Response of the Photosynthesis and Antioxidant Systems to High-Temperature Stress in Euonymus japonicus Seedlings

Song-Heng Jin, Xue-Qin Li, Bing-Song Zheng, and Jun-Gang Wang

Abstract: To uncover the adaptive mechanisms of photosynthesis under high temperatures (HTs), changes in the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco), the photosynthetic electron transport system, and antioxidant system were examined in a subtropical forest tree seedling, Euonymus japonicus. Compared with the control, the activity of rubisco and the quantum yield of photosystem (PS) II electron transport (ΦPSII) decreased in HT-treated seedlings, whereas energy-dependent quenching of chlorophyll (Chl) fluorescence (qE) increased. An analysis of the redox change of P700 and the oxygen uptake by PSI indicated that the cyclic electron transport around PSI operated at high rates in E. japonicus seedlings exposed to HT conditions. Furthermore, the levels of reactive oxygen species (ROS), the activities of superoxide dismutase, and the activities of ascorbate peroxidase were increased in HT-treated seedlings. Therefore, it is likely that an enzyme-based water-water cycle was initiated in E. japonicus seedlings in response to HT stress and that the HT-induced ROS might play an important function in HT conditions. Enhancement of cyclic electron transport around PSI and water-water cycles might help maintain a transthylakoid energy potential under HT conditions. This coordinated defense may play an important role in adaptation of E. japonicus cells to HT. For. Sci. 56(2):172–180.

Keywords: high temperature, photosynthesis, rubisco activity, cyclic electron transport around photosystem I, antioxidant system

Climatic models predict that global temperature will continue to increase in the future. The global mean temperature increased by 0.6°C from 1990 to 2000 and is projected to increase by another 1.4–5°C by 2100 (Sharkey 2005). Hence, plants growing in temperate climates will be exposed to high-temperature (HT) stress conditions. HT adversely affects plant growth and survival in a number of ways. Inhibition of photosynthesis by HT has been documented in many plant species. HT stress has been associated with interruption of electron transport, a reduction in the photochemical efficiency of PSII in cotton and wheat species (Law and Crafts-Brandner 1999). It has been shown that PSII is damaged by severe HT stress when the temperature is higher than 45°C (Gombos et al. 1994, Yamane et al. 2002). Chlorophyll fluorescence analysis and rubisco activation assays indicated that Calvin cycle activity was more sensitive to HT than the photochemical efficiency of PSII in cotton and wheat species (Law and Crafts-Brandner 1999). It has been shown that PSII is damaged by severe HT stress when the temperature is higher than 45°C (Gombos et al. 1994, Yamane et al. 1998). Although several components of the photosynthetic apparatus and associated metabolic pathways are sensitive to moderate HT stress, previous studies have demonstrated that rubisco activation is the primary site of inhibition (Feller et al. 1998, Haldimann and Feller 2004, Salvucci and Crafts-Brandner 2004). There is evidence that thylakoid membranes become permeable at moderate HT (Bukhov et al. 1999), resulting in proton leakage and a reduction in ribulose-1,5-bisphosphate (RuBP) regeneration (Wise et al. 2004). Murakami et al. (2000) suggested that the fatty acid composition of chloroplast membranes can alter the ability of plants to maintain growth at HT. However, Haldimann and Feller (2004) suggested that the thylakoid membranes of oak leaves were not significantly damaged by HT stress treatments. Therefore, exactly how HT inhibits photosynthesis remains a matter of debate.

Plants have evolved a series of mechanisms to protect their photosynthetic apparatus against the damage of HT. The acclimation of photosynthesis to increasing temperatures has been reported in different plant species. HT stress could increase the specific activity of antioxidant enzymes to overcome the increased oxidative stress (Chaitanya et al. 2002, Guo et al. 2006). Heckathorn et al. (1998) suggested that a chloroplast-localized heat shock protein protects PSII from damage under HT conditions. Most recently, Tang et al. (2007) suggested that HT stress induced an aggregation of the light-harvesting complex of PSII in spinach plants, which may represent a protective mechanism to dissipate excess excitation energy in HT-stressed plants. Previous work has shown that HT stress enhances the cyclic electron transport in different herbaceous plant species (Bukhov et al. 2000, Wang et al. 2006). However, there is little evidence for involvement of cyclic electron transport around PSI in the adaptation of higher plants, especially woody plants, to HT stress. The mechanism by which HT stress...
enhances cyclic electron transport capacity also remains unclear. Studies with the aim of better understanding of the physiological mechanisms involved in the inhibition of photosynthesis by HT stress, particularly those focusing on the role of an increase of cyclic electron transport around PSI, have concentrated on a few herbaceous plant species. Plants have large differences in their photosynthetic temperature optimum, even among closely related species. The differences in the responses of plants to HT stress are dependent on the physiologic characteristics of the plant species (Weston and Bauerle 2007). Thus far, little information is available concerning the effects of HT stress on the photosynthetic apparatus of leaves in woody plants. Because of the impact of temperature on global forest productivity, understanding photosynthetic responses to rising temperature is critical for predicting forest growth (Weston et al. 2007). This study represents a step toward elucidating the potential function of PSI-mediated cyclic electron transport in the thermal tolerance of woody plants. In this study, we examined the response of rubisco activation, cyclic electron transport around PSI, and antioxidant metabolism to HT stress in Euonymus japonicus seedlings. The results of the present study indicate that HT stress of woody plants enhances the cyclic electron transport around PSI that may arise from deactivation of rubisco. This mechanism may function in the acclimation of photosynthesis to HT stress.

Materials and Methods

Plant Materials

One-year-old seedlings of E. japonicus Thunb. were obtained from Tianman Horticulture Company in China. In March 2006, the seedlings were transplanted into plastic tubes (20.5-cm tall, 18-cm top diameter) filled with a 1:1 (v/v) mixture of coarse sand and soil. The seedlings were grown in a shaded greenhouse with natural sunlight during the day (maximum of 800 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)). The mean daytime maximum and minimum temperatures in the greenhouse were 28 and 20°C, respectively. Approximately 4 months later, HT treatments were imposed by randomly allocating seedlings to one of two growth chambers. Both growth chambers had identical 14-h photoperiods with a photosynthetic photon flux density (PPFD) at leaf height of approximately 600 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). The chambers had constant relative humidity of approximately 50% and differed only in temperature. One chamber was maintained at 25°C (day and night) throughout the experimental period as the nonstressed control. The other chamber was maintained at 38°C (day and night) as the HT treatment group. Plants were given fertilized water once per week with a half-strength Hoagland solution. Water was supplied at other times of the day in the HT growth chamber to prevent drought stress.

Measurement of Gas Exchange, Chlorophyll Fluorescence, and Redox Change of P700

The measurements were made on the second fully expanded leaf from the top of four randomly selected seedlings from each treatment. Leaf chamber temperature was maintained at equilibrium with the ambient temperature, which was equivalent to the treatment temperature. Other environmental conditions for the measurements included 600 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) of PPFD and 50 relative humidity in the sample chamber. Leaf gas exchange and chlorophyll fluorescence were measured simultaneously using a portable photosynthesis system (LI-6400; Li-Cor, Inc., Lincoln, NE) with an integrated fluorescence fluorometer (LI-6400-40 leaf chamber fluorometer; Li-Cor, Inc.) under ambient CO\textsubscript{2} concentrations and 21% O\textsubscript{2}. Actinic light (AL) supplied with light-emitting diodes (90% red light, 630 nm; 10% blue light, 470 nm) was used to record the steady-state chlorophyll fluorescence level (\( F_{\text{m}} \)) and the net photosynthetic rate (\( P_{\text{n}} \)). The minimal chlorophyll fluorescence at the open PSI center (\( F_{\text{p}} \)) and maximal chlorophyll fluorescence at the closed PSI center (\( F_{\text{m}} \)) were measured after 30 min of dark adaptation. Measurement light (630 nm, 1 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) was used to determine \( F_{\text{p}} \). An 800-ms saturating pulse (>6000 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) was applied to measure \( F_{\text{m}} \) in the dark or during AL illumination (\( F_{\text{m}}' \)). The maximum quantum yield of the primary photochemistry of PSII (\( \Phi_{\text{PSII}} \)) was calculated as \( (F_{\text{m}}' - F_{\text{p}})/F_{\text{m}} \). The quantum yield of PSI electron transport \( \Phi_{\text{PSI}} \) was calculated from measured parameters (Maxwell and Johnson 2000). The postillumination transient increase in chlorophyll fluorescence was recorded according to the procedure described by Martín et al. (2004).

The kinetics of nonphotochemical quenching formation and relaxation was obtained by measuring the chlorophyll fluorescence on the same leaves as described by Quick and Stitt (1989). The energy-dependent quenching (\( qE \)) was calculated according to Jin et al. (2008).

The redox change of P700 was measured sequentially after chlorophyll fluorescence measurement. The oxidation of P700 and its re-reduction were monitored by the absorbance changes at 810–830 nm using a chlorophyll fluorometer (PAM101; Walz, Effeltrich, Germany), with unit ED-P700DW-E, as described previously (Burrows et al. 1998). The initial rate of P700' re-reduction after far-red light (>705 nm, 6 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) was calculated. After gas exchange and chlorophyll fluorescence were measured, a leaf of the same location on the seedlings was freeze-clamped into liquid nitrogen and subsequently used for biochemical measurements.

Measurements of Full-Chain Photosynthetic Rates and PSI and PSII Activities

Intact chloroplasts were isolated from control and HT-treated plant leaves and purified as described previously (Robinson and Downton 1985). Oxygen uptake was monitored in the chloroplasts after osmotic shock with a Hansatech oxygen electrode under white saturating light (1,500 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) according to Yamamoto et al. (2006) with minor modifications. Whole electron transfer chain activity was recorded as net oxygen consumption using methyl viologen (MV) as an electron acceptor in 1 ml of buffer consisting of 40 mM sodium phosphate, pH 7.4, 1 mM NaCl, 0.6 mM Na\textsubscript{3}P, 0.12 mM MV, and 5 mM NH\textsubscript{4}Cl. PSI
activity was measured as oxygen evolution in a reaction mixture (1 ml) consisting of 5 mM NH₄Cl, 0.33 M sorbitol, 40 mM Hepes-KOH, pH 7.6, 5 mM NaCl, 5 mM MgCl₂, 1 M glycine betaine, and 1 mM KH₂PO₄. 2,6-Dichloro-p-benzoquinone (0.25 mM) was used as an electron acceptor. PSI activity was measured as oxygen uptake in a reaction mixture (1 ml) consisting of 0.1 mM KCN, 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethyleurea, 500 μM dichloro-phenolindophenol, 2 mM ascorbate, 0.67 μM nigericin, and 0.1 mM MV.

**Assays of Rubisco**

Approximately 0.20 g of leaves were ground to a powder using a chilled mortar and pestle with liquid nitrogen, a small amount of quartz sand, and insoluble polyvinylpolypyrrolidone (PVP). The powder was homogenized with 3 ml of cooled extraction buffer containing 50 mM Tricine (pH 7.8), 1 mM EDTA-2Na, 10 mM MgCl₂, 5 mM dithiothreitol (DTT), 1% (v/v) Triton-X-100, 1% PVP-40 (w/v), 0.1% (v/v) β-mercaptoethanol, and 0.2 mM leupeptin. The homogenate was centrifuged at 15,000 × g for 40 s at 4°C. The supernatant was used to determine the activation state of rubisco. The rubisco activity in the supernatant was assayed according to Sawada et al. (2003) with minor modifications. The initial rubisco activity was measured at 25°C by adding 100 μl of supernatant into 900 μl of assay buffer containing 50 mM HEPES-KOH (pH 8.0), 1 mM EDTA-2Na, 20 mM MgCl₂, 2.5 mM DTT, 10 mM NaHCO₃, 5 mM ATP, 0.15 mM NADH, 5 mM creatine phosphate, 0.6 mM RuBP, 10 units of phosphocreatine kinase, 10 units of glyceraldehyde-3-phosphate dehydrogenase, and 10 units of phosphoglycerate kinase. The total rubisco activity was assayed by adding 100 μl of the rubisco-containing supernatant into 200 μl of an activation medium containing 33 mM Tris-HCl (pH 7.5), 0.67 mM EDTA-2Na, 33 mM MgCl₂, and 10 mM NaHCO₃ and then incubating the sample at 25°C for 10 min. The percentage of rubisco activation was calculated as the ratio of the initial and total activities.

**Antioxidant Enzyme Activity Determination**

Leaf tissue (0.5 g) was ground in liquid nitrogen in 3.5 ml of 50 mM potassium phosphate buffer (pH 7.8) containing 0.2 mM EDTA, 4% (w/v) PVP-40, and 1 mM ascorbic acid. Samples assayed for superoxide dismutase (SOD) activity were extracted in the same buffer without the ascorbic acid. All enzyme extractions and centrifugations were performed at 4°C. The enzyme activity of each sample was measured four times at 25°C. Activities of SOD, ascorbate peroxidase (APX), and catalase (CAT) were assayed with methods described previously by Verma and Mishra (2005).

**Determination of Superoxide Radical (O₂⁻) Producing Rate and H₂O₂ Content**

The production rate of O₂⁻ was measured by monitoring the nitrite formation from hydroxylamine in the presence of O₂⁻ following the method of Verma and Mishra (2005). The content of H₂O₂ was measured by monitoring the A415 titanium-hydroperoxide complex following the method described by He et al. (2006).

**Statistical Methods**

Data were examined statistically using analysis of variance. Differences between means were established using Duncan’s test (P < 0.05). All experiments were performed at least four times.

**Results**

HT stress strongly reduced the photosynthetic performance in seedlings of *E. japonicus*. Compared with the controls, on the 10th day, *Pn* and *Φ*₂ of HT-treated seedlings decreased by 30.4 and 25.1%, respectively (Figure 1A and C). As HT stress progressed, *Pn* and *Φ*₂ in HT-treated seedlings declined more dramatically. However, no significant (*P > 0.05*) difference in *Fv*-*Fm* was detected between controls and HT-treated seedlings at 10 or 20 days of treatment. A decrease in the *Fv*-*Fm* ratio was apparent only after 35 days of stress (Figure 1B). *qE* increased progressively in HT-treated plants until 35 days of treatment. After 35 days, it decreased slightly (Figure 1D). On the 35th day, *qE* increased by 83.4% over the control.

The leaf rubisco activity and rubisco activation state of the control seedlings at 25°C remained rather stable during the 35 days of treatment (Figure 2). HT stress significantly (*P < 0.05*) decreased the initial rubisco activity (Figure 2A). In contrast, the total rubisco activity was barely affected by HT stress (Figure 2B). As a consequence, HT stress resulted in a significant (*P < 0.05*) reduction of the rubisco activation state, which decreased from approximately 80% at 0 days to less than 20% at 35 days of treatment (Figure 2C).

The dark re-reduction of *P₇₀₀*⁺ and the initial rate after 20 days of treatment are shown in Figure 3. Re-reduction of *P₇₀₀*⁺ was prominently enhanced by HT stress in *E. japonicus* compared with the control. At the beginning of HT stress (0 days), no significant (*P > 0.05*) difference in dark re-reduction of *P₇₀₀*⁺ was detected between HT stress and control seedlings. On the 20th day, the initial rate of *P₇₀₀*⁺ was much higher in HT-treated seedlings than in control seedlings (Figure 3B). The rate of re-reduction of *P₇₀₀*⁺ immediately after the cessation of the light is taken as a measure of the rate of electron flow that was occurring in the light (Johnson 2005, Yang et al. 2007). Thus, the activity of cyclic electron transport around PSI was enhanced in the HT-treated plants.

Figure 4 illustrates the postillumination chlorophyll fluorescence increase in the absence or presence of antimycin A. At the beginning of HT stress (0 days), no significant (*P > 0.05*) transient increases in chlorophyll fluorescence after AL illumination were detected between HT stress and control plants. After 20 days of treatment, the transient increases in chlorophyll fluorescence after AL illumination were much higher in HT-treated seedlings than in control seedlings. In higher plants and cyanobacteria, the transient increases in chlorophyll fluorescence after the termination of AL involve changes in PSI cyclic electron transport (Jin
et al. 2008, Lu et al. 2008). These results further indicate that HTs could stimulate PSI cyclic electron transport. Antimycin A inhibits ferredoxin-dependent, but not NAD(P)H dehydrogenase (NDH)-dependent, cyclic electron flow around PSI (Munekage et al. 2004, Munné-Bosch et al. 2005). After treatment with antimycin A, the transient increase in chlorophyll fluorescence after AL illumination was only slightly decreased in both groups (Figure 4).

To compare the photosynthetic capacities of *E. japonicus* seedlings grown under HT conditions, the photosynthetic O₂ evolution was examined with isolated chloroplasts. As shown in Table 1, the MV-dependent linear electron transport and PSII activity in the HT-treated plants were reduced by approximately 60%, compared with that in the control plants. Nevertheless, the PSI activity in HT-treated plants was significantly higher than that in controls (P < 0.05). Obviously, the enhancement in PSI activity should be used to increase cyclic electron transport.

Leaf SOD and APX activities remained nearly constant in control plants during the experimental period. However, under HT stress, there were remarkable increases in SOD and APX activities until 20 days of treatment (Figure 5A and B). Meanwhile, the activity of leaf CAT did not change significantly (P > 0.05) in the HT-treated seedlings compared with controls during the experimental period (Figure 5C). O₂⁻ production rate and H₂O₂ content increased much more in the HT-treated seedlings than in the controls (Figure 6).

**Discussion**

The quantum yield of PSII (which can indirectly reflect linear electron transport, Maxwell and Johnson 2000), the whole electron transport flow, and PSII activity were all lower in seedlings grown under HT conditions than in plants grown at control temperatures (Figure 1A; Table 1). However, PSI capacity was appreciably stimulated. These results indicate that PSII activity is the rate-limiting step of the linear electron transport chain under HT conditions.

In earlier studies conducted with other plant species, it was hypothesized that the loss of activated rubisco is the primary limiting factor of net photosynthesis under HT stress (Feller et al. 1998, Salvucci and Crafts-Brandner 2004). The reduced rubisco activation state under HT stress conditions is caused by the lower activity of rubisco activase (Law and Crafts-Brandner 1999, Crafts-Brandner and Salvucci 2000). In this study, HT stress inhibited the initial activity (but not the total activity) of rubisco, causing a
decrease in the activation state (Figure 4), which suggested that the suppression of rubisco activation under HT stress conditions might be largely due to inhibition of rubisco activase. A positive correlation between acclimation of photosynthesis to HTs and the activation state of rubisco has been reported (Law and Crafts-Brandner 1999, Crafts-Brandner and Salvucci 2000, Liu and Huang 2008). However, in the present study, heat tolerance did not improve with acclimation. High night temperatures accelerated respiration and could potentially inhibit protein synthesis. It is possible that inhibition of translation is responsible for the lack of acclimation observed here. However, more detailed biochemical characterization of these changes will be required to establish whether this occurs.

As expected, in HT-treated plants qE values were higher than those of control plants (Figure 1). The qE component is believed to be directly dependent on the pH gradient across the thylakoid membrane (ΔpH) (Shikanai et al. 2002). This gradient can help the thylakoid membranes cope with HT (Schrader et al. 2004). Photosynthesis uses both linear and cyclic electron flows to convert light energy into the transthylakoid proton motive force, composed of the proton gradient and the electric field. Therefore, we are left to contemplate what process generates the pH gradient necessary to support this requisite qE increase, because the linear electron transport was largely decreased in the HT stress seedlings. Analysis of the redox changes of P700 (Figure 2), the transient increase in the postillumination fluorescence (Figure 3), and the oxygen uptake by PSI showed that the cyclic electron transport around PSI was increased in the HT-treated seedlings. It has been suggested

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**Figure 2.** The effects of higher temperature stress on the initial rubisco activity, total rubisco activity, and rubisco activation state in the second fully expanded leaves of *E. japonicus* seedlings during the 35-day period. Data are means ± SE (*n* = 4). Values carrying different letters are significantly different at *P* < 0.05. FW, fresh weight.

**Figure 3.** The effects of higher temperature stress on dark re-reduction of P700⁺ after far-red (FR) illumination of the second fully expanded leaves of *E. japonicus* seedlings after 20 days of treatment. A, Kinetics of P700⁺ re-reduction in the leaves of *E. japonicus* seedlings after illumination by far-red light (>705 nm, 6 μmol m⁻² s⁻¹) under controlled and stressed conditions. Measurement temperature was equivalent to the treatment temperature. Each experiment was repeated four times, and averages were calculated. B, Effects of HT stress on the relative initial slope of P700⁺ re-reduction in *E. japonicus* seedlings. The initial slope of P700⁺ re-reduction in the control plants at 0 days of treatment is regarded as 100%. Data are means ± SE (*n* = 4). Values carrying different letters are significantly different at *P* < 0.05.

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that cyclic transport flow around PSI is responsible for enhanced proton pumping and is involved in energy dissipation when CO2 availability is reduced (Burrows et al. 1998, Shikanai et al. 2002, Munekage et al. 2004). Under high leaf temperature conditions, rubisco deactivation and the decline in initial activity have been well correlated with a decline in stromal redox status (Schrader et al. 2004). Lowered stromal redox status can lead to simulation of PSI-mediated cyclic electron flow (Sharkey 2005). Thus, we inferred that the deactivation of rubisco might contribute

![Figure 4](image)

Table 1. Effects of high temperature stress on the activities of linear electron transport, PSI and PSII in the isolated chloroplasts from the second fully expanded leaves of *E. japonicus* seedlings after 20 days of treatment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>25°C</th>
<th>38°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear electron transport activity (μmol O₂ mg Chl⁻¹ h⁻¹)</td>
<td>155 ± 19a</td>
<td>62 ± 9b</td>
</tr>
<tr>
<td>PSI activity (μmol O₂ mg Chl⁻¹ h⁻¹)</td>
<td>212 ± 23b</td>
<td>395 ± 21a</td>
</tr>
<tr>
<td>PSII activity (μmol O₂ mg Chl⁻¹ h⁻¹)</td>
<td>132 ± 10a</td>
<td>45 ± 6b</td>
</tr>
</tbody>
</table>

Data are means ± SD (n = 4). Values in the same line followed by different letters are significantly different at P < 0.05. Chl, chlorophyll.
to the decrease in stromal redox status, thereby resulting in enhanced cyclic electron transport around PSI and protecting the apparatus under the HT conditions.

There are two different pathways of cyclic electron flow around PSI in higher plant chloroplasts. One pathway is sensitive to antimycin A, whereas the other is insensitive. The insensitive pathway involves a chloroplast NDH complex (Munekage et al. 2004, Johnson 2005, Shikanai 2007). Both of the pathways have important roles in the induction of sufficient photoprotection (Munekage et al. 2004, Johnson 2005, Munne-Bosch et al. 2005, Shikanai 2007). Further studies of the pathways of cyclic transport flow around PSI in higher plants, especially under environmental stress conditions, would be interesting. In the present study, measurements of chlorophyll fluorescence after termination of AL illumination using antimycin A revealed that cyclic electron transport in the HT-treated seedlings was mainly dependent on the antimycin-insensitive pathway.

There is evidence that greater HT tolerance is correlated with increasing amounts of antioxidants and increasing activity of scavenging enzymes (Chaitanya et al. 2002). In this study, significant increases in the activities of SOD and APX were found in HT stress leaves (Figure 5). These increases were accompanied by a significantly elevated $O_2^-$ production rate and $H_2O_2$ content (Figure 6). SOD and APX are important enzymes involved in the scavenging of active oxygen species in the water-water cycle (Asada 1999). These results suggest that the enzyme-based water-water cycle may play an important function in HT conditions.

Although reactive oxygen species (ROS) were originally considered to be detrimental to cells, it is now widely recognized that redox regulation involving ROS is a key factor modulating cellular activities (Suzuki and Mittler 2006, Bechtold et al. 2008). Our experiments demonstrate that in $E. japonicus$ leaves, the $O_2^-$ production rate and the levels of $H_2O_2$ increase 1.6- and 1.8-fold, respectively, after 20 days of treatment. These effects are much less than those on rubisco and the quantum yield of PSII. It has been shown that heat damage is worse when leaves are heated in the dark rather than in the light. Thus, the accumulation of ROS in the leaves of plants is unlikely owing to inhibition of linear electron transport. $H_2O_2$ can be directly decomposed through CAT. Interestingly, in this study, leaf CAT content in HT-treated seedlings was not significantly different when
compared with that of controls throughout the experimental period. Heat stress response signal transduction pathways and defense mechanisms, involving heat shock transcription factors and heat shock proteins, are thought to be intimately associated with ROS (Penuel et al. 2003, Suzuki and Mittler 2006). Casano et al. (2001) showed that intrachloroplastic H$_2$O$_2$-mediated signaling might be involved in the photooxidative induction of increased levels of ndhB/F transcripts and NADH dehydrogenase activity. All of these results indicate that HT-induced ROS may play a key role in mediating important signal transduction events, activating stress-response pathways, and inducing defense mechanisms. However, there is limited information about the mechanism of HT-induced H$_2$O$_2$ production. There are several possible sources of H$_2$O$_2$ in plants that can be activated during abiotic and biotic stress. Which sources induce H$_2$O$_2$ accumulation remains to be established.

**Literature Cited**


