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Foliar application of 24-epibrassinolide alleviates high-temperature-induced inhibition of photosynthesis in seedlings of two melon cultivars

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Abstract

Brassinosteroids (BRs), an important class of plant steroidal hormones, play a significant role in the amelioration of various biotic and abiotic stresses. 24-epibrassinolide (EBR), an active brassinosteroid, was applied exogenously in different concentrations to characterize a role of BRs in tolerance of melon (*Cucumis melo* L.) to high temperature (HT) stress and to investigate photosynthetic performance of HT-stressed, Honglvzaocui (HT-tolerant) and Baiyuxiang (HT-sensitive), melon variety. Under HT, Honglvzaocui showed higher biomass accumulation and a lower index of heat injury compared with the Baiyuxiang. The exogenous application of 1.0 mg L^{-1} EBR, the most effective concentration, alleviated dramatically the growth suppression caused by HT in both ecotypes. Similarly, EBR pretreatment of HT-stressed plants attenuated the decrease in relative chlorophyll content, net photosynthetic rate, stomatal conductance, stomatal limitation, and water-use efficiency (WUE), as well as the maximal quantum yield of PSII photochemistry (F_v/F_m), the efficiency of excitation capture of open PSII center, the effective quantum yield of PSII photochemistry (Φ_{PSII}), photochemical quenching coefficient, and the photon activity distribution coefficients of PSI (α). EBR pretreatment further inhibited the increase in intracellular CO_2 concentration, leaf transpiration rate, minimal fluorescence of dark-adapted state, nonphotochemical quenching, thermal dissipation, and photon activity distribution coefficients of PSII. Results obtained here demonstrated that EBR could alleviate the detrimental effects of HT on the plant growth by improving photosynthesis in leaves, mainly reflected as up-regulation of photosynthetic pigment contents and photochemical activity associated with PSII.

Additional key words: brassinosteroids; chlorophyll content; chlorophyll fluorescence; gas exchange; plant growth.

Introduction

High temperature (HT), one of the main environmental, abiotic factors, leads to severe retardation in plant growth, development, and even to death. According to a recent study, each 1ºC increase in the average temperature during the growth season may reduce the crop yield by up to 17% (Lobell and Asner 2003). Photosynthetic apparatus has been identified as the most sensitive plant component to HT stress; photochemical reactions in thylakoid lamellae and carbon metabolism in the stroma of chloroplast were shown as the primary sites of injury caused by HT (Wise *et al.* 2004). The effects of warmer temperature on photosynthesis will be one of the most

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Abbreviations: BRs – brassinosteroids; C_i – intracellular CO_2 concentration; Chl – chlorophyll; D – fraction of absorbed light used in thermal dissipation in PSII antennae; E – transpiration rate; EBR – 24-epibrassinolide; F_o – minimal fluorescence of dark-adapted state; F_o' – minimal fluorescence of light -adapted state; F_m – the maximal fluorescence of dark-adapted state; F_m' – maximal fluorescence of light-adapted state; F_s – steady-state fluorescence; F_v/F_m – maximal quantum yield of PSII photochemistry; F_v'/F_m' – efficiency of excitation capture of open PSII center; g_s – stomatal conductance; L_s – stomatal limitation; NPQ – nonphotochemical quenching; P – fraction of absorbed light that is utilized in PSII photochemistry; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; q_P – photochemical quenching coefficient; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; SD – standard deviation; WUE – water-use efficiency; X – fraction of absorbed light that was neither used in photochemistry nor dissipated in PSII antenna; Φ_{PSII} – effective quantum yield of PSII photochemistry; α – photon activity distribution coefficients of PSI; β – the photon activity distribution coefficients of PSII; β/α – 1 – the relative deviation from full balance between the two photosystems.

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important determinants of the impact of global warming on the crop yield (Ainsworth and Ort 2010).

Brassinosteroids (BRs), a novel class of plant steroidal hormones, can affect a variety of physiological processes at nanomolar to micromolar concentrations (Sasse 2003), which are essential for the plant growth and development, including cell elongation, pollen tube growth, root inhibition, ethylene biosynthesis, senescence, photosynthesis, and enzyme activation (Sasse 2003, Bajguz and Hayat 2009, Hayat *et al.* 2011). In addition to their growth regulatory activities, BRs have the ability to induce tolerance in plants to salinity, drought, low temperature, heavy metals, pathogen infection, *etc.* (Bajguz and Hayat 2009, Hayat *et al.* 2010, Gudesblat and Russinova 2011). The involvement of BRs in thermotolerance has drawn much attention in the past two decades (Wilen *et al.* 1995, Dhaubhadel *et al.* 1999, Singh and Shono 2005, Kagale *et al.* 2007, Ogweno *et al.* 2008, Hayat *et al.* 2010, Janeczko *et al.* 2011, Mazorra *et al.* 2011). For example, Wilen *et al.* (1995) reported that application of EBR markedly enhanced viability of bromegrass cells following the exposure of cell suspension cultures to HT stress. Mazorra *et al.* (2011) observed that thermotolerance in tomato was independent of the endogenous BR content, but heat stress-mediated oxidative stress depended on BR concentrations.

Previous studies have shown that BRs-induced thermotolerance was associated with a higher synthesis of heat shock proteins (HSPs) (Dhaubhadel *et al.* 1999, Kagale *et al.* 2007), the regulation of reactive oxygen species (ROS) metabolism (Ogweno *et al.* 2008, Hayat *et al.* 2010), and the enhanced protection of the translational machinery from degradation following heat stress (Dhaubhadel *et al.* 1999, 2002). Another function of BRs in higher plants is their possible involvement in the regulation of photosynthesis. Exogenous BRs have been shown to diminish HT-induced depression of photosynthesis in tomato (Singh and Shono 2005, Ogweno *et al.* 2008, Mazorra *et al.* 2011), *Vigna radiata* (Hayat *et al.* 2010), and barley (Janeczko *et al.* 2011). Application of BRs enhanced CO_2 assimilation rate, carboxylation efficiency, and the activity of ROS scavenging system that lead to an upregulation of PSII activity (Ogweno *et al.* 2008, Xia *et al.* 2009, Hayat *et al.* 2010). Compared to drought and salt stress, the research of BRs-induced thermotolerance by means of regulation of photosynthesis is still limited. Furthermore, the mechanisms of BRs action in the regulation of photosynthetic processes are still far from being complete.

Melon (*Cucumis melo* L.), an important worldwide greenhouse crop, is greatly affected by HT. Melon seedlings face the hot season in the summer–autumn in south of China, resulting in premature aging and decline in the yield and quality. Therefore, the thermotolerance of developing melon seedlings is very important in agricultural production. However, only a few studies have focused on the influence of BRs on melon growth under HT stress until now. Based on the above facts, the objective of this study was to investigate whether exogenously applied EBR induced thermotolerance in melon seedlings, particularly, if EBR protected photosynthetic performance against HT stress, as assessed by chlorophyll (Chl) contents, photosynthetic gas exchange, and Chl fluorescence parameters. The physiological mechanisms of possible EBR effects on photosynthesis of HT-stressed melon seedlings were also discussed. This could improve our understanding of the mechanisms regarding the alleviation of HT damage.

Materials and methods

Plant culture and treatments: Seeds of two types of melon (*Cucumis melo* L.) cultivars, 'Honglvzaocui' (HT-tolerant) and 'Baiyuxiang' (HT-sensitive), were provided by Horticultural Research Institute, Shanghai Academy of Agricultural Sciences, China. The seeds were rinsed thoroughly with distilled water and germinated on moist filter paper in an incubator at 30°C. The germinated seeds were sown in plastic plates mixed with garden soil, perlite, and peat (2:1:1) in a growth chamber at average day/night temperature of 30/20°C (12 h/12 h), photosynthetic photon flux density (PPFD) of 400 μmol m^{-2} s^{-1}, and relative humidity of 80%. Plants were watered daily with half-strength Hoagland nutrient solution. The storage solution of EBR (*Sigma*, USA) was prepared by dissolving in absolute ethanol, stored at 4ºC, and diluted to a final concentration with distilled water. The EBR- and HT treatments started three weeks after the germination. Before the HT treatments started, plants were divided into two groups, *i.e.*, the plants treated under normal temperature (NT; 30/20°C) and those under HT (42/32°C). Both groups were sprayed with 0.05, 0.1, 0.5, 1.0, and 1.5 mg L^{-1} EBR, or only with distilled water as a control. Each plant was sprayed with 50 ml of the solution. After 2 d of the exposure to HT, the plants were collected to determine the plant growth and photosynthetic parameters. Each treatment involved three replications with ten plants per pot, which were maintained under a completely randomized block design.

For determination of fresh mass (FM), plants were harvested and divided into shoots and roots; samples were rinsed three times in distilled water, blotted on filter paper and then weighted.

Index of heat injury was calculated as Σ (number of plants at each grade × grade number)/(the highest grade × the total number of plants) × 100%.

Grade	Characteristics
0	no symptoms of heat injury
1	one or two leaves turned yellow
2	all the leaves turned yellow
3	one or two leaves wilted
4	the whole plant wilted and died

Chl quantification: The index of relative Chl content was measured as SPAD unit on the third fully expanded leaf using a Chl meter (*SPAD-502*, *Konica, Minolta Sensing Inc*., Japan) as described by Hayat *et al*. (2011). In order to show the actual Chl content, the SPAD meter was calibrated for the given leaf type according to Azia and Stewart (2001). The calibration equations is $y = -0.0003\ x^2 + 0.0466\ x + 0.0846$, where Y is Chl content [mg g^{-1}(FM)], and x is SPAD unit. The Chl content was measured by employing the calibration equations.

Gas-exchange parameters: Net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i) were measured on the third fully expanded leaf using an infrared gas analyzer portable photosynthesis system (*LI-6400*, *LICOR Inc.,* Lincoln, NE, USA). During the measurements, PPFD was set to 800 μmol m^{-2} s^{-1}, the air relative humidity was about 85%, the leaf temperature was maintained at 25°C and the ambient CO_2 concentration was about 400 $\mu L \cdot L^{-1}$. Measurements of photosynthesis were made once for each leaf and for five different leaves per treatment. The stomatal limitation (L_s) was calculated as intracellular CO_2 concentration and CO_2 concentration of air, $L_s = 1 - C_i/C_o$. WUE was calculated as a ratio between the net photosynthesis and transpiration rate, P_N/E.

Chl fluorescence: To determine the state of PSII, Chl fluorescence was measured using a portable pulse-modulated fluorometer *(PAM-2100*, *Walz*, Effeltrich, Germany). Before each measurement, leaves were dark-adapted for at least 30 min. The minimal fluorescence of dark-adapted state (F_o) was determined by a weak modulated light, which was low enough not to induce any significant variable fluorescence. A 0.8-s pulse of the saturating light of 8,000 μmol m^{-2} s^{-1} was used on dark-adapted leaves to determine the maximal fluorescence of dark-adapted state (F_m). Then the leaf was illuminated with the actinic light (red led light) of 600 μmol m^{-2} s^{-1}. When the leaf reached steady-state photosynthesis, the steady-state fluorescence (F_s) was recorded and a second 0.8-s saturating light of 8,000 μmol m^{-2} s^{-1} was applied to determine the maximal fluorescence of light-adapted state (F_m'). The actinic light was turned off, the minimal fluorescence of light-adapted state (F_o') was determined by the illumination with 3-s far-red light, photochemical quenching coefficient (q_P) was measured. The maximal quantum yield of PSII photochemistry (F_v/F_m), the effective quantum yield of PSII photochemistry (Φ_{PSII}), the efficiency of excitation capture of open PSII center (F_v'/F_m'), non-photochemical quenching (NPQ), and q_P were calculated as F_v/F_m, $(F_m' - F_s)/F_m'$, F_v'/F_m', $F_m/F_m' - 1$, and $(F_m' - F_s)/(F_m' - F_o')$, respectively (Yu *et al*. 2004).

To get more information about the divergent parameters, we calculated the fraction of the absorbed light used in PSII photochemistry (P), thermal dissipation in PSII antennae (D), and the excess excitation (X) according to Demmig-Adams *et al*. (1996). The D and P were estimated as $[1 - (F_v'/F_m')] \times 100$ and $(F_v'/F_m') \times q_P \times 100$, respectively. The fraction of the absorbed light that was neither used in photochemistry nor dissipated in the PSII antenna (X) was estimated as $(F_v'/F_m') \times (1 - q_P) \times 100$. The photon activity distribution coefficients of PSI (α), the photon activity distribution coefficients of PSII (β), and the relative deviation from the full balance between PSI and PSII ($\beta/\alpha - 1$) were calculated as $f/(1 + f)$, $1/(1 + f)$, and $(1 - f)/f$, respectively. The parameter of f was defined as the degree of openness of PSII reaction centres, and it was equal to $(F_m - F_s)/(F_m - F_o)$, where F_o is the initial fluorescence (Braun and Malkin 1990).

Statistical analysis: All data were subjected to analysis of variance and expressed as the mean ± standard deviation (SD) with a minimum of three replications. The significance of difference between the control and treatments was set at p=0.05 by *Duncan*'s *t*-test.

Results

Growth and the index of heat injury: The HT-exposure caused a significant reduction in FM of shoots and roots of both genotypes (Table 1). Under HT condition, shoot and root FM decreased by 14.6% and 21.1%, respectively, in Honglvzaocui, and by 26.8% and 37.2%, respectively, in Baiyuxiang, compared with the control. The application of 0.5–1.5 mg L^{-1} EBR had a significant positive effect on the shoot FM, but not on the root FM of Baiyuxiang, while only 1.0 mg L^{-1} EBR dramatically alleviated the decrease in FM of both cultivars. In the presence of 1.0 mg L^{-1} EBR under HT, the reduction in shoot and root FM declined to 2.4% and 9.5%, respectively, in Honglvzaocui, and to 7.0% and 20.9%, respectively, in Baiyuxiang.

Contrary to the changes in FM, EBR had significant effects on the HT-induced index of heat injury of both genotypes at the tested concentrations (0.05–1.5 mg L^{-1}), of which optimum concentration was 1.0 mg L^{-1}. The index of heat injury decreased by 45.2% and 33.3% in Honglvzaocui and Baiyuxiang, respectively, after 1.0 mg L^{-1} EBR treatment (Table 1).

Table 1. Effects of different concentration of 24-epibrassinolid (EBR) on shoot fresh mass, root fresh mass (FM), index of heat injury, and chlorophyll (Chl) content in two melon cultivars Honglvzaocui (HT-tolerant) and Baiyuxiang (HT-sensitive) under high temperature stress. Means ± SD of 3 independent measurements. Means marked with the *different letters* in each row indicate significant difference between treatments at $p<0.05$ according to *Duncan*'s multiple range test.

Parameter	Cultivar	Control	EBR concentration [mg L^{-1}]					
			0.00	0.05	0.10	0.50	1.00	1.50
Shoot FM [g per plant]	Honglvzaocui	7.10 ± 0.42^a	6.06 ± 0.22^c	6.10 ± 0.20^c	6.21 ± 0.25^c	6.40 ± 0.34^{bc}	6.93 ± 0.65^{ab}	6.20 ± 0.16^c
	Baiyuxiang	7.17 ± 0.19^a	5.25 ± 0.47^d	5.40 ± 0.34^d	5.58 ± 0.49^{cd}	6.19 ± 0.14^{bc}	6.67 ± 0.55^{ab}	6.59 ± 0.50^{ab}
Root FM [g per plant]	Honglvzaocui	0.95 ± 0.06^a	0.75 ± 0.05^c	0.76 ± 0.03^c	0.78 ± 0.01^c	0.81 ± 0.02^{bc}	0.86 ± 0.06^b	0.79 ± 0.03^{bc}
	Baiyuxiang	0.86 ± 0.046^a	0.54 ± 0.05^b	0.56 ± 0.05^b	0.59 ± 0.05^b	0.65 ± 0.05^b	0.68 ± 0.07^b	0.66 ± 0.06^b
Chl content [mg g^{-1}(FM)]	Honglvzaocui	1.30 ± 0.07^b	1.20 ± 0.02^d	1.22 ± 0.08^{cd}	1.23 ± 0.03^{cd}	1.30 ± 0.02^{bc}	1.40 ± 0.10^a	1.34 ± 0.04^{ab}
	Baiyuxiang	1.21 ± 0.06^b	1.05 ± 0.09^b	1.13 ± 0.12^b	1.15 ± 0.10^b	1.16 ± 0.11^b	1.27 ± 0.03^a	1.14 ± 0.10^{ab}
Index of heat injury [%]	Honglvzaocui		77.50 ± 2.10^a	65.00 ± 3.21^b	55.00 ± 4.00^d	55.00 ± 1.00^d	42.50 ± 3.90^e	57.50 ± 1.20^c
	Baiyuxiang		82.50 ± 2.35^a	72.50 ± 4.85^b	62.50 ± 2.00^c	57.50 ± 3.05^{cd}	55.00 ± 4.50^d	57.50 ± 2.00^{cd}

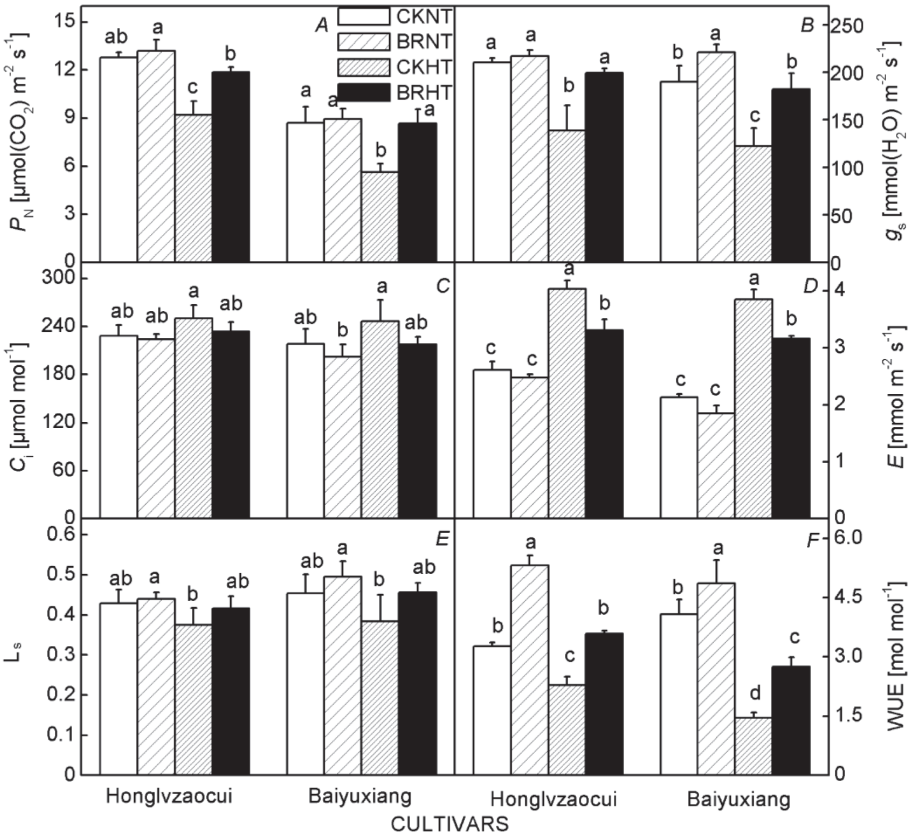

Fig. 1. Effects of 1.0 mg L^{-1} 24-epibrassinolide (EBR) on net photosynthetic rate (P_N) (*A*), stomatal conductance (g_s) (*B*), intercellular CO_2 concentration (C_i) (*C*), transpiration rate (*E*) (*D*), stomatal limitation (L_s) (*E*), and water-use efficiency (WUE) (*F*) of two melon cultivars Honglvzaocui (HT-tolerant) and Baiyuxiang (HT-sensitive) grown under high-temperature stress. CKNT – normal temperature; BRNT – normal temperature + 1.0 mg L^{-1} EBR treatment; CKHT – high temperature; BRHT – high temperature + 1.0 mg L^{-1} EBR treatment. Data are the means of five replicates with SD shown by vertical bars. Means marked with the *different letters* indicate significant difference between treatments at $p<0.05$ according to *Duncan*'s multiple range test.

Chl: HT stress caused a significant reduction in Chl content, it decreased by 7.4% and 12.6%, respectively, in Honglvzaocui and Baiyuxiang, compared with the control (Table 1). However, the exposure to EBR caused a marked enhancement in Chl content in both cultivars. In the presence of 1.0 mg L^{-1} EBR under HT stress, Chl increased by 16.3% and 20.6%, respectively, in Honglvzaocui and Baiyuxiang (Table 1). Because EBR in concentration of 1.0 mg L^{-1} showed the most significant effects on the melon seedlings under HT, it was used further on.

Gas-exchange: To examine whether EBR regulate the photosynthetic capacity under HT, we determined the effects of EBR application on gas-exchange parameters. As shown in Fig. 1, in comparison with the control, HT-stressed plants displayed a significant decrease in P_N, which decreased by 28.04% and 35.29%, respectively, in Honglvzaocui and Baiyuxiang. Foliar-applied EBR caused only small reduction in P_N, it declined to 7.18% and 0.27%, respectively, in Honglvzaocui and Baiyuxiang under HT stress. g_s and WUE of both cultivars showed similar results. Furthermore, EBR alone resulted in the remarkable increase in WUE of both cultivars and of g_s in Baiyuxiang. However, there were no significant changes in C_i and L_s in both cultivars, when the melon seedlings were treated with HT, EBR alone, or HT and EBR in a combination.

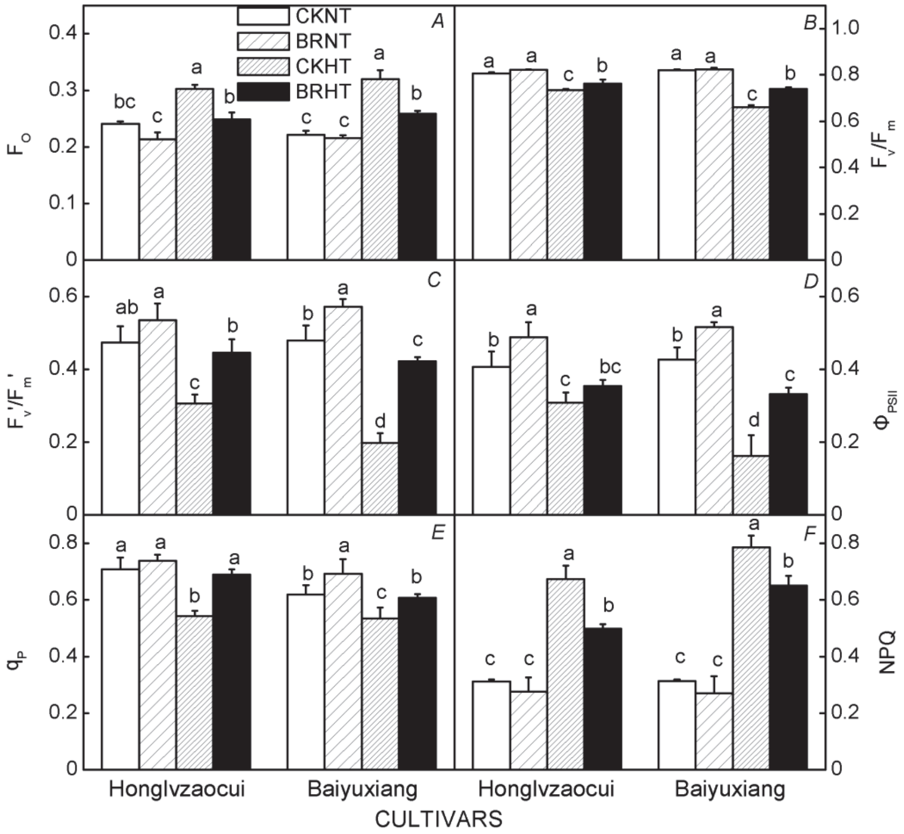

Fig. 2. Effects of 1.0 mg L^{-1} 24-epibrassinolide (EBR) on minimal fluorescence of dark-adapted state (F_o) (*A*), maximal quantum yield of PSII photochemistry (F_v/F_m) (*B*), efficiency of excitation capture of open PSII center (F_v'/F_m') (*C*), quantum efficiency of PSII (Φ_{PSII}) (*D*), photochemical quenching coefficient (q_P) (*E*), and nonphotochemical quenching coefficient (NPQ) (*F*) of two melon cultivars Honglvzaocui (HT-tolerant) and Baiyuxiang (HT-sensitive) grown under high-temperature stress. CKNT – normal temperature; BRNT – normal temperature + 1.0 mg L^{-1} EBR treatment; CKHT – high temperature; BRHT – high temperature + 1.0 mg L^{-1} EBR treatment. Data are the means of five replicates with SD shown by vertical bars. Means marked with the *different letters* indicate significant difference between treatments at $p<0.05$ according to *Duncan*'s multiple range test.

Chl fluorescence: As shown in Fig. 2, F_v/F_m, F_v'/F_m', Φ_{PSII} , and q_P of both genotypes were significantly lower in HT-stressed seedlings than those in the control, in parallel to the increase in F_o and NPQ. The treatment with EBR evidently improved F_v/F_m, F_v'/F_m', Φ_{PSII}, and q_P values, but remarkably reduced F_o and NPQ value under HT stress. For instance, the F_v'/F_m' of HT-stressed Honglvzaocui and Baiyuxiang decreased significantly by 35.3% and 58.1%, respectively, in comparison with the control. In the presence of EBR under HT stress, the reduction in F_v'/F_m' only declined to 6.1% and 11.8%, respectively, in Honglvzaocui and Baiyuxiang. Contrary to the F_v'/F_m', NPQ increased distinctly 2.2 and 2.5 times, respectively, in Honglvzaocui and Baiyuxiang under HT stress, but it increased only 1.6 and 2.1 times under HT and EBR combination treatment. Under nonstressed conditions, EBR alone caused the remarkable increase in Φ_{PSII} of both genotypes and in F_v'/F_m' and q_P of Baiyuxiang, but no significant changes were found in other parameters.

Distribution of absorbed energy: The effect of HT on a process of photosynthesis in melon seedlings was also well represented by the analysis of energy distribution. HT stress limited the fraction of light absorbed by the the excess of energy into thermal dissipation (D) in both

Table 2. Effects of 1.0 mg L^{-1} 24-epibrassinolide (EBR) on the fraction of light absorbed by PSII antenna that are used in PSII photochemistry, thermally-dissipated, and not used in photochemistry nor dissipated in the antenna (P, D, and X, respectively) in two melon cultivars Honglvzaocui (HT-tolerant) and Baiyuxiang (HT-sensitive) under high temperature stress. CKNT, normal temperature; BRNT, normal temperature + 1.0 mg L^{-1} EBR treatment; CKHT, high temperature; BRHT, high temperature + 1.0 mg L^{-1} EBR treatment. Data were presented as means ± SD of three independent measurements. Means marked with the different letters in each row indicate significant difference between treatments at $p<0.05$ according to *Duncan*'s multiple range test.

Parameter	Treatment	CKNT	BRNT	CKHT	BRHT
Honglvzaocui	P	33.51 ± 3.05^{b}	39.49 ± 3.43^{a}	16.60 ± 0.81^{c}	30.72 ± 3.13^{b}
	D	52.57 ± 4.44^{bc}	46.50 ± 4.62^{c}	69.33 ± 2.44^{a}	55.47 ± 3.70^{b}
	X	13.92 ± 2.71^{a}	14.01 ± 1.83^{a}	14.06 ± 1.71^{a}	13.81 ± 0.99^{a}
Baiyuxiang	P	29.60 ± 1.30^{b}	39.50 ± 1.50^{a}	10.55 ± 0.90^{d}	25.72 ± 1.16^{c}
	D	52.10 ± 4.10^{c}	42.87 ± 2.25^{d}	80.13 ± 2.66^{a}	57.73 ± 1.08^{b}
	X	18.30 ± 3.01^{a}	17.63 ± 3.60^{a}	9.31 ± 1.90^{b}	16.55 ± 0.19^{a}

cultivars (Table 2). The changes of distribution of absorbed energy caused by HT were reversed by EBR treatment. In particular, the values of P and D in Honglvzaocui and X in Baiyuxiang returned to the control level. Under control conditions, EBR alone caused the notable increase in P of both genotypes and of D in Baiyuxiang, but no significant changes were found in X value.

Distribution of excitation energy: Malkin *et al*. (1986) developed a method that can quantitatively estimate the excitation energy distribution between PSI and PSII. As shown in Fig. 3, the excitation energy allotted to PSII (β) and the relative deviation from the full balance between both two photosystems ($\beta/\alpha - 1$) of both genotypes were significantly higher in HT stressed plants than that in the control, which was contrary to the decreases in the excitation energy distributed to PSI (α). EBR pretreatment could maintain the balance of the excitation distribution between PSII and PSI by returning the values to the control levels in both genotypes under HT condition. Furthermore, only the application of EBR remarkably increased α value, but decreased the values of β and $\beta/\alpha - 1$.

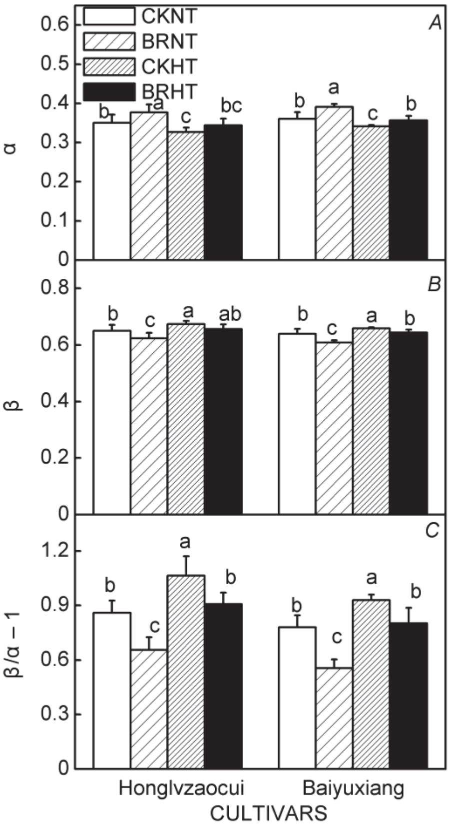

Fig. 3. Effects of 1.0 mg L^{-1} 24-epibrassinolide (EBR) on the photon activity distribution coefficients of PSII and PSI (α) (*A*), the photon activity distribution coefficients of PSII (β) (*B*), and the relative deviation from full balance between the two photosystems ($\beta/\alpha - 1$) (*C*) of two melon cultivars Honglvzaocui (HT-tolerant) and Baiyuxiang (HT-sensitive) grown under high-temperature stress. CKNT – normal temperature; BRNT – normal temperature +1.0 mg L^{-1} EBR treatment; CKHT – high temperature; BRHT – high temperature + 1.0 mg L^{-1} EBR treatment. Data are the means of five replicates with SD shown by vertical bars. Means marked with the *different letters* indicate significant difference between treatments at $p<0.05$ according to *Duncan*'s multiple range test.

Discussion

Among physiological processes in plants, photosynthesis is one of the most sensitive processes to HT, with the inhibition occurring at temperatures only slightly higher than those optimal for growth (Allakhverdiev *et al.* 2008). We found EBR to alleviate the decline in the growth of both cultivars (in terms of FM of roots and shoots and the index of heat injury) caused by HT stress, which implied that EBR played a vital role in protecting photosynthetic apparatus from HT damage. EBR-treated melon seedlings exhibited higher P_N after being exposed to HT stress than those untreated (Fig. 1). Reduced P_N can result from stomatal or nonstomatal limitations in higher plants (Farquhar and Sharkey 1982). The decrease of P_N in the melon seedlings under HT stress was likely due to the reduction in the photosynthetic activity of the mesophyll cell, rather than reflecting a change in stomata behaviour, since P_N decreased, not accompanied by any decline in C_i and an increase in L_s (Farquhar and Sharkey 1982, Sharkey and Ogawa 1987). Furthermore, the increase or decrease in g_s and E of both melon cultivars at 1.0 mg L^{-1} EBR under non-HT or HT conditions could not be related to a significant change in C_i, suggesting that g_s was not the sole factor for EBR-induced changes in photosynthesis. Similar results were found with or without EBR treatment in wheat under salt stress (Ali *et al.* 2008, Shahbaz *et al.* 2008).

Nonstomatal limitations to P_N may include changes in photosynthetic pigments, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) enzyme concentration and activity, and use of assimilation products (Dubey 2005). The present study showed that HT stress caused a significant reduction in the Chl content, which might be attributed to the increase in the activity of Chl-degrading enzyme, chlorophylase, or suppression of Chl synthesis (Hayat *et al.* 2010). Feeding EBR to HT-stressed molen seedlings significantly increased a pigment content, which supported the previous studies (Yu *et al.* 2004, Ali *et al.* 2007, Hasan *et al.* 2008, Hayat *et al.* 2010). The reason, which sounds the best in supporting the mentioned observation, is possibility that the BRs induced impact on transcription and/or translation of proteins involved in the synthesis of pigments (Bajguz 2000).

The reduction of photochemical activity is considered to be one of the nonstomatal factors that limit photosynthesis (Souza *et al.* 2004). Thus, the activity of PSII was investigated in the present study by using Chl fluorescence method, which also provides information on the ability of a plant to tolerate environmental stresses and the extent of stress damage to photosynthetic apparatus (Maxwell and Johnson 2000). In our study, the maximal quantum efficiency of PSII as F_v/F_m was significantly reduced in response to HT. However, both EBR-treated cultivars showed the less pronounced decrease in F_v/F_m ratio under HT, suggesting that exogenous EBR helped protect PSII against the over-excitation. Correspondingly, F_v'/F_m', Φ_{PSII}, and q_P exhibited the changed pattern, similar to that of F_v/F_m, indicating that improved quantum yield of PSII electron transport due to EBR treatment was attributed to the increase in the photochemical quenching and the efficiency of energy capture by open PSII reaction centres in HT-stressed seedlings. Under HT, the observed, EBR-induced increase in q_P indicated an enhancement in the rate of reductant consumption and ATP production by noncyclic electron transport relative to the rate of excitation of open PSII reaction centers (Nogués and Baker 2000). NPQ has been associated closely with the triggering excess energy dissipation by nonradiative processes, which protects somehow the photosynthetic apparatus (Gilmore 1997). A lesser decrease in F_v'/F_m' and the smaller increase in NPQ implied that EBR application resulted in a lesser dissipation of excitation energy as heat in the PSII antennae in EBR-treated plants compared with the untreated plants under HT. The results suggested that application of EBR protected the PSII against over-excitation, perhaps from a loss of integrity in the thylakoid membrane (Ogweno *et al.* 2008). It is known that the thylakoid membrane is also very sensitive to HT (Haldimann and Feller 2005).

We evaluated also the photon energy absorbed by PSII. It could be divided into three parts, expressed as P, D, and X (Demmig-Adams *et al.* 1996). HT stress caused the decrease in P value and the increase in D value more in Baiyuxiang than in Honglvzaocui, indicating that HT-sensitive melon showed a lower portion of absorbed photon energy utilized in the PSII photochemistry and it dissipated mainly the excess of energy, not utilized in photochemistry, as the thermal dissipation in the PSII antenna. Furthermore, EBR application reversed the changes of distribution of absorbed energy caused by HT, suggesting that EBR application distributed absorbed energy by more fraction of light absorbed by the PSII antenna used in photochemistry and less dissipation of excitation energy as heat in antennae, but only little by alternative ways (X). Furthermore, the significant, EBR-induced increase of photosynthesis was accompanied by an evident shift in the balance of excitation energy distribution between PSII and PSI in HT-stressed leaves (Fig. 3) . The lower $\beta/\alpha - 1$ implied that more electrons might be redistributed to PSI to form active oxygen species and to cause the over-reduction of P_{700} in melon leaves (Li *et al.* 2003). Therefore, it was speculated that EBR might keep high utilization efficiency of absorbed photon energy by maintaining the balance of excitation distribution between PSII and PSI.

In conclusion, it was shown that HT restrained the growth of two melon cultivars by the inhibition of photosynthesis. This was alleviated by the application of EBR, especially 1.0 mg L^{-1}, through maintaining photosynthetic pigments, photochemical activity, and the balance of excitation distribution between PSI and PSII. The results of this study could not only provide better understanding the physiological mechanisms of BRs-induced HT tolerance, but also to provide potential application in agriculture.

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