PHOTOSYNTHETICA 48 (4): 537-544, 2010

Chill-induced inhibition of photosynthesis was alleviated by 24-epibrassinolide pretreatment in cucumber during chilling and subsequent recovery

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Abstract

To investigate whether brassinosteroids (BRs) could be used to alleviate chill-induced inhibition of photosynthesis in cucumber (*Cucumis sativus* L) during chilling and subsequent recovery, the effects of exogenously applied 24-epibrassinolide (EBR) on gas exchange, chlorophyll fluorescence parameters, and antioxidant enzyme activity were studied. Cucumber plants were exposed to chilling under low light (12/8°C and 100 μmol m^{-2} s^{-1} PPFD) for 3 days and then recovered under normal temperature and high irradiance (28/18°C and 600 μmol m^{-2} s^{-1} PPFD) for 6 days. Chilling significantly decreased the net photosynthetic rate (P_N) and stomatal conductance (g_s), and increased rate of $O_2^{\bullet-}$ formation and H_2O_2 and malondialdehyde (MDA) content in cucumber leaves, but did not influence the optimal quantum yield of PSII (F_v/F_m). Chilling also decreased the effective quantum yield of PSII photochemistry (Φ_{PSII}) and photochemical quenching (q_P), but induced an increase in nonphotochemical quenching (NPQ), and the activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX). High irradiance (600 μmol m^{-2} s^{-1}) further aggravated the decrease in P_N, g_s, Φ_{PSII} and q_P, and enhanced the increase in reactive oxygen species (ROS) generation and accumulation in the first day of recovery after chilling. However, high irradiance induced a sharp decrease in F_v/F_m and NPQ, as well as the activities of SOD and APX on the first day of recovery. EBR pretreatment significantly alleviated chill-induced inhibition of photosynthesis during chilling stress and subsequent recovery period, which was mainly due to significant increases in g_s, Φ_{PSII}, q_P and NPQ. EBR pretreatment also reduced ROS generation and accumulation, and increased the activities of SOD and APX during chilling and subsequent recovery. Those results suggest that EBR pretreatment alleviates the chill reduction in photosynthesis and accelerated the recovery rate mainly by increasing of the stomatal conductance, the efficiency of utilization and dissipation of leaf absorbed light, and the activity of the ROS scavenging system during chilling and subsequent recovery period.

Additional key words: antioxidant enzymes; brassinosteroids; chlorophyll fluorescence; chilling stress; *Cucumis sativus* L.; photosynthesis; reactive oxygen species.

Introduction

Chilling is a major limiting factor for growth and development of many tropical and subtropical crops such as cucumber and tomato (Brüggemann *et al*. 1992, Allen and Ort 2001, Zhou *et al*. 2007). Photosynthesis is susceptible to chilling and often it is the first physiological metabolic process inhibited at chilling temperature (Berry and Björkman 1980). Under low temperatures, stomatal closure (Allen *et al*. 2000), inhibition of thylakoid electron transport and the loss of activity of Calvin cycle enzymes such as ribulose-1,5-bisphosphate

Received 28 January 2010, *accepted* 6 August 2010.
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Abbreviations: APX – ascorbate peroxidase; BRs – brassinosteroids; EBR – 24-epibrassinolide; F_m' – light-adapted maximum fluorescence; F_o – minimal fluorescence of dark-adapted state; F_m – maximal fluorescence of dark-adapted state; F_v/F_m – optimal quantum yield of PSII; FM – fresh mass; g_s – stomatal conductance; LT – low temperature; LTBR – low temperature; EBR-pretreatment; MDA – malondialdehyde; NT – normal temperature; NTBR – normal temperature/EBR-pretreatment; NPQ – nonphotochemical quenching; Φ_{PSII}, – effective quantum yield of PSII photochemistry; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; q_P – photochemical quenching coefficient; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; ROS – reactive oxygen species; SOD – superoxide dismutase.
Acknowledgments: This work was supported by the National Key Technology R&D Program (2008BADA6B02), National Natural Science Foundation of China (30860175), Program for New Century Excellent Talents in University in the China (NCET-08-0703) and Natural Science Foundation of Jiangxi Province (2007GZN1666).

carboxylase/oxygenase (Rubisco) (Brüggemann *et al*. 1992, Allen *et al*. 2000), or damage of photosystem I PSI) (Barth and Krause 1999) appear predominant.

Because of temperature sensitivity of Calvin cycle enzymes, chilling inhibits leaf net photosynthetic rate more than the photochemical activities and this causes an imbalance in the utilisation of the absorbed energy for photosynthetic carbon reduction and other processes (Powles 1984, Alam and Jacob 2002). The problem of imbalance in energy utilisation results in an excess of the absorbed light energy under chilling stress (Jung *et al*. 1998, Kościelniak and Biesaga-Kościelniak 2006). The excess energy in the photosynthetic apparatus causes formation of various reactive oxygen species (ROS), which results in an oxidative stress in plant cells (Asada 1999, Alam and Jacob 2002, Mittler 2002). Plants have evolved several mechanisms, including xanthophyll cycle-dependent energy dissipation as heat from the antenna of photosystem II (PSII), which decreases ROS production caused by excess energy in chloroplasts (Weng *et al*. 2006, Hu *et al*. 2008). Plants also possess an efficient scavenging system for ROS to protect cells from destructive oxidative stress (Mittler 2002).

Although the negative effects of chilling on photosynthesis have been well documented, there are few studies on the effect of irradiance on photosynthesis during the recovery period after chilling (Brüggemann *et al*. 1992, Allen and Ort 2001, Zhou *et al*. 2004). And some chill-induced damage symptoms can only be seen after returning the chilled plants to higher temperatures (Janda *et al*. 1996, Szalai *et al*. 1996). Martin and Ort (1985) observed that photosynthesis was fully recovered within 12 h after returning chilled tomato to 25°C in the dark, however, bright light subsequent to the chilling exposure delayed the recovery of photosynthesis. Janda *et al*. (1996) also found that F_v/F_m of maize hardly changed during chilling or when returned to a nonchilling temperature in the dark, but there was a decrease in this parameter if the plants were shifted to light after the cold treatment. It suggests that high irradiance further aggravated the chill-induced injury of photosynthetic process during the recovery period after chilling stress. So it is important to study how to alleviate the damage caused by excess absorption of light energy during the recovery period after chilling.

As a highly promising and environmentally-friendly class of plant hormone, brassinosteroids (BRs), have been widely applied in order to promote yield and to protect against environmental stress in agriculture (Khripach *et al*. 2000, Krishna 2003). BRs can induce plant tolerance to a variety of abiotic stresses, such as high- and low-temperature stress (Yu *et al*. 2002b, Ogweno *et al*. 2008), drought (Li *et al*. 1998, Zhang *et al*. 2008), salinity (Anuradha and Rao 2003, Ali *et al*. 2008), and oxygen deficiency (Ershova *et al*. 1996). However, the mechanisms for BRs-mediated stress relief are still poorly understood. In our previous study, we found that 24-epibrassinolide (EBR) was effective in increasing of photosynthesis (Yu *et al*. 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 (Ogweno *et al*. 2008). Xia *et al*. (2009) also found that EBR-induced stress tolerance was accompanied with an increase in ROS-scavenging enzyme activity. These results suggest that BRs may alleviate stress injury though amelioration of the balance in utilisation and absorption of energy in chloroplasts.

In this study, we exposed cucumber plants to chilling stress and investigated whether EBR pretreatment could induce tolerance to chilling, and whether the induced tolerance is associated with the protection of the photosynthetic process during chilling and subsequent recovery period. Accordingly, CO_2 assimilation, chlorophyll fluorescence, ROS accumulation, and activities of SOD and APX were determined in chilled cucumber leaves during chilling and the subsequent recovery period.

Materials and methods

Plants: Cucumber (*Cucumis sativus* L. cv. Jinyan No 4, from Tianjin Cucumber Institute, China) seeds were sown in a medium containing a mixture of soil and perlite (50:50, v/v) in tray in a greenhouse. Seven days later, groups of 6 seedlings were transferred into a container (40 × 25 × 15 cm) filled with half-strength Enshi nutrient solution (Yu and Matsui 1997) in a growth chamber. The environmental conditions were as follows: a 12-h photoperiod, temperature of 28/18°C (day/night), photosynthetic photon flux density (PPFD) of 600 μmol m^{-2} s^{-1} provided by high-pressure sodium lamps (*Nanjing Ningbao Lighting Appliance Industry Co., Ltd*. China).

EBR pretreatment and chilling treatment: The 24-epibrassinolide (*EBR, Sigma*, USA) and chilling treatment started when plants were in a 3-leaf stage. On the day before chilling treatment, plants were divided into two groups. One group was transferred to the growth chamber with a 12-h photoperiod and 600 μmol m^{-2} s^{-1} PPFD at 28/18°C (normal temperature, NT). The other group was transferred at the beginning of the photoperiod (6:00 h) to a cold chamber (*QHY-300BS-III, Shanghai CIMO Medical Instrument Manufacturing Co., Ltd*., China) with a 12-h photoperiod and 100 μmol m^{-2} s^{-1} PPFD and temperature of 12/8°C (low temperature, LT). Both groups of plants were sprayed with 0.1 mg(EBR) L^{-1} or distilled water (containing the same concentration of ethanol as the controls) before chilling treatments. EBR was dissolved in a minimal volume of ethanol, then made up to volume with distilled water. The chilling treatments lasted 3 days and the plants were transferred at the beginning of the photoperiod (6:00 h) to the growth

chamber for 6 days. The four treatments employed were: (*1*) control, NT/ water-pretreatment; (*2*) normal temperature/EBR-pretreatment (NTBR); (*3*) LT/ water-pretreatment; and (*4*) low temperature/EBR-pretreatment (LTBR).

Throughout the experiment, gas exchange and chlorophyll fluorescence measurements were performed at 9:00-11:00 h on the third leaf randomly with three replicates. At the same time, 0.5 g of the fresh mass (FM) of the third leaf was sampled and immediately frozen under liquid N_2, and then stored at –80°C for further analysis of MDA content, ROS, and antioxidant enzyme activities.

Gas exchange and chlorophyll fluorescence measurements: Chilled plants were transferred to the growth chamber for 2 h before measurement. Leaf gas exchange was measured by a portable photosynthesis measurement system (*CIRAS-1, PP-System*, Hitchin, Hertfordshire, UK). The air temperature, relative humidity, CO_2 concentration, and PPFD were maintained at 28°C, 85%, 360 μmol mol^{-1} and 600 μmol m^{-2} s^{-1}, respectively.

Meanwhile, chlorophyll fluorescence was measured with a portable pulse modulated fluorometer (*FMS-2, Hansatech*, King´s Lynn, Norfolk, UK) in the same leaves previously used for gas-exchange measurements. Before each measurement leaves were maintained in darkness for 30 min. Minimal fluorescence (F_o) was measured under a weak pulse of modulating light over a 0.8-s period, and maximal fluorescence (F_m) was induced by a saturating pulse of light (8,000 μmol m^{-2} s^{-1}) applied over 0.8 s. The optimal quantum yield of PSII was determined as F_v/F_m, where F_v is the difference between F_o and F_m. An actinic light source (600 μmol m^{-2} s^{-1}) was then applied to achieve a steady state of photosynthesis and to obtain F_s (steady-state fluorescence yield), after which a second saturation pulse was applied for 0.8 s to obtain light-adapted maximum fluorescence (F_m'). The quantum efficiency of PSII (Φ_{PSII}), photochemical quenching coefficient (q_P), and non-photochemical quenching (NPQ) were calculated as $(F_m' - F_s)/F_m'$, $(F_m' - F_s)/(F_m' - F_o)$, and $F_m/F_m' - 1$, respectively (Demmig-Adams *et al.* 1996).

Determination of lipid peroxidation and ROS: MDA content was measured according to the method of Hu *et al*. (2006). The leaf samples (0.5 g of FM) were homogenized with inert sand in 80:20 (v/v) ethanol/water, followed by centrifugation at 3,000 × *g* for 10 min. A diluted sample of approximately 1 ml aliquot was added to a test tube with an equal volume of either (*a*) – TBA solution containing 20% (w/v) trichloroacetic acid and 0.01% (w/v) butylated hydroxytoluene, or (*b*) + TBA solution containing the above plus 0.65% (w/v) TBA. The mixtures were then heated in boiling water (95°C) for 25 min. The reaction was ended by placing the reaction tubes in an ice bath. The samples were centrifuged at 3,000 × *g* for 10 min, and the absorption of the supernatant was read at 440, 532, and 600 nm. The MDA equivalents were calculated according to the method of Hodges *et al.* (1999).

The rate of $O_2^{\cdot-}$ formation and the H_2O_2 content were analyzed as described by Zhou *et al*. (2004). In general, $O_2^{\cdot-}$ was measured by monitoring the nitrite formation from hydroxylamine in the presence of $O_2^{\cdot-}$. The samples (0.5 g of FM) were homogenized with 3 mL of 65 mM potassium phosphate buffer (pH 7.8) and centrifuged at 5,000 × *g* for 10 min. The incubation mixture contained 0.9 mL of 65 mM potassium phosphate buffer (pH 7.8), 0.1 mL of 10 mM hydroxylamine hydrochloride, and 1 mL of the supernatant. After incubation at 25°C for 20 min, ethyl ether in the same volume was added and centrifuged at 1,500 × *g* for 5 min. The absorbance in the aqueous solution was read at 530 nm. A standard curve with NO_2^- was used to calculate the rate of $O_2^{\cdot-}$ formation from the chemical reaction of $O_2^{\cdot-}$ and hydroxylamine. H_2O_2 content was assayed diluted 2.5-fold with acetone and was measured by monitoring the absorbance at 410 nm of the titanium-peroxidase complex.

Antioxidant enzyme activity determination: In order to detect the change in the activity of SOD and APX, which are the two major ROS-scavenging enzymes in plant cells, 0.5 g of leaf FM was homogenized in 3 ml of 25 mM HEPES buffer (pH 7.8) containing 0.2 mM EDTA, 2 mM ascorbic acid (AsA) and 2% (w/v) polyvinylpyrrolidone (PVP). The homogenate was centrifuged for 20 min at 12,000 × *g* and the supernatant obtained was used for enzyme analysis. All operations were carried out at 0–4°C. An aliquot of the extract was used to determine its protein content by the method of Bradford (1976) utilizing bovine serum albumin as standard.

SOD activity was measured by the photochemical method as described by Giannopolitis and Ries (1977). One unit of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition of the rate of ρ-nitro blue tetrazolium chloride reduction at 560 nm. APX activity was measured according to the method of Nakano and Asada (1981) by monitoring the rate of ascorbate oxidation at 290 nm (using an extinction coefficient of 2.8 mM cm^{-1}). The reaction mixture contained 25 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 100 mM H_2O_2, and 0.25 mM AsA, and the enzyme aliquot.

Results

Gas-exchange parameters: Under NT growth conditions, EBR significantly increased P_N and g_s, and the increases lasted approximately 4–6 d. Chilling significantly reduced P_N and g_s, however, the reductions were significantly alleviated by pretreatment with EBR. P_N and g_s were decreased sharply in the first day of recovery after chilling in plants both untreated and treated with EBR. P_N and g_s of low temperature (LT) treated leaves did not recover to control values, however, P_N and g_s of 24-epibrassinolide treated (LTBR) leaves recovered to control values, when the plants were allowed to return to normal temperature for 3 days (Fig. 1).

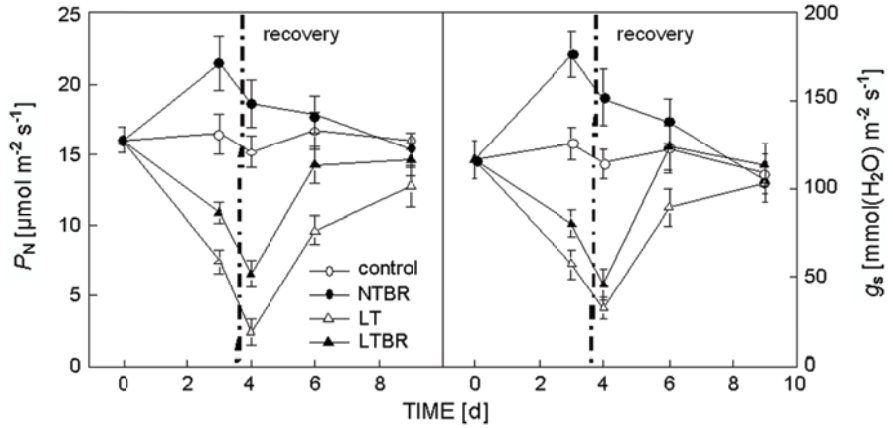

Fig. 1. Effects of 24-epibrassinolide (0.1 mg L^{-1}) on gas exchange in cucumber leaves during chilling and subsequent recovery period. The *vertical dashed line* indicates the transfer of plants to normal temperature growth conditions for recovery. Data are the means of three replicates with standard errors shown by *vertical bars*. LT – low temperature; NTBR – normal temperature/EBR-pretreatment; LTBR – low temperature/EBR-pretreatment.

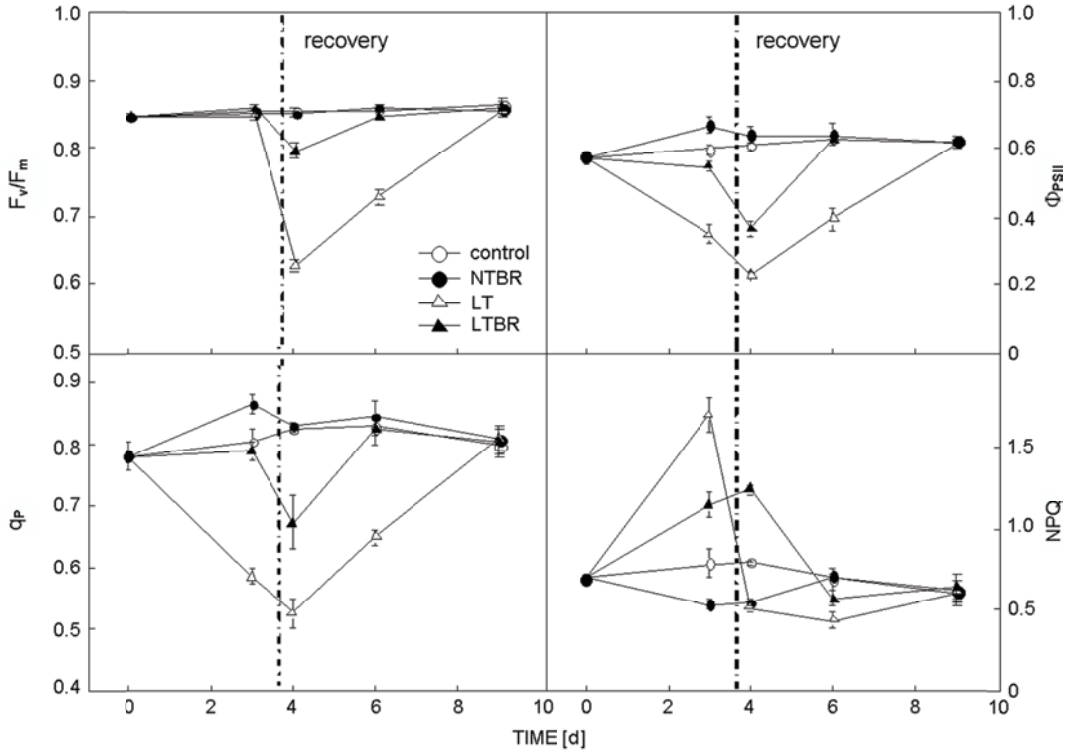

Fig 2. Effects of 24-epibrassinolide (0.1 mg L^{-1}) on optimal quantum yield of PSII (F_v/F_m), effective quantum yield of PSII photochemistry (Φ_{PSII}), photochemical quenching coefficient (q_P), and nonphotochemical quenching (NPQ) in cucumber leaves during chilling and subsequent recovery period. The *vertical dashed line* indicates the transfer of plants to normal temperature growth conditions for recovery. Data are the means of three replicates with standard errors shown by *vertical bars*. LT, NTBR, LTBR – *see* Fig. 1.

Chlorophyll fluorescence parameters: The changes induced in F_v/F_m, Φ_{PSII}, q_P, and NPQ on exposure of cucumber plants to chilling and subsequent recovery are shown in Fig. 2. Compared with control plants, EBR pretreatment increased Φ_{PSII} and q_P and decreased NPQ during the chilling treatment (3 d), but did not influence F_v/F_m. For LT plants, chilling did not induce a decrease in F_v/F_m, but significantly decreased Φ_{PSII} and q_P. On the first day of recovery, a continued decrease in F_v/F_m, Φ_{PSII}, and q_P in LT plants was observed, and they did not fully recover to the control values even after 3 d of recovery. However, only a slight decrease in F_v/F_m occurred in LTBR plants, and it recovered dramatically to the control level after 3 d of recovery. EBR pretreatment also significantly alleviated the reduction of Φ_{PSII} and q_P induced by chilling, and was beneficial to recovery after chilling. During chilling treatment, NPQ increased significantly, especially in the plants untreated with EBR. However, NPQ in LT leaves sharply decreased to lower values than in control leaves during the recovery period, and recovered completely after 6-d recovery. On the contrary, LTBR plants significantly maintained higher NPQ than control plants on the first day of recovery, and recovered to the control level after 3 days of recovery.

Membrane permeability and ROS: There were no significant differences in MDA content between plants treated and untreated with EBR under NT. Chilling increased MDA content, and the increase of MDA was maintained in the first days of recovery. Compared with LT plants, EBR decreased MDA content during chilling and subsequent recovery period. Similarly, the content of H_2O_2 and rate of $O_2^{\cdot-}$ formation were significantly increased by chilling stress, and EBR pretreatment significantly alleviated the increase in H_2O_2 content and the rate of $O_2^{\cdot-}$ formation (Fig. 3).

Activities of antioxidant enzymes: Activities of SOD and APX significantly increased after chilling stress. During the recovery period, activities of SOD and APX decreased sharply with prolongation of the recovery time to reach the control values. Compared with LT plants, EBR pretreatment significantly increased SOD and APX activities during chilling and recovery period. However, activities of SOD and APX in EBR-pretreated plants grown under NT showed no significant differences (Fig. 4).

Discussion

Decrease in P_N under stress conditions is usually associated with a decrease in the demand for ATP and NADPH, leading to an overproduction of ROS and oxidative stress (Foyer *et al*. 2002, Ort and Baker 2002, Zhou *et al*. 2004). We observed that chilling under low light significantly decreased P_N (Fig. 1) and increased ROS generation and accumulation (Fig. 3) in cucumber leaves. However, we also paid attention to the fact that there was no change in F_v/F_m in cucumber leaves on chilling under low light (Fig. 2), which implied that chilling under low light inhibited photosynthesis but did not damage the photosynthetic apparatus. This is in agreement with an early finding that PSII is relatively stable to chilling under low light (Yu *et al*. 2002a, Zhou *et al*. 2004). We also found that chilling greatly decreased Φ_{PSII} and q_P, this decrease was accompanied with significant increase of NPQ, and activities of SOD and APX (Figs. 1, 2, 4). There is evidence that protection mechanisms, such as xanthophyll cycle-dependent energy dissipation as heat from antenna in PSII, and ROS-scavenging enzymes are important to protect chloroplasts from damage under environmental stress (Demmig-Adams *et al*. 1996, Asada 1999, Hu *et al*. 2008).

Although there are many researches about effects of chilling on photosynthesis under low light, the effects of irradiance on photosynthesis during the recovery period are still very fragmentary. Chill-reduced photosynthesis can lead to a decrease in irradiance utilization and the accumulation of excessive photon energy (Powles 1984, Janda *et al*. 1996, Feng and Cao 2005). If there is still excess energy, plants will produce ROS, which cause peroxidation of membrane lipids and destruction of the photosynthetic apparatus (Foyer *et al*. 1994). So during the recovery period after chilling, exposure of chilled plants to an irradiance higher than that which can be utilized in the photosynthetic process may damage the photosynthetic apparatus, especially PSII (Janda *et al*. 1996). In our present study, the chilling-induced inhibition in P_N was further aggravated by high irradiance during the first day of recovery period (Fig. 1). Along with the decrease of P_N, high irradiance also resulted in acceleration of the decrease of F_v/F_m, Φ_{PSII}, q_P and NPQ in chilled plants (Fig. 2). Greater photoinhibition in chilled plants could be related to higher $O_2^{\cdot-}$ formation rate, and H_2O_2 and MDA content (Fig. 3). MDA is the product of membrane lipid peroxidation, and is also toxic to the photosynthetic apparatus (Feng and Cao 2005). These patterns indicated that enhanced photoinhibition or photodamage induced by high irradiance during the first day of recovery period was partly associated with decreased thermal dissipation and with increased ROS generation and accumulation, which may damage the photosynthetic apparatus (Janda *et al*. 1996, Feng and Cao 2005).

The ability of BRs to increase plant resistance against environmental stresses has been explored under laboratory-, greenhouse-, and field conditions, however, the mechanisms by which BRs induce stress tolerance remain largely unexplored (Krishna 2003). In this study, we found that EBR was involved in the protection of the photosynthesis under chilling stress in cucumber because EBR-pretreated plants exhibited higher P_N and F_v/F_m, and

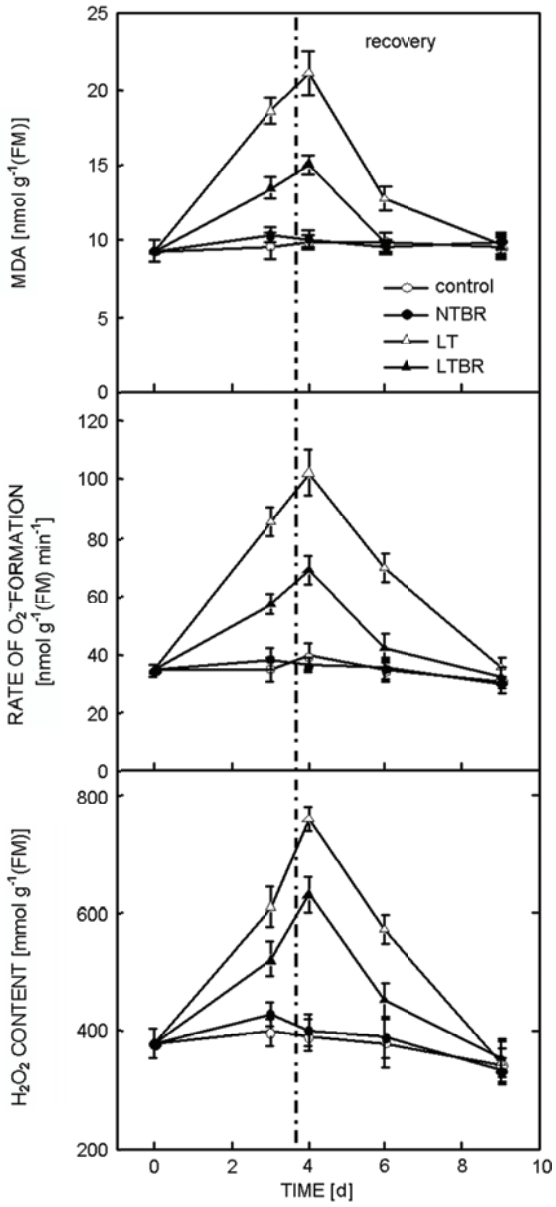

Fig 3. Effects of 24-epibrassinolide (0.1 mg L^{-1}) on malondialdehyde (MDA) content, rate of $O_2^{\cdot-}$ formation and H_2O_2 content in cucumber leaves during chilling and subsequent recovery period. The *vertical dashed line* indicates the transfer of plants to normal temperature growth conditions for recovery. Data are the means of three replicates with standard errors shown by *vertical bars*. LT, NTBR, LTBR – *see* Fig. 1.

lower MDA content in cucumber leaves during chilling stress and the subsequent recovery period (Figs. 1, 2, 3). This is in agreement with the results of Yu *et al*. (2002b) who observed that EBR-treated cucumber plants were more tolerant of chilling than untreated plants.

Chilling resulted in a significant decline in P_N, and the decrease was accompanied by a significant decrease in g_s, and high light during the subsequent recovery period enhanced the decrease of P_N and g_s (Fig. 1), which implied that stomatal limitation was responsible for this reduction in photosynthesis (von Caemmerer and Farquhar 1981). However, we found that there was a lower decrease in P_N and g_s in LTBR plants than in LT ones during chilling and subsequent recovery period (Fig. 1), suggesting that EBR could protect photosynthetic activity under chilling and subsequent recovery period with high light by ameliorated stomatal limition.

In our previous studies we reported that EBR increases photosynthesis mainly by increasing the efficiency of light utilization and the capacity of CO_2 assimilation in Calvin cycle (Yu *et al*. 2004). In our present study, we also observed that there was significantly higher Φ_{PSII} and q_P in LTBR plants than in LT ones during chilling stress and the subsequent recovery period (Figs. 1, 2). This indicated that EBR pretreatment alleviated the chill-reduced photosynthesis and may be particularly attributed to the increases in capacity of CO_2 assimilation and the efficiency of light utilization. We also found that there was a significant increase in NPQ in EBR-pretreatment plants compared to non-EBR-pretreatment plants under chilling stress conditions and the first day of subsequent recovery (Fig. 2), suggesting that the

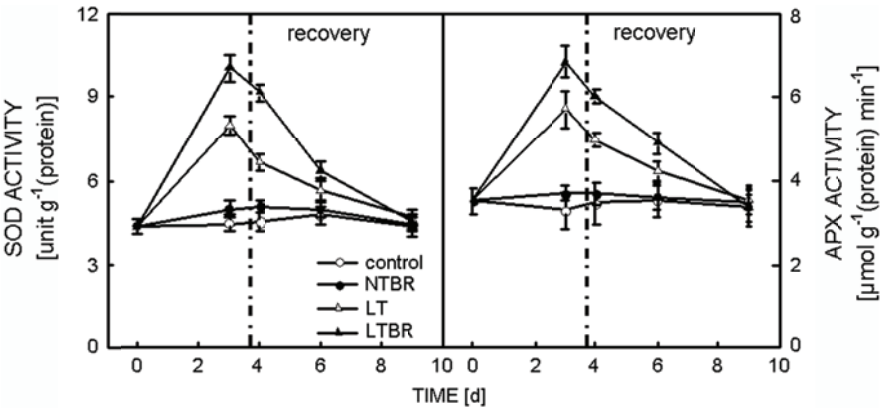

Fig 4. Effects of 24-epibrassinolide (0.1 mg L^{-1}) on the activities of SOD and APX in cucumber leaves during chilling and subsequent recovery period. The *vertical dashed line* indicates the transfer of plants to normal temperature growth conditions for recovery. Data are the means of three replicates with standard errors shown by *vertical bars*. LT, NTBR, LTBR – *see* Fig. 1.

application of EBR protected PSII against overexcitation by dissipation of excitation energy in the PSII antennae under chilling and high irradiance during the subsequent recovery period (Demmig-Adams *et al*. 1996).

The production and accumulation of ROS were apparent in the leaves of LTBR plants (Fig. 3), in spite of EBR increased efficiency of light utilization and thermal dissipation in LTBR plants during chilling and subsequent recovery period. Plants also evolved efficient antioxidant enzymes, such as SOD and APX, to scavenge ROS, these enzymes are postulated to be involved in the stress tolerance of plants (Mittler 2002). Some studies have shown that exogenous BRs can induce the expression of some antioxidant genes and enhance the activities of antioxidant enzymes such as SOD and APX (Mazorra *et al*. 2002, Ogweno *et al*. 2008, Shahbaz *et al*. 2008, Liu *et al*. 2009). Compared with chilled plants, EBR pretreatment induced a further increase in the activities of SOD and APX (Fig. 4), but reduced H_2O_2 and MDA contents (Fig. 3) in LTBR plants during chilling and subsequent recovery period. From these results, it can be suggested that EBR could protect the photosynthetic process from oxidative stress induced by chilling or high-irradiance stress through enhancing the antioxidant defense system.

In summary, our study shows that high irradiance aggravates the depression of the photosynthetic process during the recovery period after chilling. EBR pretreatment alleviated the chill-reduced photosynthesis and accelerated the recovery rate, mainly by increasing of stomatal conductance, the efficiency of light utilization and dissipation, and the activity of the ROS-scavenging system during chilling and subsequent recovery period.

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