Oxidative Stress and Diurnal Variation in Chilling Sensitivity of Tomato Seedlings

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ABSTRACT. Diurnal variation in the chilling sensitivity of 'Rutgers' tomato (*Lycopersicon esculentum* Mill.) seedlings was examined. Chilling sensitivity was highest in seedlings chilled at the end of the dark period, and these seedlings became more resistant to chilling injury on exposure to the light. The development of chilling tolerance in tomato seedlings was a response to light and not under the control of a circadian rhythm. The recovery of leaf gas exchange following chilling was faster in seedlings chilled at the end of the light period. Diurnal variation in chilling sensitivity was associated with changes in catalase and superoxide dismutase activities. An increase in catalase and superoxide dismutase activities was observed at the end of the light period. Catalase activity was significantly higher in all stages of chilling following the light period compared to those chilled after the end of the dark period. Forty-eight hours of 14 °C acclimation or pretreatment with hydrogen peroxide conferred increased chilling tolerance to tomato seedlings. Hydrogen peroxide-treated seedlings showed little evidence of a diurnal variation in chilling sensitivity. These results support a role for light and oxidative stress in conferring increased chilling tolerance to tomato seedlings.

Many plant species are damaged by exposure to low, nonfreezing temperatures (Lyons 1973). The resulting chilling injury limits crop yield and product marketability (Wang 1982). Injury symptoms are varied and reflect the myriad of metabolic processes documented to be adversely affected by low-temperature exposure (Graham and Patterson 1982; Levitt 1980; Lyons 1973). The induction of oxidative stress by low temperature has been proposed as a major contributing factor to chilling injury (Anderson et al., 1994; Prasad et al., 1994a, 1994b; Wise and Naylor 1987). Plants produce superoxide radicals, hydroxyl radicals, and H₂O₂ as normal by-products of aerobic metabolism (Scandalios 1990). These compounds are capable of damaging plant cells through their reactivity with membrane lipids, protein, and DNA. Under normal metabolic conditions, amounts of these reactive oxygen species remain low due to the action of defense enzymes such as superoxide dismutase, catalase, and peroxidases (Rabinowitch and Fridovich, 1983). In chilling-sensitive plants such as cucumber (*Cucumis sativus* L.), exposure to low temperatures in the light leads to the peroxidation of membrane lipids and the depletion of antioxidant compounds such as α -tocopherol, both indications of increased levels of reactive oxygen species (Wise and Naylor, 1987). An increase in the level of H₂O₂ during low-temperature exposure of maize (*Zea mays* L.) seedlings provided direct evidence for an increase in reactive oxygen species (Prasad et al., 1994a).

In many cases, plants can acclimate to low-temperature stress such that damage occurs at a lower temperature than in nonacclimated tissue. For example, increased chilling tolerance often occurs after a period of exposure to low, nondamaging temperatures. Acclimation to cold has been reported to involve the synthesis of proteins, membrane lipids, and metabolites that confer chilling tolerance (Graham and Patterson, 1982). The mechanism

of acclimation is largely unknown but has been postulated to involve maintenance of membrane fluidity by increasing lipid unsaturation, sterol content, and enzymatic protective systems (Levitt, 1980). More recently, a linkage between cold acclimation and resistance to oxidative stress has been postulated (Prasad et al., 1994a). Cold acclimation of maize seedlings at 14 °C for 3 d led to an increase in catalase (*Cat3*) transcripts and catalase activity (Prasad et al., 1994a, 1994b). Enhanced chilling tolerance was also associated with treatments that increased the level of antioxidants. The plant growth regulator paclobutrazol has been reported to increase antioxidant levels in tomato (*Lycopersicon esculentum* Mill.) plants, which was correlated with a reduction in chilling-induced degradation of membrane lipids (Senaratna et al., 1988). Increased activities of oxidative stress defense enzymes following treatment with the growth regulator uniconazole was associated with enhanced chilling tolerance in cucumber seedlings (Upadhyaya, 1989). Taken together, these results suggest that, during acclimation to low temperatures, moderate increases in oxidative stress increase oxidative stress defense mechanisms and the tolerance of plants to low temperatures.

Further support for a role for moderate oxidative stress in the development of chilling tolerance comes from recent studies in maize seedlings (Anderson et al., 1994; Prasad et al., 1994a, 1994b). Pretreatment of maize seedlings with H₂O₂ or other compounds that generate superoxide radicals induces chilling tolerance. These treatments led to an increase in the expression of *Cat3* mRNA and catalase enzyme activity. Thus, oxidative stress plays a dual role in low-temperature responses, as a source of injury and as a signal to increase antioxidant defenses.

In addition to developing tolerance to chilling injury following exposure to low temperatures or oxidative stress, many plants exhibit changes in the degree of chilling tolerance during a diurnal light cycle. Patterson et al. (1979) showed that 50% of tomato seedlings chilled at the end of the dark cycle were damaged within 3 d compared to 6 d for seedlings chilled at the end of the light cycle. Several chilling-sensitive species were found to be less tolerant of chilling conditions when exposed at the end of the dark cycle compared to the end of a light cycle (King et al., 1982). Diurnal changes in chilling sensitivity were not due to altered root function during chilling treatment nor to variations in dehydration during the chilling exposure resulting from diurnal differences in

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Fig. 1. Survival of tomato seedlings after the 96 h of dark chilling. Chilling was started at the time indicated. Light and shaded bars indicate light and dark periods, respectively. Each point is a mean of three, 72-seedling replications. Vertical bars represent $\pm$ SE.

1982). However, it was not clear by what means diurnal variation affected the chilling sensitivity of plants. The objective of the present study was to determine the role of oxidative stress and changes in antioxidant enzymes in the diurnal variation in chilling sensitivity in tomato seedlings.

Materials and Methods

PLANT MATERIAL. 'Rutgers' tomato seedlings were germinated under mist. The seedlings were transplanted into flats and grown in a greenhouse under natural light for 1 week. The seedlings were transferred to a growth chamber which provided a photosynthetic photon flux (PPF) of $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from a mixture of cool-white fluorescent and incandescent bulbs in 10-h photoperiods. Plants were illuminated from 0800 to 1800 HR, and day/night temperatures were set at $24/22^\circ\text{C}$, respectively. Seedlings were grown under these conditions for at least 1 week before the initiation of treatments.

DIURNAL VARIATION AND CHILLING TREATMENT. Three-week-old tomato seedlings were transferred from the growth chamber to the chilling treatment at the following time points, 0830, 1300, 1800, 2130, 0100, 0730 HR. Seedlings were chilled in a dark, 4°C growth chamber for 96 h. Seedlings were then removed from chilling and placed in the greenhouse under natural conditions. The seedlings were monitored for 1 week for survival following transfer to the greenhouse. Surviving plants recovered full turgidity within 48 h after chilling, whereas nonsurviving plants were fully desiccated and exhibited no evidence of resumed growth after 1 week.

MEASUREMENT OF PHOTOSYNTHESIS AND STOMATAL CONDUCTANCE. After 72 h of dark chilling, seedlings were transferred to the growth chamber under a 10-h photoperiod at $24/22^\circ\text{C}$. Seedlings were allowed to recover for 4 h. Then, net assimilation CO_2 rate and stomatal conductance to water vapor were measured on the first fully expanded leaf using a CIRAS-1 portable photosynthetic system (PP Systems, Haverhill, Mass.). The CO_2 concentration in the leaf cuvette was set at $350 \mu\text{mol}\cdot\text{mol}^{-1}$. Incident photosynthesis photon flux to the sample leaves was about $970 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The measuring procedure was repeated on day 2, day 3, and day 7 after removal from the chilling treatment.

CIRCADIAN VARIATION IN CHILLING SENSITIVITY OF TOMATO SEEDLINGS. To evaluate whether chilling sensitivity varied in a circadian manner, tomato seedlings were transferred to either complete darkness or continuous light at each of the following times: 0830, 1300, 1800, 2130, 0100, and 0730 HR. Seedlings were held under these conditions for 24 h and then transferred to a dark growth chamber where they were subjected to a 96-h chilling treatment at 4°C . Seedlings were monitored for their survival after transfer to greenhouse conditions as described above.

ACCLIMATION TREATMENT AND CHILLING TREATMENT. Seedlings were transferred at various times during the diurnal cycle to a dark 25°C (control) or 14°C (acclimation) growth chamber for 48 h

Fig. 2. Net CO_2 assimilation rate (upper panel) and stomatal conductance to water vapor (lower panel) measured in leaves of tomato seedlings after 72 h of dark chilling at 4°C . Three-week-old seedlings were transferred to chilling after 14 h of dark period (AD) or after 10 h of light period (AL). Seedlings were removed from chilling to recover in the growth chamber under a 10-h photoperiod at $24/22^\circ\text{C}$. Measurements were made 4 h after the removal (day 1) and at 1330 HR on days 2, 3, and 7 after chilled as described in Materials and Methods. Each point is a mean of 10 replications. Vertical bars represent $\pm$ SE.

Fig. 3. Catalase and superoxidase dismutase (SOD) activity in tomato leaves during a diurnal cycle. Leaf tissue was collected at the times indicated during a 24-h diurnal cycle before being placed in chilling conditions as described for Fig. 1. Three-week-old seedlings were incubated under a 10-h photoperiod at 24/22 °C in the growth chamber for at least 1 week before sampling. Catalase activity was measured as O_2 production and SOD activity was measured by the inhibition of cytochrome c reduction. One unit of superoxide dismutase will inhibit the rate of reduction of cytochrome c by 50% in a coupled system. Light and shaded bars indicate light and dark periods, respectively. Each point is a mean of five replications. Vertical bars represent $\pm$ SE.

before chilling at 4 °C for an additional 96 h. The seedlings were monitored for their survival following transfer to the greenhouse. Leaf tissue was collected for enzyme extraction on day 1 and day 2 during acclimation and on day 1 and day 4 of dark chilling treatment.

HYDROGEN PEROXIDE SPRAY TREATMENT. Three-week-old tomato seedlings were sprayed to runoff with either 1 or 2 M hydrogen peroxide containing 0.05% Tween-20 3 h before the designated diurnal time points. Seedlings were transferred at designated time points to chilling in the dark at 4 °C for 96 h.

EXTRACTION OF ENZYMES. Tomato leaves (1 g fresh mass) were frozen in liquid nitrogen and ground to a powder in a mortar with a pestle and extracted with 0.05 M potassium phosphate buffer (pH 7.0) containing 1 mg·mL⁻¹ dithiothreitol, 1% polyvinylpyrrolidone (w/v), and 100 μM phenylmethylsulfonyl fluoride, a protease inhibitor. Extracts were prepared from five individual plants for each treatment. The extracts were centrifuged at 10,000 g_n for 10 min, and the supernatants were strained through miracloth and used for the analysis of catalase and superoxide dismutase activities. Protein content of all crude extracts was determined by the method of Lowry et al. (1951). Bovine serum albumin was used as a standard.

CATALASE ASSAY. Catalase activity of a crude protein extract was determined as the production of O_2 (μmol·min⁻¹·mg⁻¹) by a Clark-type oxygen electrode monitor at 25 °C (Yellow Springs Inst., Ohio). The calibration was carried out by measuring fully oxygenated water at 25 °C in the cuvette before and after adding sodium dithionite, an oxygen-absorbing compound. The catalase assay was carried out in a 3-mL reaction cuvette (Yellow Springs Inst., Ohio) with constant stirring. The reaction mixture contained 50 mM potassium phosphate buffer pH 7.0 and 13 mM hydrogen peroxide. The decomposition of H_2O_2 was initiated with 30 μL of the enzyme extract.

SUPEROXIDE DISMUTASE ASSAY. Total superoxide dismutase was measured spectrophotometrically as the inhibition of the rate of cytochrome c reduction by superoxide at A_{550} , pH 7.8 as described by Foster and Hess (1980). One unit of superoxide dismutase will inhibit the rate of reduction of cytochrome c by 50% in a coupled system:

The modified assay reaction contained 216 mM potassium phosphate buffer pH 7.8, 10.7 mM ethylenediaminetetraacetate, 1.1 mM cytochrome C, 0.108 mM xanthine, and 0.05 units/mL xanthine oxidase (Sigma Chemical Company, St. Louis). The 1-mL reaction was initiated with 20 μL of the enzyme extract. The increase in absorbance at 550 nm was recorded after 5 min. The calculation of superoxide dismutase activity was based on percentage inhibition of the rate of cytochrome reduction (Foster and Hess 1980).

Results

DIURNAL VARIATION IN CHILLING SENSITIVITY OF TOMATO SEEDLINGS. Tomato seedlings acclimated to a 10-h day and 14-h night were transferred at different times during the diurnal cycle to a dark, 4 °C chamber for 96 h to assess their tolerance to chilling. All seedlings appeared normal on removal from the chilling environment (data not shown). Those seedlings placed in chilling conditions at the end of the dark period developed symptoms of chlorosis and wilting after removal from low temperature; only 23% survived (Fig. 1). In contrast, all of the seedlings that were transferred to the chilling treatment after being exposed to 5 to 10 h of light survived after transfer to greenhouse conditions. Survival was also high (67%) for seedlings that were exposed to light for only 0.5 h before chilling, although chlorosis was observed in these plants.

EFFECT OF CHILLING ON PHOTOSYNTHESIS AND STOMATAL CONDUCTANCE. To assess the effect of chilling on net gas exchange of leaves, seedlings were transferred to chilling conditions for 72 h at the end of the dark or light period of a diurnal cycle. On removal

Fig. 4. Changes in catalase activity in tomato leaf extracts during dark chilling at 4 °C. Catalase activity was measured as the production of O_2 . Three-week-old seedlings were incubated under a 10-h photoperiod at 24/22 °C in the growth chamber for at least 1 week before chilling was started. Seedlings were transferred to chilling after 14 h of dark period (AD) or after 10 h of light period (AL). Each point is a mean of three replications. Vertical bars represent $\pm$ SE.

from the chilling conditions, net CO_2 assimilation rates (Fig. 2) were significantly lower than unchilled controls, regardless of whether the chilling was imposed at the end of the light or dark cycle. However, those seedlings chilled following the light period exhibited an increase in net CO_2 assimilation rate the day after removal from low-temperature treatment relative to rates taken immediately after the chilling treatment, while those chilled following the dark period did not exhibit a similar increase. Stomatal conductance was lower on removal from chilling conditions compared with the nonchilled seedlings, regardless of whether chilling was initiated after light or dark periods. Like net CO_2 assimilation, stomatal conductance increased during the recovery phase in seedlings chilled following a light period but remained low in those chilled following the dark period.

EFFECT OF LIGHT AND CHILLING ON CATALASE AND SUPEROXIDE DISMUTASE ACTIVITIES. To determine if the diurnal variation in chilling sensitivity was associated with differences in enzymes that protect against oxidative stress, the activities of catalase and superoxide dismutase were measured. Catalase and superoxide dismutase activities were greatest at the end of the light period and decreased significantly during the dark cycle (Fig. 3). The activity

Fig. 5. Role of light in the development of chilling tolerance of tomato seedlings.

Three-week-old seedlings were incubated under a 10-h photoperiod at 24/22 °C in a growth chamber at least 1 week before sampling. Seedlings were transferred from diurnal conditions to a 24-h dark period (AD) or a 24-h light period (AL) before removal to dark chilling conditions of 4 °C for 96 h at various times during the subjective day or night. Light and shaded bars indicate subjective light and dark periods, respectively. Control seedlings were maintained in the diurnal 10-h photoperiod throughout the experiment before being removed to dark chilling conditions. Each point is a mean of three, 72-seedling replications. Vertical bars represent $\pm$ SE.

of catalase was followed during the chilling treatment for seedlings transferred at the end of the light period and dark period (Fig. 4). This revealed that catalase activity remained higher at all stages of chilling for seedlings transferred to low temperatures following the light period compared to the dark period.

CIRCADIAN VARIATION IN CHILLING SENSITIVITY OF TOMATO SEEDLINGS. To assess whether the diurnal variation in chilling sensitivity was due to an internal circadian rhythm or the result of external cues such as light, seedlings were transferred from a diurnal environment to continuous light or darkness and their sensitivity to chilling was determined at various times during the subjective day or night. Seedlings transferred to chilling at various times during a normal diurnal light and dark cycle exhibited typical changes in tolerance such that the highest survival rate was at the end of the light cycle (Figs. 1 and 5). In contrast, plants transferred to chilling temperatures at various times during exposure to constant darkness or constant light did not vary in tolerance during a 24-h cycle (Fig. 5). Those exposed to constant darkness immediately following a normal diurnal cycle were sensitive to chilling, while those exposed to constant light were completely tolerant to chilling.

DIURNAL CHANGES IN CHILLING SENSITIVITY OF ACCLIMATED TOMATO SEEDLINGS. To assess the effect of a low-temperature acclimation period on chilling tolerance, seedlings were transferred at various times during a diurnal cycle to a 14 °C chamber for 48 h before being placed in chilling conditions (4 °C, dark) for 96 h. Nonacclimated and acclimated seedlings chilled at the end of light cycle exhibited 100% survival following 96 h of dark chilling (Fig. 6). Acclimation and subsequent chilling of seedlings at the end of dark cycle exhibited 70% survival after the chilling treatment compared to 33% of nonacclimated seedlings. Acclimated seedlings exhibited diurnal variation in chilling sensitivity, although low-temperature acclimation increased survival of seedlings chilled following a dark period. These results indicate that low, nondamaging temperatures can partially increase tolerance to chilling even in the absence of light.

CATALASE ACTIVITY DURING ACCLIMATION AND DARK CHILLING. Seedlings exhibited an increase in catalase activity during the first day of 14 °C acclimation in the dark (Fig. 7). Seedlings that were acclimated after the end of light period showed higher catalase activity than seedlings that were acclimated after the end of dark period. Low-temperature acclimation following the light period was associated with increased catalase activity, which remained elevated on transfer to, and throughout the dark chilling treatment. In contrast, seedlings acclimated at 14 or 25 °C (control) at the end of dark period exhibited a tendency toward decreased catalase activity.

EFFECT OF HYDROGEN PEROXIDE TREATMENT ON DIURNAL VARIA-

Fig. 6. Diurnal variation in chilling tolerance of tomato seedlings following a period of low temperature acclimation. Three-week-old seedlings were transferred at various times during a diurnal cycle directly to a dark 25 °C growth chamber or acclimated at 14 °C in a dark growth chamber for 48 h before chilling stress. Light and shaded bars indicate light and dark periods during the diurnal cycle preceding the acclimation phase, respectively. Each point is a mean of three, 72-seedling replications. Vertical bars represent $\pm$ SE.

Fig. 7. Changes in catalase activity during acclimation and chilling. Seedlings were transferred to 14 °C acclimation conditions in the dark on day 1 following the light period (AL) or dark period (AD) of a diurnal cycle. The arrow indicates the time of transfer from 14 or 25 °C (control) acclimation conditions to 4 °C dark chilling conditions. Each point represents the mean of three replications $\pm$ SE.

TION IN CHILLING TOLERANCE. To investigate the role of oxidative stress in the development of chilling tolerance further, tomato seedlings were treated with hydrogen peroxide before exposure to low temperature. Both 1 M and 2 M H_2O_2 sprays caused an increase in the survival of seedlings after exposure to 96 h of dark chilling (Fig. 8). The protective affect of hydrogen peroxide treatment was most pronounced when seedlings were chilled following the dark period. Seedlings sprayed with 2 M H_2O_2 showed a slightly higher survival rate than seedlings sprayed with 1 M H_2O_2 . These plants exhibited damage as necrotic spots on the leaves, likely a result of the higher concentration of H_2O_2 . Interestingly, hydrogen peroxide-treated seedlings showed little evidence of a diurnal variation in chilling sensitivity regardless of the treatment concentration.

Discussion

Tomato seedlings were most sensitive to low-temperature stress if chilled at the end of a dark period. These plants became more tolerant of chilling conditions on exposure to light. Similar results were previously reported for a number of chilling sensitive plants (King et al., 1982). We have expanded on similar observations by King et al. (1982) to show that light-induced tolerance to chilling is associated with a partial recovery of photosynthetic capacity and stomatal conductance following exposure to low temperature. Inhibition of photosynthesis, particularly photosystem II activity, has been found to be an early symptom of chilling injury (Kee, 1986). In addition, Bruggemann and Dauborn (1993) reported a decrease in whole-plant photosynthetic capacity in tomato seedlings under nonphotoinhibiting, long-term chilling conditions. Our results show that the protective effects of a previous light exposure were manifested at the level of leaf gas exchange in response to chilling.

The diurnal fluctuation in chilling sensitivity resembles a circadian rhythm such as those found in almost all eukaryotes (Edmunds, 1988). Circadian rhythms are often entrained by natural day/night cycles. Our results show that the response of tomato seedlings to a diurnal light/dark cycle was a direct response to light or darkness and is not due to an internal oscillating rhythm.

Chilling stress induced an increase in production of the superoxide radicals and hydrogen peroxide, which can attack membrane lipids and inactivate many enzymes (Scandalios, 1990). The accumulation of reactive oxygen species also leads to tissue damage during injury. Because catalase is a major protectant against the accumulation and toxicity of hydrogen peroxide, several researchers have evaluated changes in catalase activities during chilling stress. For example, a reduction in catalase activity in response to low-temperature exposure in light has been observed in a number of plant species (MacRae and Ferguson, 1985; Mishra et al., 1993; Patterson et al., 1984; Schoner and Krause, 1990). Volk and Feierabend (1989) provided evidence that the decline in catalase activity in chilled rye leaves in the light was due to photoinactivation. Photoinhibition led to more rapid turnover in response to increasing light levels, but did not affect steady-state

levels of the enzyme under optimum growth temperatures due to increased production of catalase (Hertwig et al., 1992). Indeed, the expression of catalase mRNA has been shown to be upregulated during the light cycle (Redinbaugh et al., 1990). Therefore, this decrease in catalase activity under conditions of low-temperature stress was primarily due to the failure of synthesis and repair mechanisms necessary to compensate for increased turnover of the enzyme in response to light (Feierabend et al., 1992). Similar to the results of Redinbaugh et al. (1990), catalase activity in tomato seedlings was highest at the end of a light cycle and decreased in the dark under optimal growth temperatures. Catalase activity decreased in seedlings transferred to low temperature, particularly after 96 h of chilling. The elevated catalase and superoxidase dismutase (SOD) activities in seedlings during the light cycle was maintained in plants transferred to chilling conditions. The increased activity of oxidative stress defense enzymes in the light and their maintenance during exposure to low temperature may play a role in increased tolerance to low-temperature stress by limiting the accumulation of hydrogen peroxide and superoxide radicals.

Many plants have an ability to acclimate to low temperature. Our results show that acclimation at 14 °C for 48 h induces chilling

Fig. 8. Survival of tomato seedlings after 96 h of dark chilling. Three-week-old seedlings were sprayed with 1 M H_2O_2 or 2 M H_2O_2 3 h before transfer to dark chilling. Light and shaded bars indicate light and dark periods, respectively. Vertical bars represent the mean of three, 72-seedling replications $\pm$ SE.

tolerance (as indicated by poststress survival) in tomato seedlings regardless of whether seedlings were acclimated after the end of dark or light period. This is consistent with the work of Anderson et al. (1994), where growth and survival of maize seedlings were improved by prior exposure of seedlings to a 14 °C acclimation treatment before exposure to chilling stress. Here we show that an increase in catalase occurred in acclimated tomato seedlings whether exposed to acclimating temperatures at the end of a light or dark cycle. The increase in catalase activity following acclimation at 14 °C was probably indicative of increased oxidative stress resulting from low-temperature stress. Acclimation likely leads to weak oxidative stress as evidenced by increased catalase activity. This increased catalase activity could, in turn, provide protection from more severe oxidative stress during exposure to damaging low temperatures. Anderson et al. (1994) have shown that the induction of catalase in maize seedlings probably occurs at the transcriptional level, as transcripts corresponding to *cat3* increased in abundance during acclimation.

In support of a role for oxidative stress in the development of chilling tolerance, the application of H₂O₂ before chilling induced resistance to chilling injury. Treatment with H₂O₂ completely overcame the sensitivity of seedlings to low temperature when chilled at the end of the dark period. A low concentration of H₂O₂ is generated as a metabolite of enzymatic reactions during photosynthesis (Salin, 1987). Endogenous hydrogen peroxide is generally toxic at cellular concentrations of 10 mM (Salin, 1987). High concentrations of H₂O₂ can indirectly lead to lipid peroxidation and cell injury. Highly reactive compounds like H₂O₂ or reactive oxygen species might function as secondary messengers to induce the production of antioxidant compounds or increase oxidative defense enzyme activity (Greenberg et al., 1990, Prasad et al., 1994). Our results support the dual role of both a signal for induction of stress tolerance during acclimation and as a source of damage during low-temperature exposure, as proposed by Prasad et al. (1994). The current focus of our research is to address more directly the role of H₂O₂ and catalase in chilling tolerance by generating transgenic plants inhibited in catalase activity.

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