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Brassinosteroids Alleviate Heat-Induced Inhibition of Photosynthesis by Increasing Carboxylation Efficiency and Enhancing Antioxidant Systems in *Lycopersicon esculentum*

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Abstract To investigate the effects of exogenously applied brassinosteroids on the thermotolerance of plants, leaf CO_2 assimilation, chlorophyll fluorescence parameters, and antioxidant enzyme metabolism were examined in tomato (*Lycopersicon esculentum* Mill. cv. 9021) plants with or without 24-epibrassinolide (EBR) application. Tomato plants were exposed to 40/30°C for 8 days and then returned to optimal conditions for 4 days. High temperature significantly decreased the net photosynthetic rate (P_n), stomatal conductance (G_s), and maximum carboxylation rate of Rubisco (V_{cmax}), the maximum potential rate of electron transport contributed to ribulose-1,5-bisphosphate (RuBP), as well as the relative quantum efficiency of PSII photochemistry (Φ_{PSII}), photochemical quenching (q_P), and increased nonphotochemical quenching (NPQ). However, only slight reversible photoinhibition occurred during heat stress. Interestingly, EBR pretreatment significantly alleviated high-temperature-induced inhibition of photosynthesis. The activities of antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPOD), and catalase (CAT) increased during heat treatments, and these increases proved to be more significant in EBR-treated plants. EBR application also reduced total hydrogen peroxide (H_2O_2) and malonaldehyde (MDA) contents, while significantly increasing shoot weight following heat stress. It was concluded that EBR could alleviate the detrimental effects of high temperatures on plant growth by increasing carboxylation efficiency and enhancing antioxidant enzyme systems in leaves.

Keywords Antioxidant enzymes · Brassinosteroids · Chlorophyll fluorescence · Electron transport · High-temperature stress · *Lycopersicon esculentum* M. · Photosynthesis

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Introduction

The most sensitive plant component to high-temperature stress has been identified as the photosynthetic apparatus (Berry and Björkman 1980; Quinn and Williams 1985; Yordanov and others 1986; Heckathorn and others 1998). Havaux (1993) reported that the loss of photosynthetic electron transport in potato leaves was attributed to the thermoliability of photosystem II (PSII), with water splitting proving to be the most heat-sensitive component. Recently, inhibition of ribulose-1,5-carboxylase/oxygenase (Rubisco) activation has also been identified as one of the most heat-sensitive components of the photosynthetic apparatus (Law and Crafts-Brandner 1999; Crafts-Brandner and Salvucci 2000, 2002; Salvucci and Crafts-Brandner 2004). A decrease in CO_2 assimilation might result in the production of excess energy. A number of pathways are thought to cooperate in protecting the photosynthetic apparatus from photo-oxidative stress (Ort and Baker 2002). These include xanthophyll cycle–dependent energy

dissipation, cyclic electron flow, and antioxidant metabolism (Verhoeven and others 1997; Hirotsu and others 2004). Recently, reactive oxygen species (ROS) generation and its scavenging system have attracted increasing attention because of its role in the defense of plants against various stresses. There is evidence that high-temperature stress is one of the conditions that disrupts the cellular metabolic homeostasis and promotes the production of reactive oxygen species (Mittler 2002). Oxidative stress occurs when there is an imbalance in any cell compartment in the production of ROS and antioxidant defense, thereby causing damage (Mittler 2002; Scandalios 2002; Apel and Hirt 2004). Generally, plant responses to environmental stress such as high-temperature stress have been associated with the accumulation of ROS such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet-}$), and the hydrogen radical ($^{\bullet}OH$), as a result of oxidative stress (Alscher and others 1997; Anderson 2002; Apel and Hirt 2004). ROS such as H_2O_2 have detrimental effects on Rubisco (Ishida and others 1998) and the redox state also affects the expression of chloroplast and nuclear genes (Garcia-Ferris and Moreno 1994; Irihimovitch and Shapira 2000; Pfannschmidt 2003).

Brassinosteroids (BRs) are a family of over 40 naturally occurring plant steroid hormones that are ubiquitously distributed in the plant kingdom (Clouse and Sasse 1998; Bishop and Koncz 2002; Krishna 2003; Montoya and others 2005). BRs play prominent roles in various physiologic processes, including the induction of a broad spectrum of cellular responses such as stem elongation, pollen tube growth, xylem differentiation, leaf epinasty, root inhibition, induction of ethylene biosynthesis, proton pump activation, regulation of gene expression and photosynthesis, and adaptive responses to environmental stress (Clouse 1996; Clouse and Sasse 1998; Dhaubhadel and others 1999; Khripach and others 2000; Steber and McCourt 2001; Krishna 2003; Yu and others 2004; Cao and others 2005). As a new class of plant hormone, BRs have attracted increasing attention in studies addressing the adaptive response to environmental stress, particularly with respect to cold stress (Wilen and others 1995; Dhaubhadel and others 1999; Yu and others 2002), salt injuries (Dhaubhadel and others 1999; Núñez and others 2003; Ozdemir and others 2004), pathogen infection (Mandava 1988; Nakashita and others 2003), oxidative stress (Ershova and others 1996; Cao and others 2005), and thermotolerance (Kulaeva and others 1991; Wilen and others 1995; Dhaubhadel and others 1999; Mazzora and others 2002). Although great efforts have been made to develop this phytohormone as a plant growth regulator for widespread utilization in agricultural production, the mechanisms by which BRs influence plant growth, photosynthetic capacity, and stress tolerance are still poorly understood (Haubrick and Assman 2006). Several studies have shown that BR-induced thermotolerance in *Brassica napus* was associated with higher synthesis of heat shock proteins (HSPs) (Dhaubhadel and others 1999). Furthermore, BRs have also been found to be involved in the regulation of ROS metabolism (Cao and others 2005). More recently, we have found that BRs significantly increased the capacity of CO_2 assimilation by increasing the initial activity of Rubisco (Yu and others 2004). An increase in electron flux to the Calvin cycle is supposed to decrease the electron flux to O_2 and the generation of ROS. In this regard, there is an intriguing possibility that BRs could protect plants from heat stress by increasing RuBP carboxylation capacity by Rubisco and decreasing ROS generation.

In this study, we exposed tomato plants to a high-temperature stress and investigated whether application of BRs could induce tolerance to heat stress in the plants, and whether the induced tolerance to high-temperature stress is associated with the protection of the photosynthetic apparatus. Accordingly, gas exchange, chlorophyll fluorescence, and antioxidant enzymes were determined in tomato plants with or without BR pretreatment before exposure to high temperature. Chlorophyll fluorescence could detect the extent to which photosystem II is using the energy absorbed by chlorophyll and the extent to which it is being damaged by excess light, whereas analyses of the activities of antioxidant enzymes would provide information about ROS metabolism in stressed plants.

Materials and Methods

Growth Conditions and Temperature Treatments

The tomato (*Lycopersicon esculentum* Mill. cv. 9021) plants used for this experiment were sensitive to high temperatures. Seeds were obtained from the Zhejiang Academy of Agricultural Sciences, China, and were sown directly in vermiculite and germinated at 25°C in a glasshouse. Plants were watered and fertilized daily with a half-strength Enshi nutrient solution (Yu and Matsui 1997). Three weeks after sowing, groups of six seedlings were transplanted into containers (40 cm × 25 cm × 15 cm) and watered with the same nutrient solution, then transferred to a growth chamber with average day/night temperatures set at 25/18°C. The photoperiod was maintained at 12 h, with a photosynthetic photon flux density (PPFD) of 800–1000 μmol m^{-2} s^{-1}.

The 24-epibrassinolide (EBR, Sigma, USA) and temperature treatments started six weeks after germination. On the day before temperature treatments (0 day), plants were divided into two groups, normal temperature (NT) (25/

18°C) and high temperature (HT) (40/30°C); both were sprayed with 0.01, 0.1, and 1.0 mg L^{-1} EBR or distilled water as a control. EBR was dissolved in a minimal volume of ethanol and then made to volume with distilled water as described in our previous study (Yu and others 2004). Each plant was sprayed with 50 ml of solution. The high-temperature treatments lasted 8 days and the plants were allowed to recover for 4 days. The environmental conditions were as follows: 12-h photoperiod, photosynthetic photon flux density (PPFD) of 800 µmol m^{-2} s^{-1}, and temperatures of 25/18°C, respectively. On the last day of the experiment (that is, day 12), shoots were harvested, oven-dried at 82°C for 3 days, and then weighed for determination of dry weight.

Gas Exchange and Chlorophyll Fluorescence Measurements

Gas exchange parameters were determined on the third fully expanded leaves by using an infrared gas analyzer (IRGA) portable photosynthesis system (LI-6400, LiCOR Biosciences, USA). To measure the net photosynthetic rate (P_n), intracellular CO_2 content (C_i), and stomata conductance (G_s), the air temperature, relative humidity, CO_2 concentration, and PPFD were maintained at 25°C, 85%, 600 µmol mol^{-1}, and 800 µmol m^{-2} s^{-1}, respectively.

For the determination of Rubisco carboxylation capacity, leaf net CO_2 assimilation rates (A) in response to CO_2 were measured between 1600 and 10 µmol m^{-2} s^{-1}. A/C_i curves were measured according to the method of von Caemmerer and Farquhar (1981), in which the leaf temperature and PPFD were maintained at 25°C and 1800 µmol m^{-2} s^{-1}. Estimations of the maximum carboxylation rate of Rubisco (V_{cmax}) and maximum rates of RuBP regeneration (J_{max}) were made by fitting a maximum-likelihood regression below and above the inflexion of the A/C_i response according to the method described by Ethier and Livingston (2004).

To determine the state of PSII, chlorophyll fluorescence was measured using a portable pulse-modulated fluorometer (FMS-2, Hansatech, UK) with the same leaves previously measured for gas exchange. Before each measurement, leaves were dark-adapted for at least 30 min. The minimal fluorescence (F_0) was determined under a weak pulse of modulating light over 0.8 s, with maximal fluorescence (F_m) induced by a saturating pulse of light applied over 0.8 s. The maximal quantum efficiency of PSII was determined as F_v/F_m. An actinic light source was then applied not only to achieve a steady state of photosynthesis, but also to obtain F_s (steady-state fluorescence yield), after which a second saturation pulse was applied for 0.7 s to obtain (F_m') light-adapted maximum fluorescence. Quantum efficiency of PSII (Φ_{PSII}), efficiency of excitation capture by open PSII center (F_v'/F_m'), nonphotochemical quenching coefficient (NPQ), and photochemical quenching coefficient (q_P) were calculated as $(F_m' - F_s)/F_m'$, F_v'/F_m', $(F_m/F_m') - 1$, and $(F_m' - F_s)/(F_m' - F_0)$, respectively (Genty and others 1989; Bilger and Björkman 1990; van Kooten and Snel 1990).

Activities of Antioxidant Enzymes

To detect the change in the activity of ROS-scavenging enzymes, leaf samples were taken at 0, 4, 8, and 12 days after high-temperature treatment. Enzyme extract was obtained from 0.3 g of leaves, which were homogenized in 3 ml 25 mM HEPES buffer (pH 7.8) containing 0.2 mM EDTA and 2% (w/v) polyvinylpyrolidone (PVP). The homogenate was centrifuged for 20 min at 12,000g. The resulting supernatant was used for enzyme analysis. The procedure for enzyme extraction was carried out at 0–4°C. Protein content was determined according to the method of Bradford (1976), which utilizes bovine serum albumin as the standard.

A photochemical method published by Giannopolitis and Reis (1977) was used to determine superoxide dismutase (SOD). One unit of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition in the rate of p-nitro blue tetrazolium chloride reduction at 560 nm. Ascorbate peroxidase (APX) was determined according to the method of Nakano and Asada (1981) by monitoring the rate of ascorbate oxidation at 290 nm (E = 2.8 mM cm^{-1}). The reaction mixture contained 25 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 20 mM H_2O_2, and 0.25 mM ascorbic acid (AsA). The method published by Cakmak and Marschner (1992), with some modifications, was used to determine guaiacol peroxidase (GPOD) activity. The contents of the reaction mixture were as follows: 25 mM phosphate buffer (pH 7.0), 0.05% guaiacol, 10 mM H_2O_2, and enzyme extract. The increase in absorbance at 470 nm, caused by guaiacol oxidation (E = 26.6 mM cm^{-1}), was used to measure activity. Catalase (CAT) was measured in a reaction mixture containing 25 mM phosphate buffer (pH 7.0), 10 mM H_2O_2, and the enzyme. The decomposition of H_2O_2 was determined at 240 nm (E = 39.4 mM cm^{-1}) (Cakmak and Marschner 1992).

Hydrogen Peroxide (H_2O_2) and Malonaldehyde (MDA) Content

The content of H_2O_2 was determined by monitoring the A_{410} of titanium peroxide complex according to the methods described by Patterson and others (1984). The thiobarbituric acid (TBA) test, which determines malonaldehyde (MDA) as

an end-product of lipid peroxidation in the leaves, was used to measure MDA. Leaves were homogenized and centrifuged in a potassium phosphate buffer (pH 7.8) for 20 min at 12,000*g*, with 1 ml of the supernatant incubated in boiling water for 30 min. The tubes were placed in an ice bath to stop the reaction, after which the samples were centrifuged at 1500*g* for 10 min and the absorption was read at 532 nm. The value for nonspecific absorption at 600 nm was measured simultaneously and then subtracted from OD_{532}. The extinction coefficient of 155 mM cm^{-1} was used to calculate the amount of the MDA–TBA complex.

Statistical Analysis

All data were subjected to analysis of variance and expressed as the mean ± standard error of three replications. The significance of difference between the control and treatments was set at $p = 0.05$ by Student's *t*-test.

Results

EBR Effects on Plant Growth and Gas Exchange Parameters

Under optimal growth conditions, EBR at the tested concentrations (0.01–1.0 mg L^{-1}) had no significant effects on gas exchange because net CO_2 assimilation (P_n), stomatal conductance, and intracellular CO_2 concentrations for the EBR treatment did not differ from those of untreated plants on day 4 after EBR treatment. Heat stress significantly reduced P_n; this reduction, however, was significantly alleviated by pretreatment with EBR at 0.01–1.0 mg L^{-1}. On day 4 after heat treatment, P_n values increased by 14.8%, 26.6%, and 10.2 % for 0.01, 0.1, and 1.0 mg L^{-1} EBR treatments, respectively (Table 1). Clearly, EBR showed a concentration-dependent effect on P_n at high temperatures.

Similar to the changes in P_n, EBR at the tested concentrations had little effect on plant dry matter accumulation under optimal growth conditions. However, after exposure to high temperature, EBR treatment significantly increased accumulation of shoot dry matter. Dry matter increased by 40.6%, 78.8%, and 23.6% for 0.01, 0.1, and 1.0 mg L^{-1} EBR treatments, respectively (Table 1). Because 0.1 mg L^{-1} EBR treatment showed the most significant effects, only the results stemming from that treatment are presented in the following experiments.

The rate of CO_2 assimilation (P_n) decreased sharply with prolongation of the high-temperature treatment (Fig. 1A). On day 4 and day 8 the reduction in P_n was 25.1% and 65.3%, respectively, for untreated plants under high temperatures (CKHT). P_n did not fully recover to values close to control, even when the plants were allowed to return to optimal growth conditions for 4 days. However, plants treated with EBR under high temperatures (BRHT) showed significantly higher P_n than untreated plants (CKHT). They also significantly maintained higher stomata conductance (G_s) than the untreated plants; that is, G_s decreased by 13.5% and 60.3% on day 4 and day 8, respectively (Fig. 1B), for BRHT compared with 27.0% and 93.0% for CKHT under the same period. There was no significant difference between the treatments at 4 days for intracellular CO_2 concentrations (C_i), but at 8 days, C_i for CKHT treatment was significantly lower than EBR-treated leaves (Fig. 1C) and did not recover to the control value.

Table 1 Effects of 24-Epibrassinolide on CO_2 Assimilation (P_n) and Plant Biomass at Different Temperature Regimes after 8 Days of Heat Treatment and 4 Days of Recovery

Temperature (°C)	EBR concentration (mg L^{-1})	P_n (μmol m^{-2} s^{-1})	Dry weight (g $plant^{-1}$)
25/18	0	20.2 ± 1.21a	16.2 ± 2.89a
	0.01	20.4 ± 1.24a	17.5 ± 1.82a
	0.1	21.5 ± 3.18a	18.8 ± 1.34a
	1.0	20.3 ± 1.15a	16.8 ± 0.46a
40/30	0	10.3 ± 1.24c	8.25 ± 0.90b
	0.01	17.6 ± 0.37b	11.6 ± 1.41b
	0.1	19.4 ± 1.45a	14.7 ± 0.32a
	1.0	16.9 ± 0.29b	10.2 ± 1.02b

Data are the mean of independent measurements of four replicates ± standard deviation. Values followed by different letters are significantly different at 0.05% level

The response of net photosynthesis to intracellular CO_2 was further investigated by analyzing A/C_i curves, which allowed for the determination of the maximum carboxylation rate of Rubisco (V_{cmax}) and the maximum potential rate of electron transport contributed to ribulose-1,5-bisphosphate (RuBP) (J_{max}) and the stomatal limitation to CO_2 (l) (Table 2). Similarly, EBR treatment under optimal growth conditions had no effects on V_{cmax} and J_{max}. For heat-stressed plants, V_{cmax} and J_{max} significantly decreased, whereas l increased. EBR treatment, however, increased V_{cmax} and J_{max} by 12.3% and 21.0% but decreased l by 75.2%, respectively (Table 2). The results indicated that the rate-limiting enzyme Rubisco in the Calvin cycle and other enzymes involved in RuBP regeneration were protected by EBR pretreatment and functioned well under heat stress.

EBR Effects on Chlorophyll Fluorescence Parameters

The maximum quantum efficiency of photosystem II (PSII), as given by F_v/F_m, remained 0.83 and was not

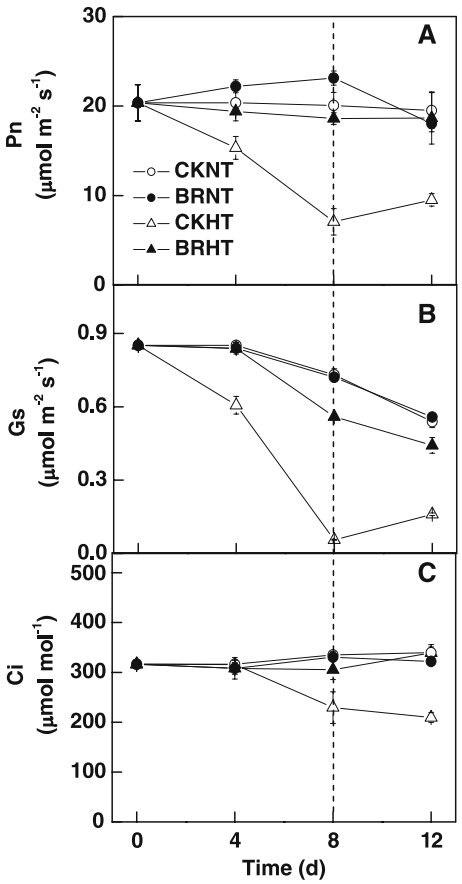

Fig. 1 Effects of 24-epibrassinolide (0.1 mg L^{-1}) and growth temperature on the photosynthetic rate P_n (**A**), stomatal conductance G_s (**B**), and intercellular CO_2 concentration C_i (**C**) in tomato leaves. The vertical dashed line indicates the transfer of plants to normal conditions for recovery. Data are the mean of three independent measurements of three plants ± standard deviation shown by vertical error bars

influenced by EBR treatment under optimal growth conditions. F_v/F_m started to decline slightly after 4 days of high-temperature treatment. Such declines, however, were not observed in EBR-treated plants (Fig. 2A). Accordingly, slight photoinhibition occurred in heat-stressed plants (CKHT) but was prevented by EBR pretreatment (BRHT).

Φ_{PSII} is a product of the proportion of open PSII reaction centers (q_P) and the efficiency of the excitation energy capture by open PSII reaction centers (F_v'/F_m') (Genty and others 1989). Φ_{PSII}, q_P, and F_v'/F_m' remained fairly uniform throughout the experiment for all treatments except CKHT, where Φ_{PSII} was reduced by 34.0% 8 days after heat treatment. The reduction of Φ_{PSII} was attributed to decreases in both q_P and F_v'/F_m' (Fig. 2B). Interestingly, EBR treatment significantly alleviated the heat-induced reductions in Φ_{PSII}, q_P, and F_v'/F_m' (Fig. 2B, C, D).

NPQ was not influenced by EBR treatment under optimal growth conditions but was significantly increased by heat stress. However, no such increase was found in EBR-treated plants (Fig. 2E). For CKHT plants, NPQ significantly increased by 4 days and 8 days by more than 50%. At 8 days there was no significant difference between the EBR treatments and the untreated plants under normal temperature (CKNT); however, there was a significant difference between the three treatments and CKHT, even after 4 days of recovery (Fig. 2E). Accordingly, heat-stressed plants had less PSII photochemical quenching and higher nonphotochemical quenching, and the improved photosynthetic CO_2 assimilation via EBR application in heat-stressed plants resulted in an increased PSII efficiency.

Table 2 Effects of 24-Epibrassinolide (0.1 mg L^{-1}) on the Maximum Carboxylation Rate of Rubisco (V_{cmax}), the Maximum Potential Rate of Electron Transport Contributed to Ribulose-1,5-bisphosphate (RuBP) (J_{max}), and the Stomatal Limitation (l) to CO_2 in Tomato Leaves as Influenced by High Temperatures

Treatment	V_{cmax} (μmol m^{-2} s^{-1})	J_{max} (μmol m^{-2} s^{-1})	l (%)
CKNT	90.0 ± 3.1 a	175.9 ± 9.3 a	28.0 ± 3.5 c
BRNT	90.2 ± 5.1 a	177.8 ± 10.2 a	31.0 ± 2.9 b
CKHT	73.1 ± 2.4 c	131.6 ± 3.0 c	47.8 ± 3.3 a
BRHT	81.7 ± 2.0 b	158.8 ± 2.4 b	27.3 ± 3.4 c

Results are expressed as the mean of three independent measurements of three plants ± standard deviation. Values followed by different letters are significantly different at 0.05% level

EBR Effects on the Activities of Antioxidant Enzymes, Hydrogen Peroxide, and MDA Contents

Activities of SOD, APX, GPOD, and CAT significantly increased after heat stress. The increases in SOD and GPOD were gradual up to 4 days, doubling at 8 days for high-temperature treatments, whereas APX and CAT increased up to three times from 0 days to 4 days (Fig. 3). CAT activity, in contrast, increased moderately under high-temperature stress after 4 days (Fig. 3D). In general, EBR increased SOD, APX, GPOD, and CAT activities under both high- and normal-temperature treatments.

Changes in antioxidant enzymes were accompanied by significant changes in H_2O_2 and MDA contents. H_2O_2 content significantly increased under a high-temperature stress regime, and nearly doubled after 4 days of CKHT treatment (Fig. 4A). EBR treatment decreased H_2O_2 content under both optimal growth conditions and high-temperature stress conditions. There was also no significant difference in H_2O_2 content between BRHT and CKNT after 4 days of recovery. Similarly, the contents of MDA were significantly reduced in plants treated with EBR under high temperatures (Fig. 4B). It is clear that heat

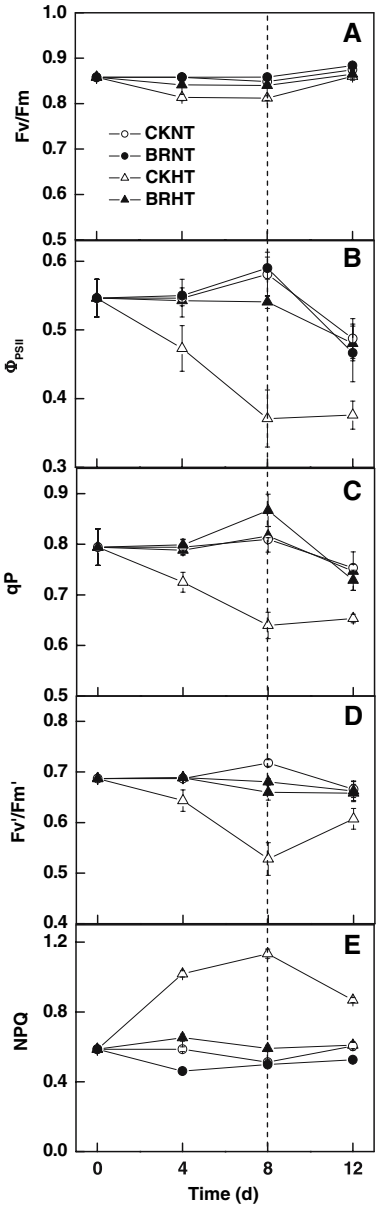

Fig. 2 Effects of 24-epibrassinolide (0.1 mg L^{-1}) and growth temperatures on F_v/F_m (**A**), Φ_{PSII} (**B**), q_P (**C**), F_v'/F_m' (**D**), and NPQ (**E**) in tomato leaves. The vertical dashed line indicates the transfer of plants to normal conditions for recovery. Data are the mean of three independent measurements ± standard deviation shown by vertical error bars

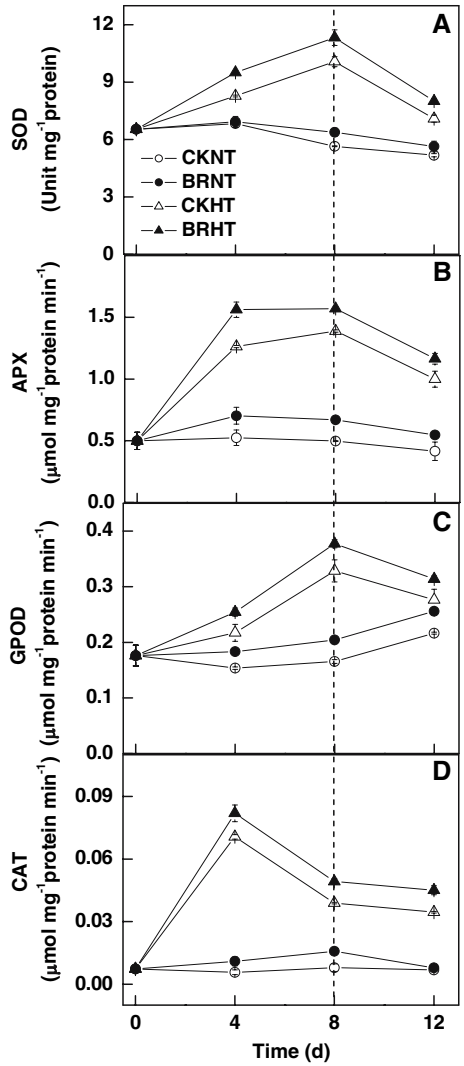

Fig. 3 Changes in the activities of SOD, APX, GPOD, and CAT in tomato leaves as influenced by growth temperatures and 24-epibrassinolide (0.1 mg L^{-1}) application. The vertical dashed line indicates the transfer of plants to normal conditions for recovery. Data are the mean of three independent measurements ± standard deviation shown by vertical error bars

stress increased lipid peroxidation in plants and this was significantly alleviated by EBR treatment.

Discussion

In our previous studies we reported that EBR could ameliorate the effects of chill-induced inhibition of photosynthesis and the enhancement of photosynthesis in *Cucumis sativus* under optimal growth temperatures (Yu and others 2002, 2004). In the present study we found that BRs are also involved in the protection of the photosynthetic apparatus under conditions of high-temperature stress in tomato because EBR-treated plants exhibited higher biomass accumulation and P_n after exposure to high-temperature stress (Table 1). This is in agreement with early findings that EBR-treated tomato plants are more tolerant of high temperature than those that are untreated (Dhaubhadel and others 1999; Singh and Shono 2005).

High-temperature stress resulted in a significant decline in P_n, and the decrease was accompanied by a significant decrease in C_i (Fig. 1), implying that both stomatal and nonstomatal factors were responsible for this reduction in

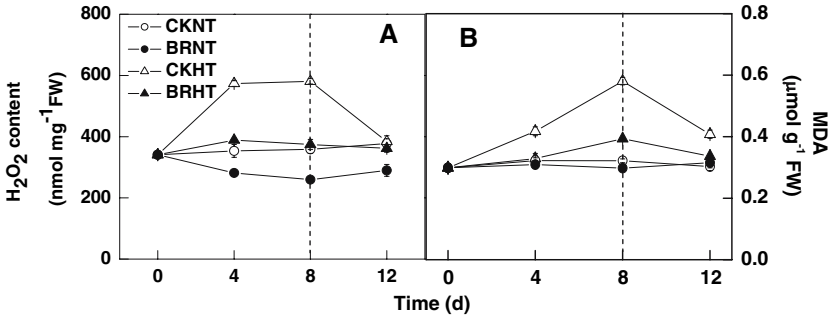

Fig. 4 Changes in the contents of H_2O_2 and lipid peroxidation (expressed as MDA content) in tomato leaves as influenced by growth temperatures and 24-epibrassinolide (0.1 mg L^{-1}) application. The vertical dashed line indicates the transfer of plants to normal conditions for recovery. Data are the mean of three independent measurements ± standard deviation shown by vertical error bars

photosynthesis (von Caemmerer and Farquhar 1981). There is evidence that photosynthetic performance is largely determined by the Rubisco activation state at high temperature (Takahama and Oniki 1992; Sharkey 2006; Yamori and others 2006). The inactivation or loss of Rubisco would reduce the carboxylation efficiency (V_{cmax}), whereas a reduction in J_{max} is associated with a diminution of other Calvin-cycle enzymes (for example, sedoheptulose-1,7-bisphosphate, a key regulatory enzyme of the Calvin cycle) (Allen and others 1997; Nogués and Baker 2000; Ölcer and others 2001; Lefebvre and others 2005). In our previous studies we reported that EBR increases photosynthesis mainly by increasing the Rubisco activation state (Yu and others 2004). In agreement with these early findings, there were significant decreases in V_{cmax} and J_{max} (Table 1), indicating that EBR greatly increased the carboxylation efficiency and the ability to regenerate RuBP under high-temperature stress. RuBP regeneration can be limited by either the inability of electron transport to supply reductants and ATP or by an inactivation or loss of Calvin-cycle enzymes other than Rubisco (Baker and others 1997). Because change in V_{cmax} was associated with change in PSII efficiency (Table 2, Fig. 2B), we could attribute the change in Φ_{PSII} to the change in carboxylation efficiency resulting from a downstream regulating mechanism. A slight increase in q_P for BRHT could be attributed to an increase in the rate of reductant consumption and ATP produced by noncyclic electron transport relative to the rate of excitation of open PSII reaction centers (Nogués and Baker 2000). We also found that there was a less pronounced decrease in F_v'/F_m' in EBR-treated plants than in non-EBR-treated plants under high-temperature-stress conditions (Fig. 2D), suggesting that the application of EBR resulted in less dissipation of excitation energy in the PSII antennae (Horton and others 1996; Gilmore 1997). As observed by Leakey and others (2003), NPQ increased markedly in heat-stressed leaves (Fig. 2). The significantly reduced and stable values for the BRHT indicate that the application of EBR protected the PSII against overexcitation under high-temperature stress, which could have caused damage, perhaps from a loss of integrity in the thylakoid membrane, compared with the control under high temperatures. It is known that the thylakoid membrane is also very sensitive to high temperature (Haldimann and Feller 2005).

In addition to nonradiative dissipation, the ROS scavenging system is also believed to play an important role in protecting cells from oxidative damage. As observed by Rivero and others (2004), heat stress induced overall increases in the activities of SOD, APX, GPOD, and CAT and the contents of H_2O_2 and MDA, suggesting that heat stress resulted in lipid peroxidation in these plants. Several Calvin-cycle enzymes within chloroplasts are extremely sensitive to high levels of H_2O_2, causing reductions in CO_2 fixation and foliar biomass (Willenkens and others 1997; Zhou and others 2004, 2006). Interestedly, EBR treatment induced further increases in the activities of SOD, APX, GPOD, and CAT, but it reduced H_2O_2 and MDA contents in the leaves of tomato plants subjected to high-temperature treatment. All these results suggest that EBR could protect the photosynthetic apparatus from oxidative stress induced by high-temperature stress. It has been shown that BRs can induce the expression of some antioxidant genes and enhance the activities of antioxidant enzymes such as SOD, POD, and CAT (Mazzora and others 2002; Núñez and others 2003; Cao and others 2005). However, it is still unclear whether BRs directly or indirectly modulate the responses of plants to oxidative stress (Cao and others 2005). Nevertheless, although BRs and ROS are thought to act as secondary messengers for the induction of antioxidant defenses in stressed plants (Mazzora and others 2002), the relationship between BRs and ROS in stress-signal transduction still remains unclear. Detailed studies at the molecular level would be necessary to elucidate the mechanism by which endogenous and exogenous BRs regulate the stress response.

In conclusion, this study showed an interesting activity of BRs in the stress response that should be important not

only for a basic understanding of the role of the hormone but also for potential use of the chemical in agriculture. We have found that high-growth temperatures resulted in reduced carboxylation efficiency, increased antioxidant enzyme activity, and lipid peroxidation, leading to a downregulation of PSII activity. EBR pretreatment effectively alleviated the heat-induced growth inhibition, mainly by increasing carboxylation efficiency and increasing the activity of the ROS scavenging system.

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