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Activity of enzymatic antioxidant defense systems in chilled and heat shocked cucumber seedling radicles

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Chilling whole cucumber seedlings that had 10-mm long radicles for 4 days at 2.5°C significantly inhibited subsequent radicle growth both by increasing the time it took the seedlings to recover from chilling and attain a linear rate of radicle growth, and by decreasing the subsequent rate of linear growth. Exposing cucumber seedlings to 45°C for up to 20 min had no effect on subsequent radicle growth, while longer exposures produced reductions in growth. A heat shock at 45°C for 10 min induced the optimal protection to 4 days of chilling at 2.5°C by reducing chilling inhibition from 60 to 42%. Two hours after being chilled, heat shocked or heat shocked and then chilled, there was no difference in protein content of the apical 1 cm of the seedling radicle among these treatments and the non-heat shocked, non-chilled control. Two days after treatment, the protein content was still similar in tissue that had been heat shocked or heat shocked and chilled, while it was significantly reduced in tissue that had been chilled. In general, 2 h after treatment, the activity of the 5 antioxidant enzymes examined in this study [superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC 1.11.1.11), guaiacol peroxidase (GPX; EC 1.11.1.7) and glutathione reductase (GR; EC 1.6.4.2)] were reduced by chilling and unaffected or increased by heat shock. When heat shock was followed by chilling, there was a consistent effect of the heat shock treatment on preventing the loss of enzyme activity following chilling. This protective effect of the heat shock treatment was even more pronounced after 2 days of recovery at 25°C for SOD, CAT and APX. In contrast, the activity of GR and GPX was substantially higher in chilled tissue than in tissue that had been heat shocked before being chilled. Elevated levels of GR and GPX therefore appear to be correlated with the development of chilling injury, while elevated levels of SOD, CAT and APX appear to be correlated with the development of heat shock-induced chilling tolerance.

Introduction

Plants have evolved antioxidant pathways that are usually sufficient to protect them from oxidative damage during periods of normal growth and moderate stress (Asada and Takahashi 1987, Hauptmann and Cadenas 1997). When severely stressed, however, the production of reactive oxygen species (ROS) can exceed the capacity of the antioxidant system to neutralize them and oxidative damage can occur. Both enzymatic and non-enzymatic systems protect tissue from activated oxygen species generated as the result of external environmental stresses, such as chilling, drought and air pollution. Members of the enzymatic antioxidant defense system include superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC 1.11.1.11), phenolic peroxidases, such as guaiacol peroxidase (GPX; EC 1.11.1.7), and the ascorbate/glutathione cycle that includes glutathione reductase (GR; EC 1.6.4.2). The superoxide radicle (O_2^-) is dismutated to H_2O_2 by SOD, and CAT, APX and GPX metabolize H_2O_2 to H_2O. APX requires reduced ascorbate and GPX requires a phenolic compound like guaiacol to function. GR functions in the regeneration of reduced ascorbate after it is converted to monodehydroascorbate by APX.

Chilling can lead to an increased concentration of toxic oxygen compounds in susceptible plant tissue (Wise and Naylor 1987, Hodgson and Raison 1991). There appears to be a relationship between antioxidant enzyme activity and

Abbreviations – APX, ascorbate peroxidase; CAT, catalase; GPX, guaiacol peroxidase; GR, glutathione reductase; ROS, reactive oxygen species; SOD, superoxide dismutase.

chilling tolerance. In chilling-tolerant cucumber cultivars, the activity of SOD and APX was higher than in chilling-sensitive cultivars (Shen et al. 1999). The activities of CAT, APX and GR were higher in chilling-tolerant mandarin fruits stored at low temperature than in susceptible fruit (Sala 1998). The chilling tolerance of rice cultivars was also closely linked to the cold stability of CAT and APX (Saruyama and Tanida 1995). Chemical treatments that increase chilling tolerance also increase antioxidant enzyme activity. Pinhero et al. (1997) suggested that increased activities of SOD and APX in leaves and roots of paclobutrazol-treated chilling-susceptible maize cultivars contribute to their enhanced chilling tolerance. The pretreatment of maize plants with salicylic acid at normal growth temperature also induces both antioxidant enzymes and increase chilling tolerance (Janda et al. 1999).

Pre-conditioning and acclimation at near chilling temperatures increase the chilling tolerance of many crops (Saltveit and Morris 1990). For example, Wang (1995a,b, 1996) suggested that the protective effect of pre-conditioning in zucchini squash resulted from an increase in the activity of antioxidant enzymes, such as SOD, CAT, GR and APX. Chilling tolerance can be induced in cucumber seedlings by prior exposure to such abiotic shocks as chilling, ethanol, salts and heat shock (Jennings and Saltveit 1994, Mangrich and Saltveit 2000). The mechanism by which heat shock induces chilling tolerance is not fully understood. However, there is increasing evidence that heat shock produces an oxidative stress that induces genes and promotes the synthesis of enzymes involved in oxidative stress defense (Morgen et al. 1986).

In the present work, we investigated the effect of a heat shock treatment that confers chilling tolerance to cucumber seedlings on the activity of antioxidant enzymes. Activity of the enzymes APX, CAT, GR, GPX and SOD was measured in extracts from cucumber seedlings subjected to injurious chilling treatments, protective heat shock treatment and combinations of these treatments.

Materials and methods

Plant material

Cucumber (*Cucumis sativus* L., cv. Poinsett) seeds were obtained from a local vendor. Five grams of seeds was imbibed in 1 l of aerated water overnight at 20°C. Imbibed seeds were transferred to moist paper toweling overlying capillary cloth that was sandwiched between two 15×30 cm Plexiglas plates (6 mm thick) that were held together with rubber bands. The seeds were individually positioned in 3 parallel horizontal rows about 4 cm apart so the radicle would emerge downward. The plates were held in a vertical position at 25°C in a humid, dark, ethylene-free atmosphere for about 36 h or until the radicles were about 10 mm long.

Germinating seeds with 10 ± 1-mm long radicles were removed from the large Plexiglas sandwich and gently transferred to moist paper toweling overlying capillary cloth and sandwiched between 7×13 cm Plexiglas plates (3 mm thick) as before. The seeds were individually positioned in a horizontal row about 1 cm below the top. Each smaller plate held 7–10 seedlings and was treated as a unit of replication. The plates were positioned vertically in a $20 \times 26 \times 14$ cm tall white translucent plastic tub and loosely covered with aluminum foil. The trays were either held at 25°C for the initial measurements of radicle growth or chilled at 2.5°C in the dark before being moved to 25°C for the growth measurements. All manipulations were preformed under dim, florescent room lighting.

Application of heat shock treatments

Each small plate of cucumber seedlings with 10 ± 1 mm long radicles was placed in a plastic bag and immersed in water at 25 or 45°C for 0–120 min. The 25°C treatment was the non-heat shock control. The bags were left open at the top and the open weave of the capillary cloth allowed adequate ventilation of the plates so that internal carbon dioxide levels did not exceed 0.1% (data not shown). The bagged plates were then held for 15 min in 20°C water before being removed from the bags and placed in plastic tubs lined with wet capillary cloth.

Measurement of chilling injury

The extent of chilling injury of the cucumber seedlings was measured as the subsequent linear growth of the radicle after chilling (Rab and Saltveit 1996) by a method modified from that previously described (Jennings and Saltveit 1994). Radicle length was measured with a transparent ruler to the nearest mm before and after treatment, after chilling and periodically during growth at 25°C. In some experiments, the small plates were disassembled and the radicles were gently straightened before measurement. The growth measurements for each seedling were regressed over time and the slope and correlation coefficient calculated. Only growth rates with R^2 values greater than 0.99 were used in the analysis.

Preparation of enzyme extract

Radicle tips (1 cm in length) were excised from the seedlings before any treatment (i.e., control) or after 2 h or 2 days of recovery at 25°C after the radicles had been chilled for 4 days at 2.5°C (i.e., chilled), had been heat shocked at 45°C for 10 min (i.e., heat shocked) or had been heat shocked at 45°C for 10 min before being chilled at 2.5°C for 4 days (i.e., heat shocked and chilled). The 0.5 g fresh weight (ca. 150 radicle tips) was homogenized at 4°C in 1 ml of extraction buffer [50 m*M* potassium phosphate buffer (pH 7.0), 1% Triton X-100 and 7 m*M* 2-mercaptoethanol] with mortar and pestle. The homogenate was then centrifuged at 25000 *g* for 20 min and the supernatant was used as the crude extract for the APX, GPX and GR assay. For all other enzyme measurements, 0.5 g of radicles were extracted as described above with 4.5 ml of 0.05 *M* Tris-HCl buffer (pH 7.5) 3 m*M* $MgCl_2$ and 1 m*M* EDTA.

Enzyme assay

SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of NBT using the method of Dhindsa et al. (1981). The 3 ml reaction mixture contained 50 m*M* phosphate buffer (pH 7.8), 13 m*M* methionine, 75 μ*M* NBT, 2 μ*M* riboflavin, 0.1 mM EDTA and 50 μl of enzyme extract. Riboflavin was added last and the tubes were shaken and placed 30 cm below a light bank consisting of two 15 W fluorescent lamps for 10 min. The absorbance by the reaction mixture was read at 560 nm.

CAT activity was assayed by measuring the rate of disappearance of H_2O_2 using the method of Maehly and Chance (1959). The reaction mixture contained 2.5 ml of 50 m*M* phosphate buffer (pH 7.4), 0.1 ml of 1% H_2O_2 and 50 μl of enzyme extract diluted to keep measurements within the linear range of the analysis. The decrease in H_2O_2 was followed as a decline in absorbance at 240 nm.

APX activity was determined according to the method of Chen and Asada (1989) with minor modification. The 1-ml reaction mixture was composed of 50 m*M* phosphate buffer (pH 7.0) containing 0.1 m*M* EDTA, 0.5 m*M* ascorbate, 1.54 m*M* H_2O_2 and 50 μl of enzyme extract. The oxidation of ascorbate was followed by the decrease in the absorbance at 240 nm.

GPX activity was determined according to Upadhyaya et al. (1985). The reaction mixture contained 2.5 ml of 50 m*M* phosphate buffer (pH 6.1), 1 ml of 1% H_2O_2, 1 ml of 1% guaiacol and 20 μl of enzyme extract. The increase in absorbance at 420 nm was followed for 1 min.

GR activity was assayed by measuring the decrease in absorbance at 334 nm due to the oxidation of NADPH (Klapheck et al. 1990). The 1-ml reaction mixture contained 0.1 *M* Tris-HCl (pH 8.0), 1 m*M* EDTA, 0.1 m*M* NADPH, 1 m*M* GSSG and 50 μl of enzyme extract at 30°C.

Protein content was determined using bovine serum albumin as a standard, according to the method of Bradford (1976). All enzyme activity was calculated per milligram of protein per minute and expressed as a percentage of the control.

Statistical analysis

All experiments were repeated at least twice with similar results. Data were subjected to an analysis of variance and means and standard errors calculated.

Results

Growth of non-chilled cucumber seedling radicles and chilled radicles that had recovered from chilling-induced inhibition of growth were both linear for the duration of the experiment (Fig. 1). As the length of chilling at 2.5°C increased from 1 to 6 days, the days to recover linear growth increased gradually from 0.02 ± 0.01 for the non-chilled control to 0.96 ± 0.02 days for seedlings chilled for 4 days, and then rapidly increased to 2.6 ± 0.3 and 3.0 ± 0.4 days following 5 and 6 days of chilling, respectively (Fig. 2A). The rate of radicle growth remained relatively constant for the first 3 days at 0.98 ± 0.08 mm h^{-1} before declining to 0.82 ± 0.07 and 0.78 ± 0.09 mm h^{-1}following 4 and 6 days of chilling (Fig. 2B). The combined reduction in growth caused by the reduced rate of radicle elongation and the delay in recovery of linear growth gave rise to the observed increased severity in chilling injury between 4 and 5 days of chilling (Fig. 1). All subsequent chilling durations were confined to 4 days at 2.5°C.

Heat shock treatments at 45°C for up to 20 min did not significantly reduce subsequent radicle growth, but exposures for 30–120 min resulted in a linear reduction in the rate of subsequent radicle elongation (Fig. 3). Seedlings were heat shocked at 45°C for 5–30 min (Fig. 4). Subsequent radicle growth for 2, 3, 4 or 5 days at 25°C after a

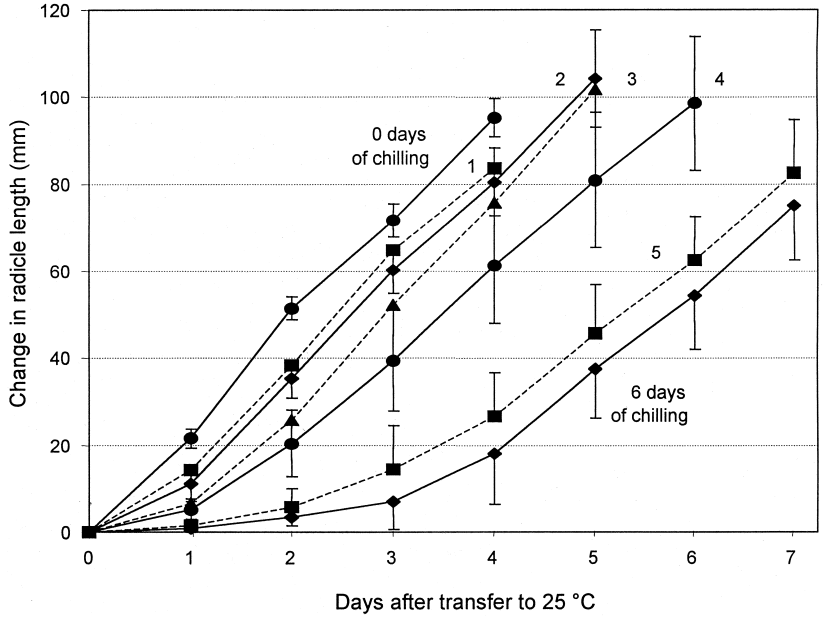

Fig. 1. Radicle elongation of whole cucumber seedlings at 25°C following chilling at 2.5°C for 0–6 days. Values are the mean ± SE (n = 10).

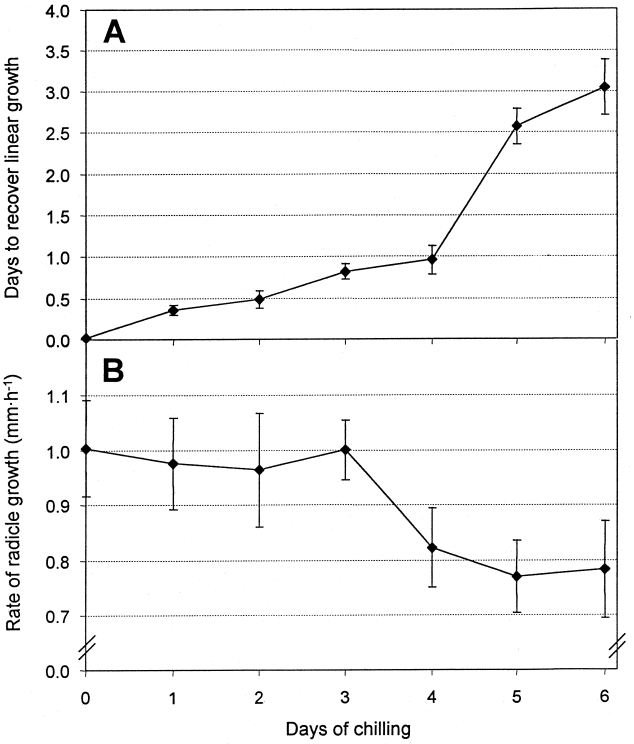

Fig. 2. The time at 25°C needed for radicles of whole cucumber seedlings to recovery from chilling at 2.5°C for 0–6 days and resume linear growth (A) and the rate of the linear portion of radicle growth following chilling and recovery (B). Values are the mean ± SE (n = 10).

0–30 min heat shock and 4 days of chilling at 2.5°C was fitted by a series of quadratic equations that had R^2 values around 0.94 ± 0.05. The duration of heat shock that produced the maximum rate of subsequent radicle growth (i.e., the maximum induced chilling tolerance) was determined by solving for the first derivative of each quadratic equation. The greatest growth occurred in those radicles previously heat shocked for 10.3 ± 0.2 min before chilling. Chilling for 0 or 4 days at 2.5°C reduced subsequent radicle growth by 60% from 1.07 ± 0.04 to 0.44 ± 0.10 mm h^{-1}, respectively. In contrast, seedlings heat shocked for 10 min before chilling had radicles that grew at 0.62 ± 0.04 mm h^{-1}. Chilling reduced the growth of non-heat shocked radicles by 60% and the growth of heat shocked radicles by 42% of the non-chilled control seedlings. The mode of action by which this 30% reduction in chilling-induced inhibition of radicle growth was caused by the optimum heat shock treatment was further studied by measuring how these treatments affected the activity of antioxidant enzymes.

The protein content of the 1-cm radicle tips remained relatively constant at 6.5 ± 0.4 mg g^{-1} fresh weight for all treatments except for a 43% decline to 3.7 ± 0.5 mg g^{-1} fresh weight for the following 2 days of recovery from chilling (Fig. 5). The protein content was not reduced by heat shock or during chilling, but it was reduced during the 2 days following chilling. Tissue that had been heat shocked and then chilled did not exhibit this reduction in protein content.

In general, the activity of the 5 enzymes (SOD, CAT, APX, GR and GPX) were lower than the control 2 h after chilling, but the activity of the same enzymes were the same or higher than the control 2 h after heat shock (Figs 6 and 7). Chilling had only a slight effect on the activity of SOD (89 ± 9% of the control) and CAT (84 ± 2%), while it had more of an effect on GPX (67 ± 17%), APX (65 ± 3%) and GR (58 ± 19%) activity. In contrast, heat shock had a slight effect on SOD (99 ± 4% of the control) and GPX (97 ± 7%), while it had more of an effect on CAT (115 ± 9%), APX

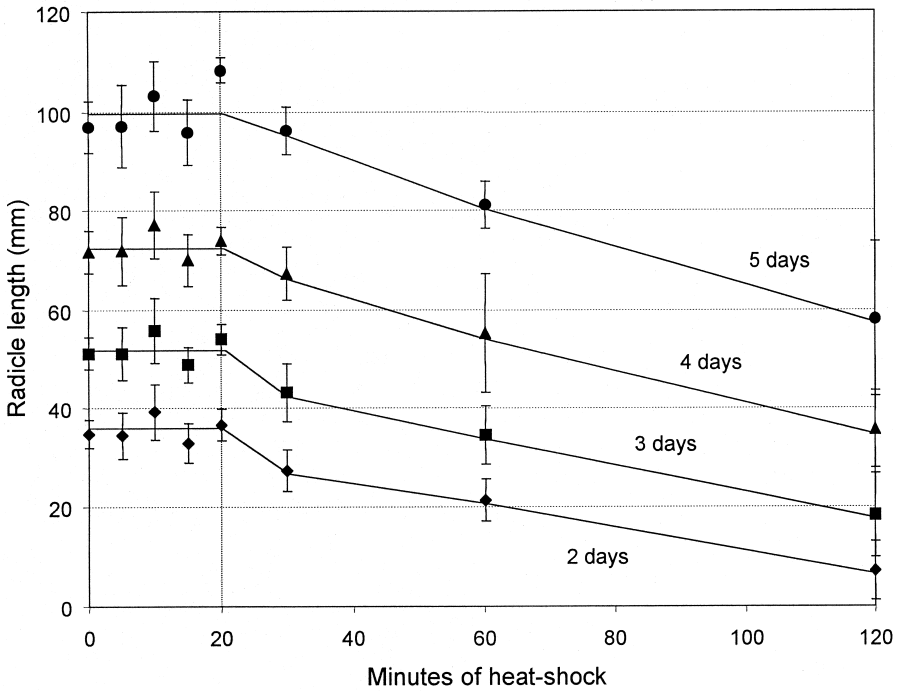

Fig. 3. Radicle length of whole cucumber seedlings measured after 2, 3, 4 and 5 days at 25°C following 0–120 min of heat shock at 45°C. Values are the mean ± SE (n = 10).

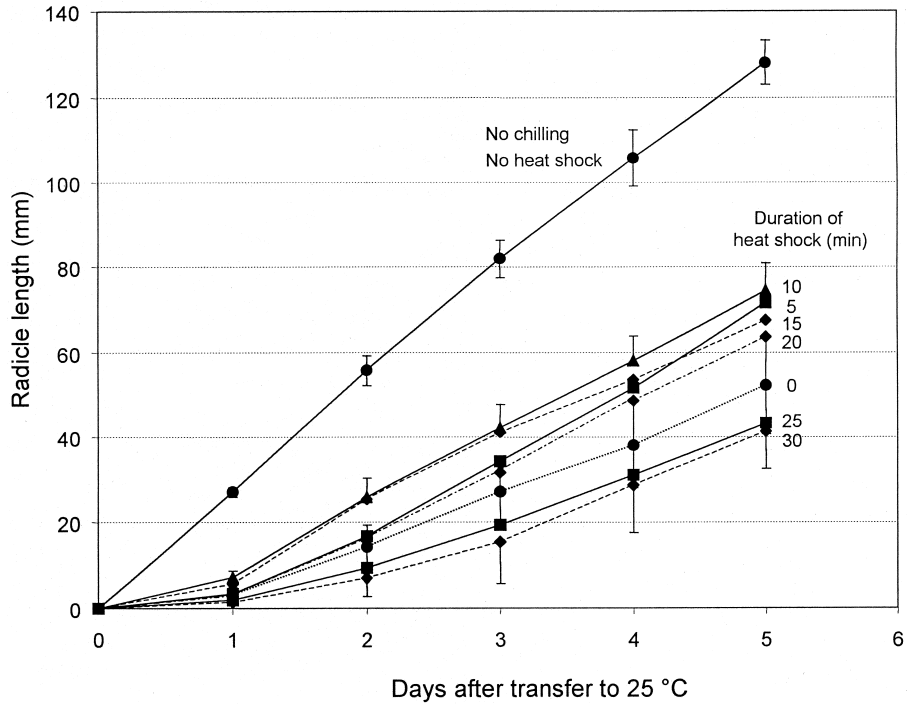

Fig. 4. Radicle length of whole cucumber seedlings transferred to 25°C following 0–30 min of heat shock at 45°C and 4 days of chilling at 2.5°C. Values are the mean ± SE (n = 10).

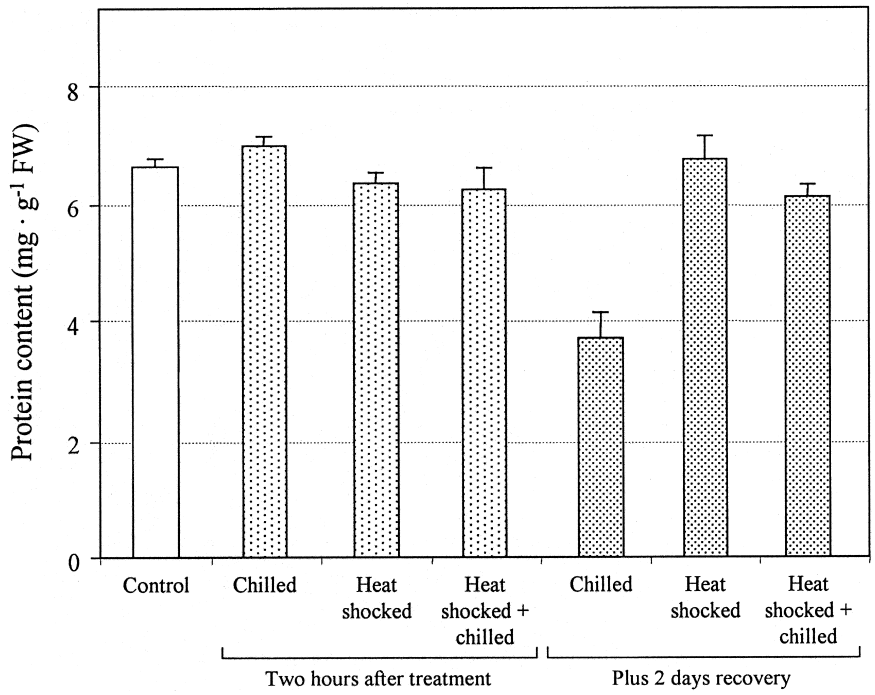

Fig. 5. Protein content of the apical 1 cm excised from radicles of whole cucumber seedlings subjected to combinations of heat shock and chilling treatments. Control seedlings were not chilled or heat shocked, chilled seedlings were held at 2.5°C for 4 days, while heat shocked seedlings were held at 45°C for 10 min and heat shocked and chilled seedlings were first heat shocked and then chilled. The seedlings were assayed 2 h or 2 days after application of the treatments. Values are the mean ± SE (n = 4).

(139 ± 6%) and GR (166 ± 4%). In all cases, a heat shock before chilling reduced the loss of activity during chilling. The protective effects of the heat shocked ranged from a slight 3% increase in the activity of SOD (92 ± 5% of the control) over the chilled tissue to more substantial increases of 29, 32, 37 and 96% for CAT (108 ± 5%), APX (86 ± 3%), GPX (92 ± 4%) and GR (111 ± 10%), respectively.

The pattern of enzyme activity changed when the seedlings were held for 2 days of recovery at 25°C after the treatments (Figs 6 and 7). Chilling decreased the activity of CAT (72 ± 4%) and APX (66 ± 14%), slightly increased the activity of SOD (138 ± 6%), while it substantially increased the activity of GR (890 ± 21%) and GPX (1550 ± 120%). A heat shock had a less dramatic effect on enzyme activities. It slightly reduced the activity of SOD (to 84 ± 9% of control) and CAT (96 ± 2%), while it increased the activity of GR (106 ± 9%), APX (113 ± 11%) and GPX (116 ± 12%).

When preceded with a heat shock, the activities of all 5 enzymes in chilled tissue were substantially greater than the control after 2 days of recovery at 25°C. Compared to the non-heat shocked, non-chilled control seedlings, tissue that had recovered from being heat shocked and chilled had activities of GR, SOD, APX, CAT and GPX that were 147 ± 7, 159 ± 8, 189 ± 5, 213 ± 14 and $1430 \pm 130\%$ of the controls, respectively. This combined treatment resulted in enzyme activity for SOD, CAT and APX after recovery that was substantially higher than found in tissue receiving just chilling or heat shock separately. While heat shocked and chilled seedlings still had levels of GR and GPX activity higher than those found in control tissue and in tissue that had recovered from the heat shock, the levels were the same or lower than that found in recovered chilled tissue.

Discussion

Many stresses, such as chilling and heat shock, induce the synthesis and accumulation of ROS that have been associated with injury development. Exposure to sub-lethal levels of some stresses induces both higher levels of antioxidant enzyme activity and stress tolerance. In this paper, we show that a correlation exists between the ability of a heat shock treatment to induce chilling tolerance and elevated levels of certain antioxidant enzymes.

SOD plays a key role in cellular defense against ROS. Expression of a cytosolic SOD gene was induced in tobacco seedlings by heat shock and chilling (Hérouart et al. 1994). A 42°C heat shock for 2 h also increased SOD activity in rye leaves (Kurganova et al. 1997). The induction of an antioxidant protective system in chloroplast could be a primary response of plant cell to heat shock. In contrast, SOD levels in our cucumber radicles were relatively similar 2 h after the treatments, and 2 days after the chilling or heat shock treatments only showed a slight increase (30%) or decrease (16%), respectively. However, the combined treatment of heat shock (that protected seedlings from chilling injury) and chilling resulted in a 60% increase in SOD activity after 2 days of recovery. The 30% increase in SOD following chilling was insufficient to confer chilling tolerance. However, another possibility is that the limiting reaction conferring chilling tolerance may not be SOD activity, but some subsequent detoxifying step. The product of SOD activity is

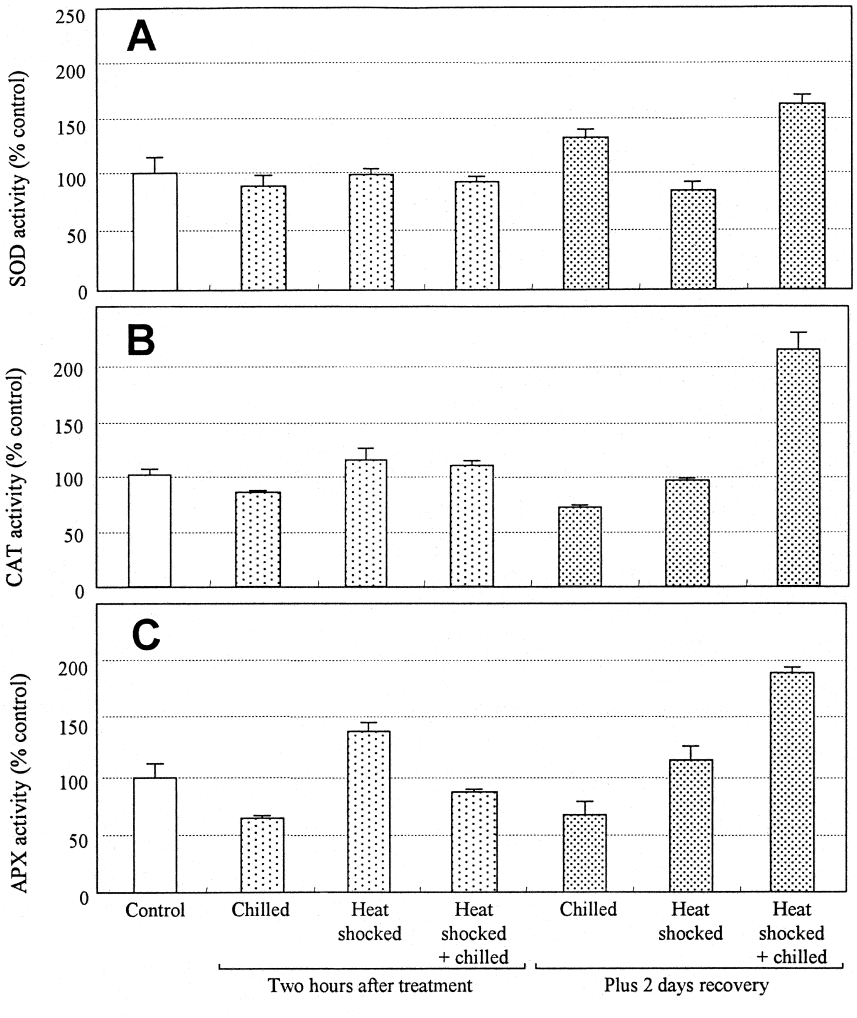

Fig. 6. Activity of (A) SOD, (B) CAT and (C) APX in aqueous extracts of the apical 1 cm of radicles from whole cucumber seedlings that were subjected to combinations of heat and chill treatments. Control seedlings were not chilled or heat shocked, chilled seedlings were held at 2.5°C for 4 days, while heat shocked seedlings were held at 45°C for 10 min and heat shocked and chilled seedlings were first heat shocked and then chilled. The seedlings were assayed 2 h or 2 days after application of the treatments. Values are the mean $\pm$ SE ($n = 4$).

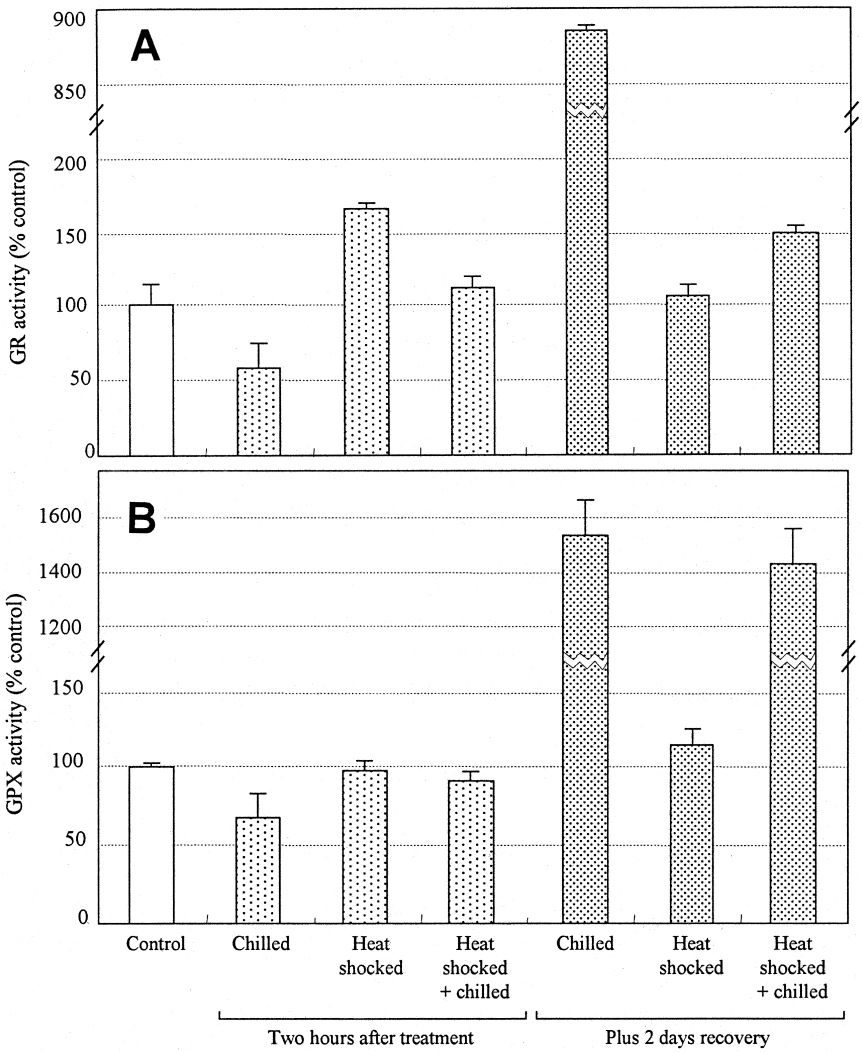

Fig. 7. Activity of (A) GR and (B) GPX in aqueous extracts of the apical 1 cm of radicles from whole cucumber seedlings that were subjected to combinations of heat and chill treatments. Control seedlings were not chilled or heat shocked, chilled seedlings were held at 2.5°C for 4 days, while heat shocked seedlings were held at 45°C for 10 min and heat shocked and chilled seedlings were first heat shocked and then chilled. The seedlings were assayed 2 h or 2 days after application of the treatments. Values are the mean ± SE (n = 4).

H_2O_2, which is still toxic and must be eliminated by conversion to H_2O by subsequent reactions.

H_2O_2 is a product of many cellular reactions, e.g., photorespiration and respiration, as well as the activity of SOD. Detoxification of H_2O_2 is enhanced under the same stressful conditions that enhance the production of ROS. We studied 3 enzymes (APX, CAT and GPX) that convert H_2O_2 to H_2O. CAT activity increased in *Quercus suber* L. seedling after a heat shock at 45°C for 1 h (Faria et al. 1999). However, CAT activity seldom decreased under severe conditions, which resulted in injury, such as at 48°C for 1.5 h (Zhu et al. 1996) or 55°C for 1.5 h (Dat et al. 1998). Levels of APX mRNA in leaves or cell suspensions of Arabidopsis increased after treatment with high temperature (Storozhenko et al. 1998). High temperatures also increased peroxidase activity in the flavedo of mandarin fruit (Martinez-Tellez and Lafuente 1997), reduced peroxidase activity and lignin levels in tomato seedling roots (Zacheo et al. 1995) and decreased peroxidase activity in iceberg lettuces (Loaiza-Velarde et al. 1997). Peroxidase's genes were highly expressed upon chilling (4°C) suspension cultures of sweet potato, but expression was reduced by prior acclimation at 1°C reduced chilling injury (Kim et al. 1999).

Elevated activity of certain antioxidant enzymes is associated with chilling tolerance. The activity of CAT and APX appear to control tolerance against oxidative stress in rice seedlings (Saruyama and Tanida 1995, Hodges et al. 1997). Treatments with paclobutrazol (Pinhero et al. 1997) or salicylic acid (Janda et al. 1999) that reduced chilling injury also enhanced antioxidant enzyme activity. The activity of SOD and APX was higher in chilling-tolerant cucumber cultivars than in chilling-sensitive cultivars (Shen et al. 1999). CAT, APX and GR activities were higher in tolerant mandarin fruits after low temperature storage (Sala 1998). The tolerance of rice cultivars to chilling injury was closely linked to the cold stability of CAT and APX (Saruyama and Tanida 1995).

Activity of both APX and CAT was substantially increased by the same heat shock and chilling treatment that resulted in induced chilling tolerance. Heat shock increased their activity 2 h after treatment, but their activity had decreased to near control levels when measured after 2 days of recovery. In contrast, when chilling followed the heat shock, levels of APX and CAT were 89 and 113% higher, respectively, than the controls. GPX activity was also very high following the protective treatments, but its activity was

not substantially different from the level induced by the injurious chilling treatment itself.

GR activity was also substantially increased by the injurious chilling treatment. The combined protective treatment slightly increased GR activity, but its likely contribution to chilling tolerance was negated by the much greater rise in GR activity cause by chilling alone. Kaminaka et al. (1998) suggested that expression of the rice cytosolic GR gene was regulated via an ABA-mediated signal transduction pathway during environmental stresses, such as chilling, drought and salinity. Transcript levels of GOR2 (a second GR cDNA) increased in the recovery (post-stress) phases of both drought and chilling by about 10- and 3-fold, respectively (Stevens et al. 1997).

A hypothesis has been proposed and elaborated by Saltveit (1996, 2001) that the induced synthesis of heat shock proteins monopolizes the cell's protein synthetic capacity thus greatly reducing the cell's ability to synthesize proteins that are induced by subsequent stresses. While this thesis is supported by data showing that the wound-induced synthesis of phenylalanine ammonia lyase in lettuce is almost eliminated by a prior or subsequent heat shock that induces the production of copious amounts of hsps (Loaiza-Velarde et al. 1997), the increased activity of the antioxidant enzymes studied here suggests that de novo protein synthesis is not greatly diminished in heat shocked and/or chilled cucumber seedling radicles. Rather, it appears that the selective synthesis of certain antioxidant enzymes may be partially responsible for the ability of heat shock to increase chilling tolerance.

The contribution of newly synthesized antioxidant enzymes to chilling tolerance is also consistent with the report that heat shock treatments are also effective in reducing chilling injury when administered after chilling (Saltveit 2000). It has been suggested that plants suffer from post-chilling oxidative stress when they are transferred from chilling to ambient conditions (Saruyama and Tanida 1995). Shen et al. (1999) suggested that higher antioxidant enzyme activity in chilling-tolerant cultivars than in sensitive cultivars during the re-warming period was important in defending against post-chilling oxidative stress. Hariyarda and Parkin (1991) found that lipid fluorescent pigments accumulate in both the phospholipid and glycolipid fractions of thylakoid lipids extracted from re-warmed cucumber seedlings after only 1 or 2 days of chilling at 4°C, indicating that peroxidation of thylakoid lipids was induced during the re-warming period and not during the period of chilling. The heat shock-induced synthesis of antioxidant enzymes, either before or after chilling, would therefore confer the same level of protection if damage occurred not during chilling, but during recovery after chilling. In contrast, heat shock-induced synthesis of hsps would only be protective if applied before chilling, since the accepted role of hsps is to protect molecules from thermal denaturation and assist their re-folding and activation after stress (Vierling 1991).

The increased synthesis and accumulation of ROS may be a contributing factor in the development of chilling injury. The ability of heat shock treatments to enhance or preserve antioxidant enzyme activity in cucumber seedlings may contribute to the ability of this treatment to increased chilling tolerance. Elevated levels of GR and GPX therefore appear to be correlated with chilling injury since they were highest in injured tissue. In contrast, elevated levels of SOD, CAT and APX appear to be correlated with heat shock-induced tolerance to chilling since activity of these enzymes were low in injured tissue but high in tissue that had been induced by a heat shock to be chilling-tolerant. Interestingly, heat shock by itself did not induce protective levels of these enzymes, which only appeared when the heat shock and chilling treatment was followed by 2 days of recovery at 25°C.

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