Plant Pathol. J. 23(4) : 300-307 (2007)

The Plant Pathology Journal

Expression of Lily Chloroplastic Cu,Zn Superoxide Dismutase Enhances Resistance to *Erwinia carotovora* in Potatoes

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(Received on October 12, 2007; Accepted on November 13, 2007)

Previously, a chloroplast-localized Cu,Zn superoxide dismutase (chCu,ZnSOD) was isolated from lily and the sense- and antisense- sequences of the lily chCu,ZnSOD were used to transform potato plants. Two selected lines, the sense- and anti-sense strand of transgenic plants, were further characterized for resistance to *Erwinia carotovora*, which is a severe pathogen affecting potato plants. Only the sense-strand transgenic potato, which contained less $O_2^{\cdot-}$ and more H_2O_2 than wild-type and antisense-strand transgenic plants, showed increased resistance to *E. carotovora*. Additional studies using $O_2^{\cdot-}$ or H_2O_2 scavengers in wild-type, sense-strand, and antisense-strand transgenic plants suggest that resistance to *E. carotovora* is induced by reduced $O_2^{\cdot-}$ and is not influenced by H_2O_2. To the best of our knowledge, this report is the first study suggesting that resistance to *E. carotovora* is enhanced by reduced $O_2^{\cdot-}$, and not by increased amounts of H_2O_2.

Keywords **:** *Erwinia*, potato, resistance, SOD, superoxide anion

Exposure of plants to unfavorable environmental conditions, such as fluctuations in temperature, water availability, air pollutants, and salt stress, can increase the production of reactive oxygen species (ROS) (Apel and Hirt, 2004), including superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\cdot}OH$), and singlet oxygen ($^1O_2$), etc. Several cellular components are susceptible to ROS damage as follows: lipids are degraded by peroxidation of unsaturated fatty acids in membranes; proteins, including enzymes are denatured; carbohydrates, especially polysaccharides are hydrolyzed; and, nucleic acids undergo "nicking", cross-linkage and scission of the DNA strands. To protect cells from ROS-induced damage, plants have developed very efficient antioxidant systems (Gutteridge and Halliwell, 2000). Among these antioxidant systems, superoxide dismutase (SOD; superoxide: superoxide oxidoreductase, EC. 1.15.1.1: SOD) is the first protective enzyme in line to detoxify ROS by converting two $O_2^{\cdot-}$ into H_2O_2 and O_2. Superoxide dismutases (SODs) provide an essential defense mechanism against superoxide anion radicals and are involved in protecting cells from oxygen toxicity. SOD is found in diverse organisms, including all oxygen-consuming organisms, aero-tolerant anaerobes, and some obligate anaerobes (Fink and Scandalios, 2002). Three different kinds of SOD, identified in plants, are distinguished by their covalently linked catalytic metal ions and by their cellular locations as follows: manganese SOD in mitochondria, iron SOD in chloroplasts, and copper-zinc SOD (Cu,ZnSOD) in chloroplasts and cytosol (Fink and Scandalios, 2002). These different SODs and their isozymes probably arose because of the need to deactivate $O_2^{\cdot-}$ produced in these locations, as $O_2^{\cdot-}$ is very reactive and cannot readily cross biological membranes (Takahashi and Asada, 1983). Recently, there is an increasing interest in physiological responses that are mediated by changes in ROS levels. The different ROS species act as signal transducers and exert different physiological effects. In response to pathogen attack, H_2O_2 has been proven to be a major signal molecule, inducing increased tolerance (Apel and Hirt, 2004; Wu et al., 1997). Auxin-induced growth inhibition is accompanied by a reduction in the $O_2^{\cdot-}$ production and $^{\cdot}OH$ formation that are essential for normal root growth. Theoretically, the concentration of ROS can be regulated by adding certain chemicals, including H_2O_2, or by altering antioxidant activity (Apel and Hirt, 2004). The cellular balance between SODs and other H_2O_2-scavenging enzymes is crucial in determining steady-state levels of $O_2^{\cdot-}$ and H_2O_2 (Mittler et al., 2004). As Mittler et al. (2004) suggested, transformation of potato plants with sense- and antisense-sequences of a lily chloroplast-localized Cu,ZnSOD (chCu,ZnSOD) (Park et al., 2006) resulted in different $O_2^{\cdot-}$ and H_2O_2 contents in the transgenic plants compared with the wild-type (WT) plants (Kim et al., 2007). In a sub-

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sequent study by our group, two representative lines were selected as the SS4 line (sense-transgenic potato) and the SA1 line (antisense-transgenic potato) and further characterized. The SS4 line exhibited increased expression of chCu,ZnSOD and greater resistance to oxidative stress than the non-transgenic control plants. In contrast, the SA1 line was more susceptible to oxidative stress than the WT plants (Park et al., 2006). The roles of ROS as signal transducers have been extensively studied because plants appear to control various physiological processes by regulating the amount of ROS. These processes include pathogen defense (Alvarez et al., 1998; Levine et al., 1994), tolerance to abiotic stresses (Prasad et al., 1994), root development (Joo et al., 2001), senescence (Zimmermann and Zentgraf, 2005), and stomatal behavior (McAinsh et al., 1996). Especially in response to pathogen attack, ROS are produced via enhanced activity of the following enzymes: plasma membrane-bound NADPH-oxidase, cell wall-bound peroxidase, and amine oxidases in apoplasts during the early infection stage (Apel and Hirt, 2004). It has been shown that H_2O_2 released during the oxidative burst in response to pathogen attack orchestrates a localized hypersensitivity response, in which H_2O_2 acts as a second messenger, mediating systemic expression of vari- ous defense-related genes (Levine et al., 1994; Orozco-Cardenas et al., 2001). Transgenic potato plants expressing high levels of H_2O_2 were more resistant to pathogen attack (Wu et al., 1997).

A key step in the induction of plant resistance is timely recognition of the invading pathogen. Plants have the ability to recognize pathogen-derived molecules, also known as, exogenous elicitors (Van der Biezen and Jones, 1998; Montesano et al., 2003). ROS increase tolerance to biotic and abiotic stresses; however, the role of $O_2^{\cdot-}$ levels in this process has not been proven. Previously, the transgenic lines developed by our research group were used to determine the effects of different compositions of $O_2^{\cdot-}$ and H_2O_2 on the resistance of potato leaves to *Erwinia carotovora*. *E. carotovora* causes soft rot in numerous fleshy fruits, vegetables, and ornamentals by creating an osmotically fragile cell (Agrios, 1997). The soil-dwelling microbe invades potatoes and other crops either in the field or in storage and causes affected tissue to become soft and watery and then turn slimy and foul-smelling. It produces extracellular pectic enzymes that destroy pectin and, to a lesser extent, also produces an extracellular cellulase that degrades cellulose. The present study investigated how the regulation of $O_2^{\cdot-}$ and H_2O_2 levels in potato plants affected the response to the potato pathogen, *E. carotovora*. To the best of our knowledge, this study is the first to reveal that the concentration of $O_2^{\cdot-}$ in plants affects *E. carotovora* resistance.

Materials and Methods

Plant material and growth conditions. Transgenic lines of *Solanum tuberosum* L. cv. Desiree were made using the sense- and the anti-sense sequences of lily chCu,ZnSOD as previously described (Park et al., 2006). Briefly, the sense- and anti-sense lily chCu,ZnSOD cDNA in the PMBP Ti binary vectors were transformed into *Agrobacterium tumefaciens,* strain LBA4404, and introduced into the leaf disc of the cv. Desiree plants using the leaf disc transformation method (Choi et al., 1999). The transgenic potato lines were cultured in a growth room maintained at 25±2°C with a 16 h photoperiod, providing approximately 60 μmol m^{-2} s^{-1} photon flux density from a fluorescent lamp (Osram Korea, Seoul, Korea).

Pathogen strains and plant treatments of various chemicals modifying ROS *Erwinia carotovora* subsp. carotovora strain SCC1 (Rantakari et al., 2001) was propagated in YEP medium at 28°C. The plants were infected with 10 μL of diluted cell solution of *E. carotovora* subsp. carotovora SCC1 culture (~10^6 to 10^7 cfu/plant) on the middle of leaf area after making a small wound with a toothpick. Infected plants were incubated at 200 to 250 μmol m^{-2} s^{-1} photon flux density at ~80% humidity in a growth room with a 16-h light period. Growth of *E. carotovora* in leaves 0, 24, 48, and 72 h after inoculation was determined by checking colony-forming units (CFU) of ten individual plants in four independent experiments. The leaf tissue was homogenized to extract the bacteria and diluted to plate for counting CFU of ten individual plants each time point in four independent experiments (Kim and Jeun, 2007). WT, SS4, and SA1 plants were treated with various chemicals to evaluate the involvement of $O_2^{\cdot-}$ in the mechanism of resistance to *E.carotovora*. Potato leaves were incubated for 24 h with 100 μM $CuCl_2$, 10 μM diphenylene iodonium (DPI), and 10 μM paraquat, and then inoculated *E. carotovora* for 72 h. Potato leaves were incubated for 24 h with 1 mM H_2O_2, 1 mM KI, 5 mM SA, and 500 μM Me-JA and then inoculated with *E. carotovora* and incubated for 72 h (Chung et al., 2007). Percentage of infected leaves was calculated by counting completely macerated leaves after 3 d from ten independent experiments with 9 to 13 individual leaves.

Detection of superoxide anion and hydrogen peroxide in leaves and tubers. The amount of superoxide anion in leaves was quantified using the nitroblue tetrazolium (NBT) staining method (Jabs et al., 1996) with minor modifications. The amounts of superoxide anion in wild-type and transgenic potato leaves were visualized by incubating the leaves in 10 mM citrate buffer at pH 6

containing 50 mM NBT for 1 hr. The stained leaves were immersed in 90% methanol at 70°C until the chlorophylls in the leaves were completely removed. Hydrogen peroxide on tubers was determined by a chemiluminescence assay (Miura et al., 1995) with minor modifications. Approximately 10 μl of a 2.5 mM of luminol solution in 10 mM Tris-HCl buffer, pH 7.4 with or without hyphal wall components (HWC elicitor) was uniformly applied to a 1-cm^2 surface on the tuber cut. HWC elicitor from mycelia of *Phytophthora infestants* was prepared according to the method described previously (Doke and Tomiyama, 1980). The emitted chemiluminescence was detected on X-ray film in the dark at 20°C. The images were analyzed using a densitomeric analyzing software program (GeneGenius, USA).

Northern blot analysis. Total RNAs were isolated from the samples using the phenol/SDS method (Ausubel et al., 1987). Twenty five μg of total RNA was separated by electrophoresis on a 1% agarose gel containing 2.2 M formaldehyde and then was transferred to a nylon membrane. DNA probes were amplified using PCR from the PR-2 and -3 genes of pepper cDNAs selected from the EST database (http://plant.pdrc.re.kr/ks200201/pepper.html). The membranes were hybridized with probes labeled with digoxigenin (DIG) using the PCR DIG Probe Synthesis Kit (Roche Molecular Biochemicals, Mannheim, Germany). Hybridization was done using DIG Easy Hyb buffer for 16 h at 42°C. The membranes were washed twice in 2×SSC, 0.1% (w/v) SDS at room temperature for 5 min each, and then were washed twice in 0.1× SSC, 0.1% SDS at 65°C for 5 min each. Target genes were detected by chemiluminescence as described in the manufacturer's instructions (Roche Molecular Biochemicals, Germany).

Results

Expression of lily chCu,ZnSOD enhanced resistance to *E. carotovora* in potato plants. The resistances of WT, SS4, and SA1 leaves were compared by inoculating the plants with different concentrations of *E. carotovora*. Only SS4 plants showed increased resistance to *E. carotovora* (Fig. 1). When the WT, SS4, and SA1 plants were inoculated with 10^6 of *E. carotovora* for 3 days, the SS4 had significant reductions in leaf macerated area (13.3%) than the WT (53.3%) and SA1 (46.7%) plants (Fig 1A and B). The SS4 plants also had significantly smaller macerated areas than the WT and the SA1 plants when they were

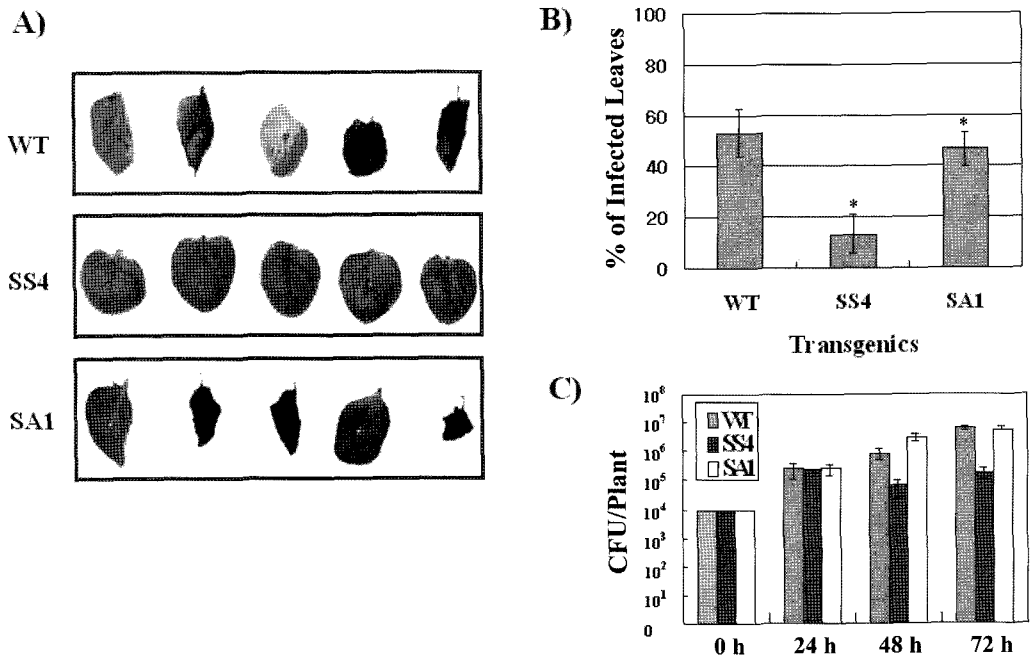

Fig. 1. Plants overexpressing lily chCu,ZnSOD were resistant to *E. carotovora* infection in normal light. (A) The leaves of wild-type (WT), SS4, and SA1 plants were inoculated with 10 μL of bacterial suspension (1×10^6 μL^{-1}) of *E. carotovora* in normal light (200 to 250 μmol m^{-2} s^{-1} photons) and photographed 72 h later. (B) Results for resistance were obtained from four independent experiments. Percentage±SD of leaves completely macerated after 72 h was calculated from four independent experiments with 10 individual leaves. (C) Growth of *E. carotovora* in leaves 0, 24, 48, and 72 h after inoculation. Colony-forming units of ten individual plants were determined from each time point in four independent experiments. An asterisk indicate a significant difference in the mean values between WT and transgenic plants at $P<0.001$, respectively, as determined by the Anova's *t*-test.

inoculated with 10^7 of *E. carotovora* for 3 days (data not shown). CFU of *E. carotovora* were counted 24, 48, and 72 h after inoculating the leaves of the potato plants with *E. carotovora* (Fig. 1C). SS4 plants had significantly lower CFU compared with WT and SA1 plants 48 and 72 h after inoculation. Interestingly, SS4 plants had fewer CFU at 48 and 72 h after inoculation than they did 24 h after inoculation (Fig. 1C). SA1 had significantly higher numbers of CFU than WT 36 h after inoculation; however, there was no difference in the number of CFU between SA1 and WT 72 h after inoculation.

Inhibition of spread of *E. carotovora* by pharmacological experiments. The WT, SS4, and SA1 plants were treated with various chemicals to evaluate the involvement of O_2^- and/or H_2O_2 in the mechanism of resistance to *E. carotovora*. $CuCl_2$ is a superoxide anion scavenger (Liszkay et al., 2004) and DPI is a NAD(P)H oxidase inhibitor (Cross and Jones, 1986). Treatment with these two chemicals should have reduced the amount of O_2^- in the leaves. When $CuCl_2$ and DPI were applied to the WT, SS4, and SA1 plants, there were significant decreases in the percentage of macerated leaves (Fig. 2). Treatment with 100 mM $CuCl_2$ decreased percent macerated leaves on the WT (57% → 15%), SS4 (14.2% → 0%), and SA1 plants (41.3% → 14.6%). DPI treatment also reduced the percent macerated leaves on the WT (57% → 8%), SS4 (14.2 → 6%), and SA1 plants (41.3 → 14%). In contrast to the effect of $CuCl_2$ and DPI, paraquat induces O_2^- production in chloroplasts (Gupta et al., 1993). Paraquat treatment had no effect on macerated leaf area in SS4 (14.2% → 21.4%) and SA1 plants (41.3% → 42%), but significantly decreased macerated leaf area in the WT plants (57% → 21.4%). The effect of H_2O_2 on resistance to *E. carotovora* was evaluated by treatment with H_2O_2 and KI, a H_2O_2 scavenger. Hydrogen peroxide treatment increased the amount of H_2O_2 and KI treatment decreased the amount of hydrogen peroxide in potato plants. The treatments, however, did not affect resistance to *E. carotovora* in the WT, SS4, and SA1 plants (Fig. 3). When the WT and transgenic lines were treated with Me-JA and SA, only SA induced increased resistance in the WT, SS4, and SA1 plants. Compared with SA, MeJA did not induce any tolerance in the WT, SS4, and SA1 plants (Fig. 3).

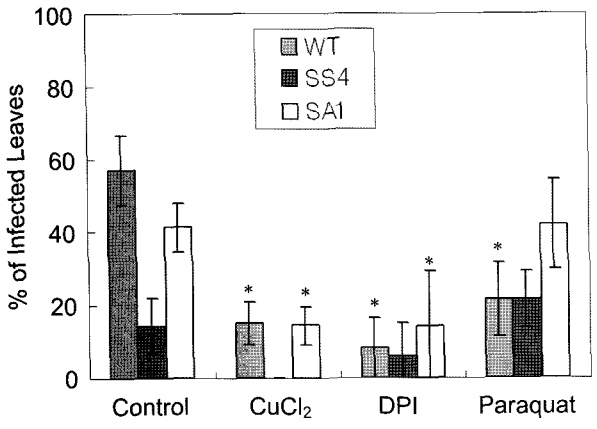

Fig. 2. Effects of superoxide anion concentration on resistance to *E. carotovora* infection in normal light. Wild type (WT), SS4, and SA1 plants were treated with various chemicals to evaluate the involvement of O_2^- in the mechanism of resistance to *E. carotovora*. $CuCl_2$ is a superoxide anion scavenger and diphenylene iodonium-HCl (DPI) is a NAD(P)H oxidase inhibitor. Potato leaves were incubated for 24 h with $CuCl_2$, DPI, and paraquat, and then inoculated *E. carotovora* for 72 h. Percentage±SD of leaves completely macerated after 3 d was calculated from ten independent experiments with 9 to 13 individual leaves. An asterisk indicate a significant difference in the mean values between WT and transgenic plants at $P<0.001$, respectively, as determined by the Anova's *t*-test.

Changes in superoxide anion and H_2O_2 levels with treatment of various chemicals modifying ROS. To verify that the superoxide anion scavengers and generators were functioning properly to control the level of superoxide anion, the amount of superoxide anion in leaves was detected using the NBT-staining method. Treatment with DPI and $CuCl_2$ resulted in dramatic reductions in superoxide anion in WT and SA1 plants (Fig. 4). Treatment with paraquat increased levels of superoxide anion detected in the SA1 and SS4, but not in the WT, plants. Treatment with H_2O_2 and KI, an H_2O_2 scavenger, did not change superoxide anion in SS4 and SA1 plants, while a decrease was detected

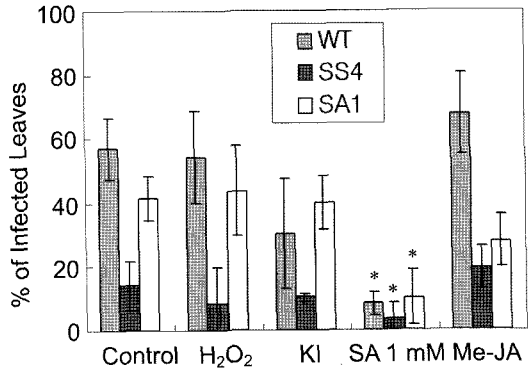

Fig. 3. Effects of H_2O_2 concentration on resistance to *E. carotovora* infection in normal light. Wild type (WT), SS4, and SA1 plants were inoculated with *E. carotovara* in normal light (200 to 250 μmol m^{-2} s^{-1} photons). Potato leaves were incubated for 24 h with H_2O_2, KI, SA, and Me-JA, and then inoculated with *E. carotovora* and incubated for 72 h. Percentage±SD of leaves completely macerated after 3 d was calculated from ten independent experiments with 9 to 13 individual leaves. An asterisk indicate a significant difference in the mean values between WT and transgenic plants at $P<0.001$, respectively, as determined by the Anova's *t*-test.

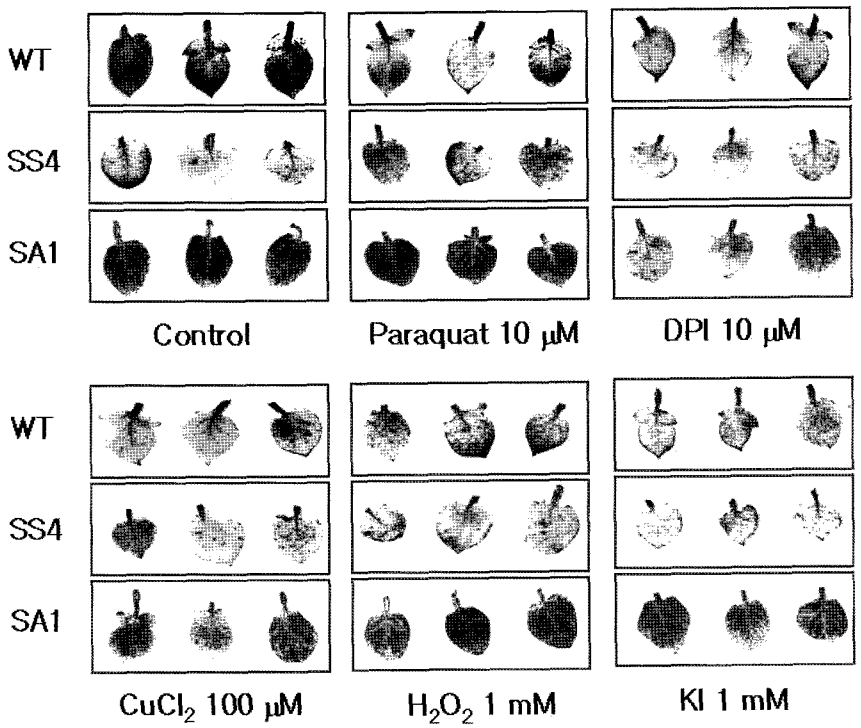

Fig. 4. Accumulation of superoxide anions in the leaves of wild type (WT), SS4, and SA1 plants and in response to treatment with various ROS-generators or -scavengers. Potato leaves were incubated for 24 h with 100 μM of $CuCl_2$, 10 μM of DPI, 10 μM of paraquat, 1 mM of H_2O_2, and 1 mM of KI; superoxide was quantified with NBT.

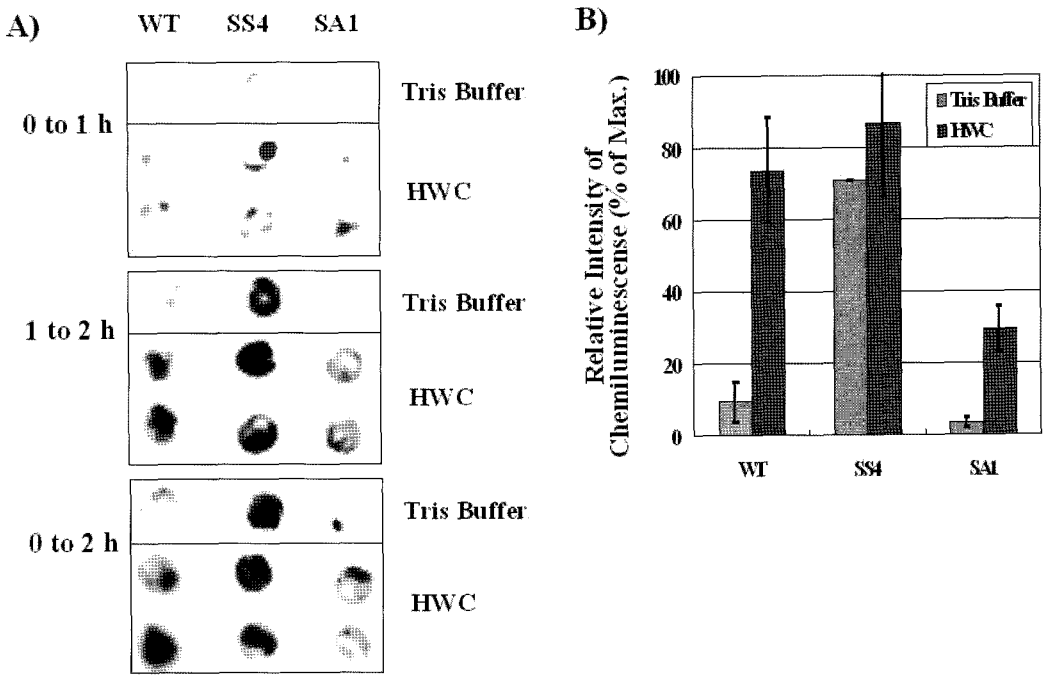

Fig. 5. An autophotogram of potato tuber discs treated with an HWC-elicitor with luminol. (A) Imaging analysis as a function of chemiluminescence of a local oxidative burst on the surface of potato tuber discs for detection of H_2O_2. X-ray film was exposed to discs at different time intervals, such as from 0 to 1h, to 2 h, and from 1 h to 2 h, after exposure to 5 mM Tris-HCl buffer (pH 7.4) containing luminol (100 μM/ml) with and without an HWC elicitor (1 mg/ml). (B) Graph showing the relative chemiluminescence intensities for the WT, SS4, and SA1 plants (n=4). The bars indicate mean±SD.

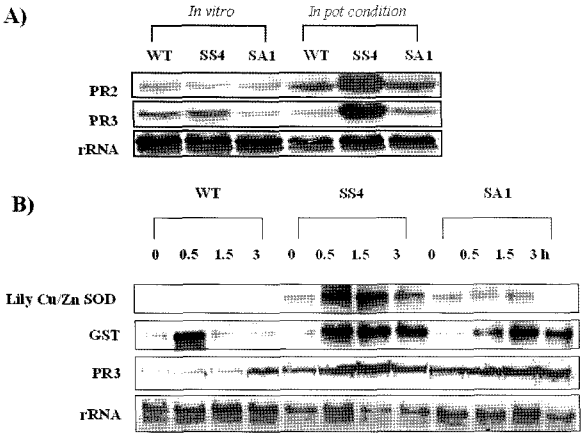

Fig. 6. Expression of the lily chCu,ZnSOD, GST, and PR genes in transgenic potatoes under *E. carotovora* infection. RNA isolated from leaves of the wild type (WT) and transgenic potatoes (SA1, SS4) grown *in vitro* and in-pot and was analyzed by Northern blotting. DNA probes were amplified using PCR from the PR-2 and –3 genes of pepper cDNAs selected from the EST database. The separated total RNA on the nylon membrane was hybridized to the (A) PR-2 and PR-3 and B) Lily Cu/Zn SOD, GST, PR-3 probes.

in WT plants (Fig. 4).

To investigate the changes in specific ROS during pathogen infection, H_2O_2 was detected on tubers using a chemiluminescence assay in response to treatment with a fungal elicitor (HWC). In our previous paper, high amounts of superoxide anion were detected in SA1 plants by ESR spectroscopy after treatment with elicitors (Kim et al., 2007). Without elicitor treatment, the relative chemiluminescence intensity was highest in SS4 plants, while the intensities of WT and SA1 plants remained unchanged at low levels. After elicitor treatment, large amounts of H_2O_2 were generated in WT and SS4, but not in SA1 plants (Fig. 5). These results indicate that high amounts of H_2O_2 were generated during pathogen infection not only in SS4, but also in WT, plants.

Differential expression of pathogen-related (PR) genes. To determine if the elevated levels of H_2O_2 in SS4 plants could induce PR genes, we tried to detect PR genes in leaves grown *in vitro* and under in-pot conditions. Expression of *PR-2* and *PR-3* genes was not induced in *in vitro* cultured WT or transgenic plants, even though the level of H_2O_2 in SS4 was already elevated. However, in pot-cultivated plants, expression of *PR-2* and *PR-3* genes was elevated in SS4 plants (Fig. 6A). Expression of different genes, lily Cu,ZnSOD, PR-3, and glutathione S-transferase (GST), was determined in transgenic plants after inoculation with *E. carotovora*. Overexpression of the introduced lily Cu,ZnSOD gene was detected in SS4 plants after inoculation with pathogens. Induced expression of GST genes was detected in all plants; the induction level and duration were highest in SS4 plants. SS4 plants exhibited the highest expression of the PR-3 gene compared with WT and SA1 plants.

Discussion

Previously, we transformed potato plants with the sense- and the antisense-sequence of lily chCu,ZnSOD (Park et al., 2006). The concentration of $O_2^{\cdot-}$ and H_2O_2 in cells is determined by the balance between SODs and other H_2O_2-scavenging enzymes (Apel and Hirt, 2004; Mittler et al., 2004). Because the level of chCu,ZnSOD expression was altered in the transgenic plants compared with that of WT, it was predicted that they also would have different concentrations of $O_2^{\cdot-}$ and H_2O_2. The amounts of $O_2^{\cdot-}$ and H_2O_2 measured by NBT and DAB staining, in the transgenic plants were altered relative to those in the WT plants. SA1 plants (transgenics with antisense chCu,ZnSOD) had 21.7% and 100% more $O_2^{\cdot-}$ concentrations than WT and SS4 plants (transgenics with sense chCu,ZnSOD), respectively. SS4 plants had the highest concentrations of H_2O_2 and the lowest concentrations of $O_2^{\cdot-}$ compared with WT and SA1 plants (Kim et al., 2007). The transgenic plants were subjected to additional characterization because ROS composition and specific ROS species can change the response to many external stimuli, including exposure to pathogens (Apel and Hirt, 2004). SS4, which contained lower amounts of $O_2^{\cdot-}$ and higher amounts of H_2O_2 than WT and SA1, showed increased resistance to *E. carotovora* (Fig. 1). Because it could not be determined whether decreased $O_2^{\cdot-}$ or increased H_2O_2 in SS4 was responsible for the increased resistance to *E. carotovora*, we used various treatments for increasing $O_2^{\cdot-}$ and H_2O_2, or for decreasing $O_2^{\cdot-}$ and H_2O_2, respectively. Treatments with $O_2^{\cdot-}$ scavenging chemicals, which decreased the amounts of $O_2^{\cdot-}$ in the WT, SS4, and SA1 plants, significantly increased resistance to *E. carotovora*. However, treatments with H_2O_2 did not induce an additional increase in resistance to *E. carotovora*. These data strongly suggest that resistance to *E. carotovora* is induced by reduced amounts of $O_2^{\cdot-}$, and is not influenced by the amounts of H_2O_2. Moreover, high amounts of H_2O_2 were generated during pathogen infection not only in SS4, but also in WT plants (Fig. 5). These data also suggest that, in WT plants, high amounts of H_2O_2 could not induce resistance to *E. carotovora* in potato. There are many reports indicating increased pathogen resistance due to increased levels of H_2O_2 (Lamb and Dixon, 1997; Levine et al., 1994; Orozco-Cardenas et al., 2001; Wu et al., 1997). In the early stages of infection, oxidative bursts, which are produced via enhanced activity of plasma membrane-bound NADPH-oxidase, cell wall-bound peroxidase, and apoplastic amine

oxidases, mediate systemic expression of various defense-related genes, thereby delivering the signal of a perceived pathogen attack. Chloroplasts are believed to be the major ROS production site that affects the balance between defense pathways in plants (Kariola et al., 2005). Accumulation of ROS has been implicated as a signal for activation of plant defense responses and has been shown to modulate SA- and JA-dependent defenses (Lamb and Dixon, 1997). There are increasing evidences that high amounts of ROS induce tolerance to pathogens; however, except for H_2O_2, the roles of specific ROS species in regulating defense pathways remain to be identified. Our data indicate that tolerance to *E. carotovora* is induced by a reduction in $O_2^{\cdot-}$, resulting from either high levels of chCu,ZnSOD expression or treatment with $O_2^{\cdot-}$ scavengers. Without increased ROS production, the SAR response remains deactivated and defense is directed toward the JA-dependent pathway (Kariola et al., 2005). However, even in the absence of elevated ROS, SS4 or treatment of $O_2^{\cdot-}$ scavengers, such as DPI or $CuCl_2$, had increased resistance to *E. carotovora*. Therefore, the results of the present study imply that low amounts of $O_2^{\cdot-}$ might turn on a defense pathway, although, at present, it is not clear if the pathway is the SA-dependent SAR pathway or not.

These data clearly indicate that different ROS species may have unique physiological roles, demonstrating that low amounts of $O_2^{\cdot-}$ improve resistance to *E. carotovora* in potato plants. This study is the first report that regulation of pathogen resistance is determined by reduced $O_2^{\cdot-}$ levels, and not by increased amounts of H_2O_2. It is of interest to determine if there is an unknown pathway, activated by reduced amounts of $O_2^{\cdot-}$, which increases pathogen resistance.

Acknowledgements

This research was supported by grants from the KRIBB Research Initiative Program and the Plant Diversity Research Center of 21st Century Frontier Research Program funded by Ministry of Science and Technology of Korean government.

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