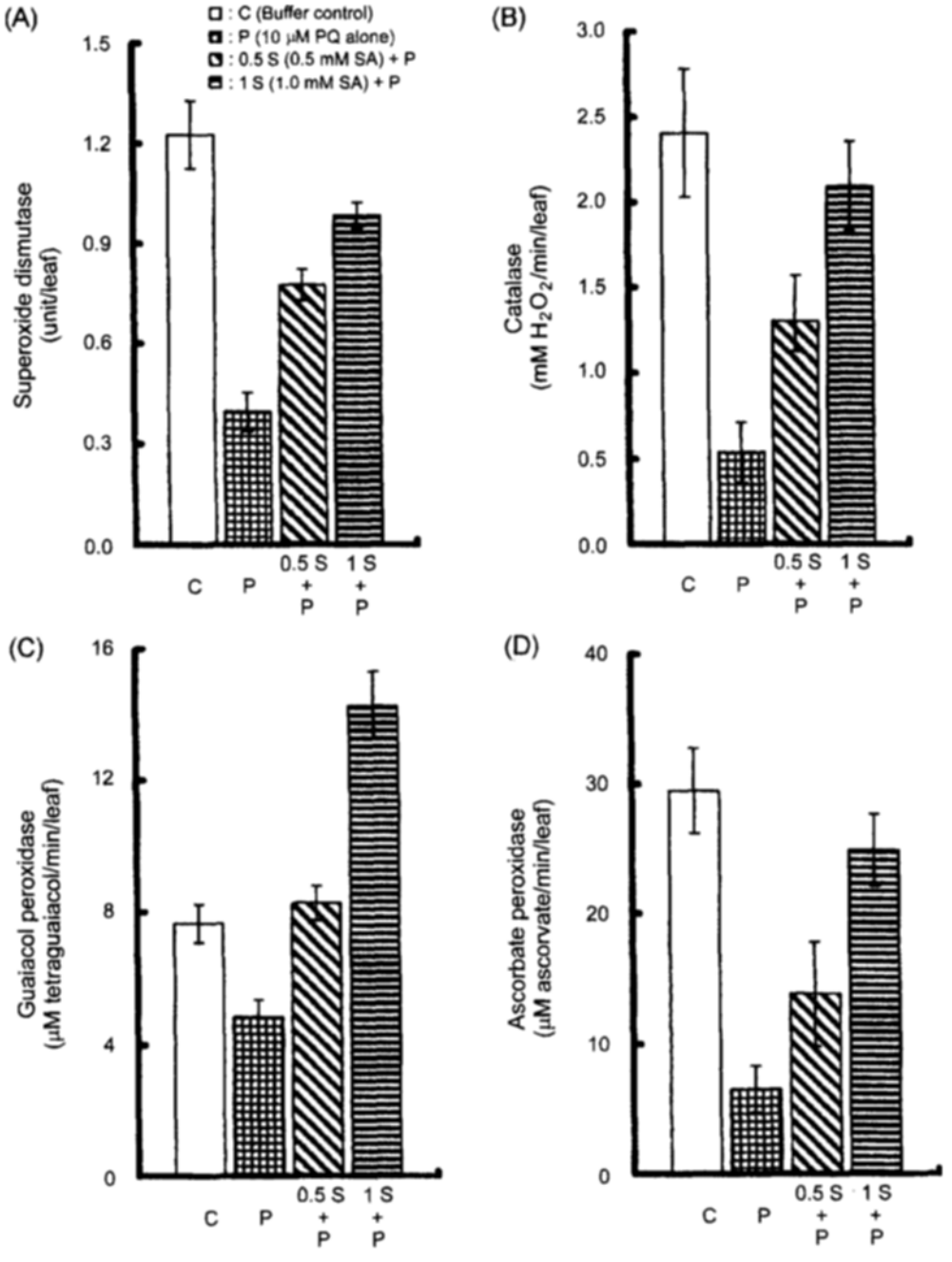


**Figure 3.** Effects of 12-h SA pre-treatment on changes in activities of superoxide dismutase (A), catalase (B), guaiacol peroxidase (C), and ascorbate peroxidase (D) in leaves of 21-d-old *A. thaliana* exposed to 10 μM PQ for 1 d. Data shown represent mean ± SE of three independent experiments. Symbols are the same as those in Figure 2.

addition of $H_2O_2$ to young maize plants, before the onset of a cold treatment, might improve their chilling tolerance, probably because of the increased activity by antioxidant enzymes. Plants treated with SA also can accumulate in-vivo $H_2O_2$ (Chen et al., 1993; Fauth et al., 1996; Rao et al., 1997). These reports lead us to speculate that SA may have acted by enhancing $H_2O_2$ levels in order to induce defense responses during PQ stress in our *Arabidopsis* plants. Hence, one of our study objectives was to explore the existence of a link among SA, $H_2O_2$ levels, and the induction of antioxidant enzymes for protection from PQ toxicity.

**Table 1.** Changes in $H_2O_2$ levels, and catalase and SOD activities in leaves of *A. thaliana* treated with SA alone for 12 h, without subsequent PQ application.

| Treatment | $H_2O_2$ (ng/leaf) | Catalase (mM $H_2O_2$/min·leaf) | SOD (unit/leaf) |
|---|---|---|---|
| Buffer control | 60.9 ± 3.8 (100) | 3.24 ± 0.12 (100) | 1.00 ± 0.01 (100) |
| 0.5 mM SA | 71.3 ± 3.7 (117) | 2.40 ± 0.02 (74) | 1.04 ± 0.04 (104) |
| 1.0 mM SA | 75.2 ± 2.1 (124) | 2.09 ± 0.09 (65) | 1.13 ± 0.05 (113) |

Mean values ± SE are shown.
Values (%) in parentheses indicate changes relative to buffer control.

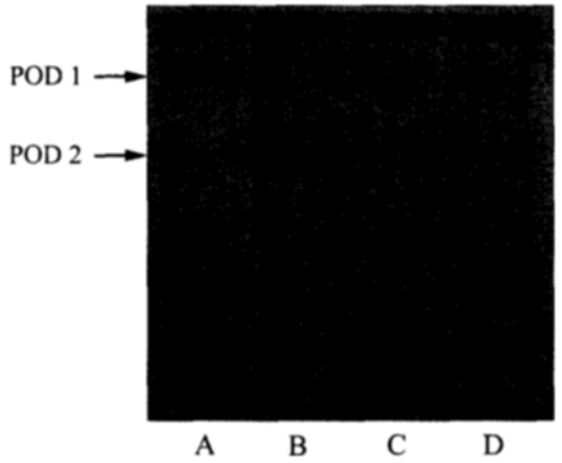


**Figure 4.** Native PAGE gels stained for peroxidase isozymes in *A. thaliana*. **Lane A,** leaves treated with 2 mM Na-phosphate (pH 7.0) buffer alone; **Lane B,** leaves treated with 10 μM PQ alone; **Lane C,** leaves pre-treated for 12 h with 0.5 mM SA before exposure to 10 μM PQ for 1 d. **Lane D,** leaves pre-treated for 12 h with 1.0 mM SA before exposure to 10 μM PQ for 1 d. Note that the "POD 1" isozyme band in Lane A completely disappeared in Lane B (10 μM PQ alone), but began to reappear in Lane C (0.5 mM SA) and showed strong recovery in its staining intensity in Lane D (1.0 mM SA). The weak "POD 2" band in Lane A also was significantly enhanced in Lanes B, C, and D.

After the 12-h SA pre-treatment, but before the application of PQ, we noted an ~35% decrease in catalase activity but a small increase (~13%) in the activity of SOD (an $H_2O_2$-generating enzyme). This resulted in an ~23% increase in the $H_2O_2$ content compared with levels measured in the control plants. These observations provide support for the proposal that SA-mediated induction of guaiacol peroxidase activity in our experiments may have involved a protective accumulation of $H_2O_2$ against PQ stress.

Based on these results, therefore, we assume that, because of the weak increase in SOD content and the inactivation of catalase by SA, the ensuing moderate increase in $H_2O_2$ levels can cause a large induction of guaiacol peroxidase activity, thereby leading to enhanced PQ tolerance in *A. thaliana* plants. Moreover, because pre-treatment with SA also effectively retarded the rapid decreases in SOD and APX brought on by PQ exposure, further investigation is required into the possible protective roles for these other antioxidant enzymes.

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## LITERATURE CITED

Al-Hakimi AMA, Hamada AM (2001) Counteraction of salinity stress on wheat plants by grain soaking in ascorbic acid, thiamin or sodium salicylate. Biol Plant 44: 253-261

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1-5

Asada K (1984) Chloroplasts: Formation of active oxygen and its scavenging. Methods Enzymol 105: 422-429

Babbs CF, Pham JA, Coolbaugh RC (1989) Lethal hydroxyl radical production in paraquat-treated plants. Plant Physiol 90: 1267-1270

Bernt E, Bergmeyer HU (1974) Inorganic peroxides, *In* HU Bergmeyer, ed, Methods of Enzymatic Analysis, Vol 4. Academic Press, pp 2246-2248

Chen Z, Silva H, Klessig DF (1993) Active oxygen species in the induction of plant systematic acquired resistance by salicylic acid. Science 262: 1883

Dat JF, Lopez-Delgado H, Foyer CH, Scott IM (1998) Parallel changes in $H_2O_2$ and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. Plant Physiol 116: 1351-1357

Dhindsa RS, Plumb-Dhindsa P, Thorpe TA (1981) Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J Exp Bot 52: 93-101

Fauth M, Merten A, Hahn MG, Jeblick W, Kauss H (1996)

Competence for elicitation of hydrogen peroxide in hypocotyls of cucumber is induced by breaching the cuticle and is enhanced by salicylic acid. Plant Physiol 110: 347-354

Foyer CH, Lopez-Delgado H, Dat JF, Scott IM (1997) Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. Physiol Plant 100: 241-254

Giannopolitis CN, Ries SK (1977) Superoxide dismutases. 1. Occurrence in higher plants. Plant Physiol 59: 309-314

Janda T, Szalai G, Páldi E (1999) Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize (*Zea mays* L.) plants. Planta 208: 175-180

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage $T_4$. Nature 227: 680-685

Lopez-Delgado H, Dat J, Foyer CH, Scott IM (1998) Induction of thermotolerance in potato microplants by acetylsalicylic acid and $H_2O_2$. J Exp Bot 49: 713-720

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193: 265-275

Martinez CA, Loureiro ME, Oliva MA, Maestri M (2001) Differential responses of superoxide dismutase in freezing resistant *Solanum curtilobum* and freezing sensitive *Solanum curtilobum* subjected to oxidative and water stress. Plant Sci 160: 505-515

Pancheva TV, Popova LP, Uzunova AN (1996) Effects of salicylic acid on growth and photosynthesis in barley plants. J Plant Physiol 149: 57-63

Polle A, Otter T, Seifert F (1994) Apoplastic peroxidases and lignification in needles of Norway spruce (*Picea abies* L.). Plant Physiol 106: 53-60

Prasad TK, Anderson MD, Martin BA, Stewart CR (1994) Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. Plant Cell 6: 65-74

Rao MV, Davis RD (1999) Ozone-induced cell death occurs via two distinct mechanisms in *Arabidopsis*: The role of salicylic acid. Plant J 17: 603-614

Rao MV, Paliyath G, Ormrod DP (1996) Ultraviolet-B radiation and ozone-induced biochemical changes in the antioxidant enzymes of *Arabidopsis thaliana*. Plant Physiol 110: 125-136

Rao MV, Paliyath G, Ormrod DP, Murr DP, Watkins CB (1997) Influence of salicylic acid on $H_2O_2$ production, oxidative stress, and $H_2O_2$-metabolizing enzymes. Plant Physiol 115: 137-149

Raskin I (1992) Role of salicylic acid in plants. Annu Rev Plant Physiol Plant Mol Biol 43: 439-463

Sharma YJ, León J, Raskin I, Davis KR (1996) Ozone-induced responses in *Arabidopsis thaliana*: The role of salicylic acid in the accumulation of defense related transcripts and induced resistance. Proc Natl Acad Sci USA 93: 5099-5104

Yalpani N, Enyedi AJ, León J, Raskin (1994) Ultraviolet light and ozone stimulate accumulation of salicylic acid, pathogenesis-related proteins and virus resistance in tobacco. Planta 193: 372-376