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24-epibrassinolide improved chilled tomato photosynthetic performance by stabilizing electron transport chain and function of photosystem II

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Abstract

To explore the protective mechanisms of brassinosteroids in the chill-induced photoinhibition in tomato (*Solanum lycopersicum*), we studied the effect of foliar sprayed 24-epibrassinolide (EBR, 0.1μM) on the gas exchange, chlorophyll fluorescence characteristics, and chlorophyll *a* fluorescence transient in tomato seedlings under chilling stress (a temperature of 8 °C and an irradiance of 200 μmol m^{-2} s^{-1}) for 4 d. Results showed that chilling significantly inhibited CO_2 assimilation and induced photoinhibition of photosystem II (PS II). However, photosystem I (PS I) was relatively tolerant to chilling stress, which was due to the downregulation of PS II activity and increase of cyclic electron transport around PS I (CEF). Chilling led to the inactivation of PS II reaction centers (RCs) and blocked the electron transport at the PS II acceptor side, but did not affect the oxygen-evolving complex (OEC) on the donor side of PS II. Exogenous EBR could alleviate chill-induced PS II photoinhibition mainly by the increase of CO_2 assimilation and thermal dissipation of excitation energy in the PS II antennae, while the protective effect of CEF was relatively smaller. This study demonstrated that EBR maintained the stability of the electron transport chain and the function of PS II in chilled tomatoes. EBR promoted the absorption (ABS/CS), trapping (TR_o/CS), and electron transport (ET_o/CS) per leaf area in tomatoes under chilling stress, which was due to increasing the density of active reaction centers (RC/CS), rather than the activity of active RCs.

Keywords: 24-epibrassinolide, chilling stress, electron transport chain, photosynthesis, *Solanum lycopersicum.*

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Abbreviations: ABS/CS - absorption flux per CS; ABS/RC - absorption flux (exciting PS II antenna of Chl *a* molecules) per RC; AQY - apparent quantum yield; BRs - brassinosteroids; CEF - cyclic electron transport around PS I; c_i - intercellular CO_2 concentration; CS - cros section; E - transpiration rate; EBR - 24-epibrassinolide; ET_o/CS – electron transport flux per CS; ET_o/RC - electron transport flux (further than Q_A^-) per RC; ETR - electron transport rate; F_m - maximal fluorescence yield; F_o - minimal fluorescence yield; F_v/F_m - maximal quantum yield of PS II photochemistry; g_s - stomatal conductance; M_o - approximated initial slop (in ms^{-1}) of the fluorescence transient normalized on the maximal variable fluorescence F_v; NPQ - nonphotochemical quenching coefficient; OEC - oxygen-evolving complex; OJIP curve - Chl *a* fluorescence transient; PI_{ABS} - performance index for energy conservation from photons absorbed by PS II until the reduction of intersystem electron acceptors; P_m - maximum P700 oxidation; P_N - net photosynthetic rate; $P_{N,max}$ - maximum net photosynthetic rate; PPFD - photosynthetic photon flux density; PS I - photosystem I; PS II - photosystem II; qP - photochemical quenching coefficient; RC/CS - density of Q_A-reducing PS II RCs per CS; RCs - PS II reaction centers; ROS - reactive oxygen species; TR_o/CS - trapped energy flux per CS; TR_o/RC - trapped energy flux (leading to Q_A reduction) per RC; V_J - relative variable fluorescence at the J-step; W_K - normalized relative variable fluorescence at the K step; Y(I) - effective photochemical quantum yield of PS I; Y(II) - effective PS II quantum yield; Y(NA) - quantum yield of non-photochemical energy dissipation of reaction centers due to PS I acceptor side limitation; Y(ND) - quantum yield of non-photochemical energy dissipation in reaction centers due to PS I donor side limitation; Y(NPQ) - quantum yield of regulated energy dissipation; Y(NO) - quantum yield of nonregulated energy dissipation; φ_{Eo} - quantum yield for electron transport (ET); φ_{Po} - maximum quantum yield for primary photochemistry; Ψ_o - probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-.

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Introduction

In the east and north of China, the combination of chilling and low irradiance in winter is the typical limiting factor for the growth and development of most greenhouse crops, such as tomato and cucumber, originating from tropical and subtropical regions (Hu *et al.* 2006, Qian *et al.* 2020, Zhang *et al*. 2020b). Photosynthesis is the major physiological metabolic process inhibited at chilling temperatures (Allen and Ort 2001). Under chilling experienced at low irradiance, stomatal closure and loss of activity of Calvin cycle enzymes (*e.g.* sedoheptulose-1,7-bisphosphatase (SBPase), fructose-1,6-bisphosphatase (FBPase), and ribulose 1,5-bisphosphate carboxylase (Rubisco)) appear predominant (Brüggemann *et al.* 1994, Artuso *et al.* 2000, Allen and Ort 2001, Ding *et al.* 2016). As the energy metabolism center in green cells, chloroplasts absorb light energy and use it to drive a series of electron transfers, resulting in the synthesis of reducing power (ATP and NADPH) required for carbon assimilation. The decrease in carbon assimilation is usually associated with a decrease in the demand for reducing power, resulting in the accumulation of NADPH in the chloroplast, under chilling conditions (Ort and Baker 2002, Hu *et al.* 2010). The accumulation of excessive reducing power in chloroplasts leads to over-reduction of the photosynthetic electron transport chain, which eventually promotes the production of reactive oxygen species (ROS) and photoinhibition (Ort and Baker 2002, Zhao *et al.* 2020). Chilling hinders the thylakoid electron transport and causes the impairment of photochemistry of photosystem II (PS II), even damage to photosystem I (PS I) (Kee *et al.* 1986, Hu *et al.* 2006, Hu *et al.* 2010, Huang *et al.* 2016). For instance, the primary photosynthetic processes associated with PS II functioning respond to chilling treatment sensitively in alfalfa (Lang *et al.* 2020). Chilling under low irradiance limited the PS I functionality by blocking electron flow further than Q_A in common fig (Mlinarić *et al*. 2021). However, chilling also induces xanthophyll cycle-dependent energy dissipation as heat from the antenna of PS II, cyclic electron flows around PS I (CEF), and water-water cycle to reduce the accumulation of excess energy in the chloroplast (Zhou *et al.* 2004, Hu *et al.* 2008, Huang *et al.* 2011, Takahashi *et al.* 2011). Therefore, the imbalance in energy metabolism in the chloroplast is the main reason for photoinhibition and even photooxidative damage, which suggests that the effective measure of the energy balance in the chloroplast can play an important role in chilling tolerance.

Brassinosteroids (BRs) are a group of phytohormones that increase tolerance to various stresses in plants and have been widely applied in agriculture to promote yield and protect against environmental stresses (Krishna 2003, Anwar *et al.* 2018). Xia *et al.* (2009b, 2011) reported that BRs treatment can active the continuous production of H_2O_2, and the autopropagative nature of the reactive oxygen species signal mediates BRs-induced systemic tolerance. Exogenous BRs enhance the antioxidant system capacity of tomato under drought (Behnamnia *et al.* 2009) and temperature stresses (Ogweno *et al.* 2007, Cui *et al.* 2016), rice under salinity (Sharma *et al.* 2013), and eggplant under chilling (Wu *et al.* 2015) to enhance stress tolerance. BRs also can ameliorate the reductions in photosynthesis caused by abiotic stresses (Ahammed *et al.* 2020). For instance, EBR alleviates heat-caused inhibition of photosynthesis in melon seedlings by improving the photosynthetic pigment content, stomatal conductance, and photochemical activity of PS I (Zhang *et al.* 2013). EBR pretreatment improves the plastoquinone pool oxidation and the efficiency of PS II photochemistry of potato under salinity stress (Kolomeichuk *et al.* 2020). In pepper, EBR can alleviate drought-induced photoinhibition by increasing the efficiency of light utilization and dissipation of excitation energy in the PS II antennae (Hu *et al.* 2013). In our previous studies, we also found that EBR pretreatment alleviated chill-reduced inhibition of photosynthesis in cucumber particularly attributed to the increase of efficiency of utilization and dissipation of leaf absorbed radiation (Hu *et al.* 2010). These results indicate that BRs can protect crops against photoinhibition by regulating chloroplast energy metabolism under stress conditions. In fact, BRs application under normal conditions can directly promote photosynthesis by enhancing the activation of Rubisco and increasing the quantum yield of PS II (Yu *et al.* 2004, Xia *et al.* 2009a). Mumtaz *et al.* (2020) also discovered that BRs signaling reduction transcriptionally impairs chlorophyll synthesis, quantum photo harvesting, and light energy transfer, leading to a decrease in photosynthetic capacity in *altered brassinolide sensitivity1* (*abs1*) tomato mutant.

Zhang *et al*. (2020a) reported that the application of BR could enhance the photosynthetic potential of tung trees by maintaining the stability of the leaf structure, morphology, and function, and alleviating the damage caused by cold injury. Tomato mutants of BR biosynthesis (*Dwf*) and related signaling through BRASSINAZOLE-RESISTANT1 (*bzr1*) are more sensitive to PS II and PS I photoinhibition with decreased cyclic electron flow around PS I and lower nonphotochemical quenching, which demonstrates that BRs act as a positive regulator of photoprotection in response to chilling stress (Fang *et al*. 2019). Li and Zhang (2015) also proposed that BRs alleviated chill-induced photoinhibition in pepper mainly by improving photochemical reaction efficiency rather than by promoting heat dissipation. Because the redox state of the photosynthetic electron transport chain affects stress-induced photoinhibition, it is important to explore the role of BRs in the protection of components of the photosynthetic electron chain to fully understand the protective mechanisms of BRs. EBR could significantly attenuate the dissolution of the chloroplast plasma membrane and the deformation of the lamellar structure in chloroplasts of maize seedlings under chilling stress (Sun *et al.* 2020). Li *et al*. (2015) observed that EBR increases the proportion of open PS II reaction centers and oxidation state of Q_A to improve the activity of PS II reaction center and enhance the efficiency of radiation energy transfer and conversion in pepper seedlings under chilling stress. Notably, transcriptome analysis also revealed that EBR upregulated the transcripts encoding proteins of the PS II oxygen-evolving complex, PS I subunit, light-harvesting

chlorophyll protein complexes I and II, and ferredoxin under chilling stress (Zhao *et al.* 2019). Therefore, we speculate that BRs may play a key role in the stability of the photosynthetic electron transport chain under chilling stress, which would optimize the activity and efficiency of the photosynthetic system and alleviate the chill-induced photoinhibition.

Tomato is considered sensitive to chilling stress during all stages of plant development due to its tropical/subtropical origin (Caffagni *et al.* 2014, Ding *et al.* 2016), and generally suffers chilling injury when it is exposed to temperature below 10 °C (Park *et al.* 2004, Ding *et al.* 2016). In the present study, we examined the effects of EBR on gas exchange, chlorophyll fluorescence, and chlorophyll *a* fluorescence transients in tomato leaves under chilling conditions. This study aimed to explore the protective role of BRs on the photosynthetic apparatus of chill-exposed tomato plants.

Materials and methods

Plant growth and treatments: Experiments were conducted at Jingganshan University, Jiangxi Province, China. Seeds of tomato (*Solanum lycopersicum* L. cv. Zhongshu No. 4), an important tomato cultivar in China, were purchased from the Xingyun vegetable seed breeding center in Qing County, Hebei Province. Seeds were sown in grass peat in a tray in the artificial climate chamber. Two weeks later, seedlings were transferred into a pot (15 × 15 cm) filled with grass peat and watered daily with a half-strength Enshi nutrient solution (Yu and Matsui 1997). The environmental conditions in the climate chamber were as follows: day/night temperatures of 28/18 ± 1 °C, photosynthetic photon flux density (PPFD) of approximately 500 μmol m^{-2} s^{-1}, 12-h photoperiod, and air humidity of 75 %. The 24-epibrassinolide (EBR, *Sigma*, St. Louis, USA) and chilling treatment started when seedlings were in a 6-leaf stage. On the day before chilling treatment, plants were divided into two groups. One group was cultured in the artificial climate chamber with a 12-h photoperiod and PPFD 500 μmol m^{-2} s^{-1} at 28/18°C (normal temperature, NT). The other group was transferred at the beginning of the photoperiod (7:00 h) to the artificial climate box (*ZRY-YY1000*, *Safu experimental apparatus technology*, Ningbo, China) with a 12-h photoperiod, PPFD 200 μmol m^{-2} s^{-1}, and temperature of 8 °C (low temperature, LT). Both groups of plants were sprayed with 0.1 μM EBR or distilled water (containing the same concentration of ethanol and *Tween* as the controls) 1 day before and then 2 days after chilling treatments. EBR was dissolved in a minimal volume of ethanol, and then made up to volume with distilled water. The four treatments employed were: *1*) normal temperature (NT): plants were cultured in the artificial climate chamber (28/18°C) and sprayed with distilled water; *2*) normal temperature with EBR treatment (NTBR): plants were cultured in the artificial climate chamber (28/18°C) and sprayed with 0.1 μM EBR; *3*) chilling treatment (LT): plants were cultured in the artificial climate box (8 °C) and sprayed with distilled water; *4*) chilling with EBR treatment (LTBR): plants were cultured in the artificial climate box (8 °C) and sprayed with 0.1 μM EBR. There were five replications per treatment. The gas exchange, chlorophyll fluorescence, and chlorophyll *a* fluorescence transient were determined 4 d after treatment. All measurements were carried out on the first fully expanded leaf with five replicates from each treatment.

Gas exchange parameters were recorded from 8:00 - 12:00 h using the *LI-6400XT* portable photosynthesis system (*Li-Cor,* Lincoln, NE, USA). The measurements were performed at a PPFD of 500 μmol m^{-2} s^{-1}, a reference CO_2 concentration of 400 μmol mol^{-1}, relative humidity of 70 %, an air temperature of 28 °C, and a flow rate of 500 μmol s^{-1} inside the Infrared Gas Analyzer (IRGA) chamber. Net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (c_i) were recorded when steady-state conditions were reached. The net photosynthetic rate in response to irradiance (P_N-PPFD curve) was measured according to Hu *et al.* (2013). P_N was determined at 13 levels of PPFD (1 400, 1 000, 800, 600, 400, 200, 150, 100, 50, and 0 μmol m^{-2} s^{-1}). The leaf was exposed to the highest PPFD of 1 400 μmol m^{-2} s^{-1} for 1 200 s before P_N was determined, thereafter, the leaf was exposed to a series of decreasing irradiance for 120 s at each level. The apparent quantum yield (AQY) and maximum net photosynthetic rate ($P_{N, max}$) were analyzed with the P_N-PPFD curve according to Ye (2007).

Chlorophyll *a* fluorescence and P700 were synchronously measured with the Fluo + P700 Measuring Mode of the *Dual-PAM-100/F* (*Walz*, Effeltrich, Germany) according to the instruction manual. The induction curve recording mode was selected on the slow kinetics window and dark-light induction curves were recorded. Before each measurement, leaves were dark-adapted for at least 30 min. First, minimal fluorescence yield (F_o) and maximal fluorescence yield (F_m) of the dark-adapted state were measured. Then, actinic radiation of 500 μmol m^{-2} s^{-1} was applied for 240 s to obtain light-adapted chlorophyll fluorescence and P700 parameters. The determined parameters included: maximal quantum yield of PS II photochemistry (F_v/F_m), effective PS II quantum yield Y(II), quantum yield of regulated energy dissipation Y(NPQ), quantum yield of nonregulated energy dissipation Y(NO), photochemical quenching coefficient qP and nonphotochemical quenching coefficient NPQ, maximum P700 oxidation P_m, effective photochemical quantum yield of PS I Y(I), quantum yield of non-photochemical energy dissipation in reaction centers due to PS I donor side limitation Y(ND), and quantum yield of non-photochemical energy dissipation of reaction centers due to PS I acceptor side limitation Y(NA). The electron transport rate (ETR) was calculated as ETR(I) or ETR(II) = 0.5 × 0.84 × PPFD × Y(I) or Y(II), where 0.5 is the fraction of absorbed radiation reaching PS I or PS II, 0.84 is the leaf absorptance, and PPFD is the photosynthetic photon flux density of actinic radiation. The cyclic electron transport around PS I (CEF) was estimated by the ETR(I)/

Table 1. Formulae and explanation of the technical data of the OJIP curves and the selected JIP-test parameters used in this research.

Parameters	Definition
Data extracted from the recorded fluorescence transient OJIP	
F_t	fluorescence at time t after onset of actinic illumination
$F_o \cong F_{20\mu s}$	minimal reliable recorded fluorescence, at 20 μs (O step) of OJIP
$F_K \equiv F_{300\mu s}$	fluorescence at 300 μs (K-step)
$F_J \equiv F_{2ms}$	fluorescence at 2 ms (J-step)
$F_I \equiv F_{30ms}$	fluorescence at 30 ms (I-step)
F_P (= F_m)	maximal recorded fluorescence, at the peak P of OJIP
Basic parameters calculated from the extracted data	
$W_K = (F_K\text{-}F_o)/(F_J\text{-}F_o)$	the normalized relative variable fluorescence at the K step
$V_J \equiv (F_J - F_o)/(F_m\text{ -}F_o)$	relative variable fluorescence at the J-step
$M_o \equiv 4(F_{300\mu s} - F_o)/(F_m\text{ -}F_o)$	approximated initial slop (in ms^{-1}) of the fluorescence transient normalized on the maximal variable fluorescence F_V
Quantum yields and efficiencies	
$\varphi_{Po} = TR_o/ABS = 1 - F_o/F_m$	maximum quantum yield for primary photochemistry
$\varphi_{Eo} = ET_o/ABS = [1 - (F_o/F_m)] \times (1 - V_J)$	quantum yield for electron transport (ET)
$\Psi_o = (1 - V_J)$	probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-
Fraction of OEC = $[1 - (V_K/V_J)]_{treatment}$ / $[1 - (V_K/V_J)]_{control}$	an estimation about OEC (oxygen evolving complexes)
Specific energy fluxes (per RC: Q_A^- reducing PS II reaction center), in ms^{-1}	
$ABS/RC = M_o \times (1/V_J) \times (1/\varphi_{Po})$	absorption flux (exciting PS II antenna Chl a molecules) per RC
$TR_o/RC = M_o \times (1/V_J)$	trapped energy flux (leading to QA reduction), per RC
$ET_o/RC = M_o \times (1/V_J) \times (1 - V_J)$	electron transport flux (further than QA^-), per RC
Phenomenological energy fluxes (per excited cross section (CS))	
ABS/CS = Chl/CS	absorption flux per CS, approximated by F_M
$TR_o/CS = \varphi_{Po} \times (ABS/CS)$	trapped energy flux per CS
$ET_o/CS = \varphi_{Po} \times \psi_o \times (ABS/CS)$	electron transport flux per CS
Density of RCs	
$RC/CS = \varphi_{Po} \times (V_J/M_o) \times (ABS/CS)$	density of Q_A-reducing PS II RCs per CS
Performance indexes	
$PI_{ABS} = (RC/ABS) \times [\varphi_{Po}/(1\text{- }\varphi_{Po})] \times [\psi_o/(1\text{- }\psi_o)$	performance index for energy conservation from photons absorbed by PS II until the reduction of intersystem electron acceptors

ETR(II), CEF = ETR(I)/ETR(II) (Yamori *et al.* 2011).

Chl *a* fluorescence transient measurement and JIP-test analysis: Chl *a* fluorescence transient (OJIP curve) was measured by a plant efficiency analyzer (*Handy PEA*, *Hansatech Instruments*, Norfolk, UK). Leaves were dark-adapted for 30 min using special leaf clips. OJIP curve was induced by red actinic light (wavelength at peak 650 nm; 2 000 μmol m^{-2} s^{-1}) and 2 s of transient fluorescence was recorded. An average of five replicative data was taken to draw each OJIP curve. Based on the model of "Theory of Energy Fluxes in Biomembranes", the OJIP curves were analyzed using the JIP-test (Strasser *et al.* 2004). This analysis took into consideration several basic fluorescence data at 20 μs (F_o, step O), 300 μs (F_K, step K), 2 ms (F_J, step J), 30 ms (F_I, step I), and maximum yield (F_m, which is equal to F_P, step P). Detailed parameters are listed in Table 1, in which formulas, equations, and definitions of JIP-test parameters are listed according to Chen *et al.* (2014) and Tsimilli-Michael (2019).

Statistical analyses: The results were reported as means ± SEs. Statistical analyses were carried out with *PASW Statistics 18* (*SPSS*, Chicago, IL, USA). After checking for normal distribution (Shapiro-Wilk test) and homogeneity of variance (Levene's test), the significance of the results was checked by a *post-hoc* Fisher's least significant difference (LSD) test *via* one-way analysis of variance (*ANOVA*). The Dunnett's T3 test was used in the case of non-homogeneous variances. Significant differences among different treatments were reported at $P < 0.05$.

Results

Chilling significantly decreased P_N, g_s, and E, and increased c_i. Compared with chilling treatment, EBR significantly

Table 2. Effects of 24-epibrassinolide (0.1 μM) and chilling (8 °C) on gas exchange characteristics in leaves of tomato after 4 d of treatment. NT - normal temperature; NTBR - normal temperature with EBR treatment; LT - chilling treatment; LTBR - chilling with EBR treatment. Data are the means of five independent measurements with standard errors. Values followed by different letters are significantly different at the 0.05 % level.

Parameters	NT	NTBR	LT	LTBR
P_N [μmol m^{-2} s^{-1}]	6.98±0.48 b	8.84±0.45 a	0.33±0.04 d	1.79±0.22 c
g_s [mol m^{-2} s^{-1}]	0.10±0.01 b	0.14±0.03 a	0.03±0.01 c	0.03±0.01 c
c_i [μmol mol^{-1}]	275.0 ±6 b	284.0 ±6 b	378.0±14 a	275.0 ±21 b
E [mmol m^{-2} s^{-1}]	1.18±0.11 a	1.47±0.16 a	0.38±0.07 b	0.29±0.07 b
$P_{N, max}$ [μmol m^{-2} s^{-1}]	7.03±0.35 b	9.54±0.81 a	0.68±0.12 d	1.92±0.16 c
AQY [μmol mol^{-1}]	0.03±0.002 b	0.04±0.002 a	0.009±0.001 c	0.013±0.001 c

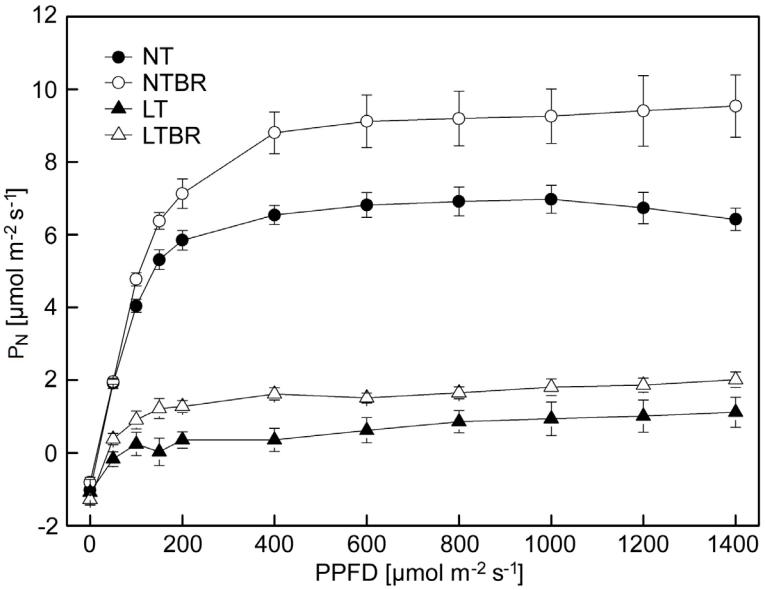

Fig. 1. Effects of 24-epibrassinolide (0.1 μM) and chilling (8 °C) on net photosynthetic rate in response to radiation (P_N-PPFD curve) in leaves of tomato after 4 d of treatment. NT - normal temperature; NTBR - normal temperature with EBR treatment; LT - chilling treatment; LTBR - chilling with EBR treatment. Data are the means of five independent measurements with standard errors.

increased P_N by 5.5 fold but decreased c_i by 27.4 %. Under natural temperature, EBR also significantly increased P_N and g_s by 26.6 and 40.0 %, respectively, but had no significant changes on E and c_i (Table 2). The P_N-PPFD curves of the four treatments were shown in Fig. 1. Under natural temperature, EBR significantly increased $P_{N, max}$ and AQY by 35.7 and 20.6.%_, respectively. Chilling sharply decreased $P_{N, max}$ and AQY by 90.3 and 73.5 %, however, only the reduction of $P_{N, max}$ was significantly alleviated by EBR treatment (Table 2).

Compared with NT, F_v/F_m declined by 47.0 % in LT and 34.0 % in LTBR, suggesting that exogenously supplied EBR could alleviate the chill-induced photoinhibition of tomato plants. Moreover, the quantum yield of PS I and PS II was significantly affected by chilling. Under chilling stress, Y(II) and Y(NPQ) were decreased by 65.6 and 39.5 % respectively, while Y(NO) was increased by 153.9 % compared to NT. Chilling also decreased qP and NPQ by 40.0 and 74.8 % but increased ETR(I)/ETR(II) by 92.4 %. At the same time, chilling decreased Y(I) and Y(NA) by 35.2 and 48.3 %, and increased Y(ND) by 44.8 % compared to NT, but did not affect P_m. EBR treatment significantly alleviated the quantum yield of PS I and PS II under normal temperature and chilling treatment. Compared with chilling stress, EBR treatment increased Y(II) and Y(I) by 62.3 and 25.8 %, respectively. EBR also significantly increased qP and NPQ and decreased Y(NO), Y(ND), and ETR(I)/ETR(II) in tomato plants under chilling stress. Under normal temperature, EBR treatment increased Y(II), Y(I), qP, and P_m, and slightly decreased Y(NPQ), NPQ, and Y(ND), but did not affect F_v/F_m, Y(NO), Y(NA), and ETR(I)/ETR(II) (Table 3).

The OJIP curves obtained from NT and NTBR plants displayed a typical OJIP shape. OJIP curves from chilled plants showed a sharp depression at the J-, I-, and P-step, which were alleviated by EBR treatment (Fig. 2). Chilling significantly decreased performance index (PI_{ABS}), quantum yields (φ_{Po}, φ_{Eo}, and ψ_o), but increased M_o and V_J. Chilling also decreased phenomenological energy fluxes per excited cross section (ABS/CS, TR_o/CS, ET_o/CS) and

Table 3. Effects of 24-epibrassinolide (0.1 μM) and chilling (8 °C) on chlorophyll fluorescence and P700 parameters in leaves of tomato after 4 d of treatment. NT - normal temperature; NTBR - normal temperature with EBR treatment; LT - chilling treatment; LTBR - chilling with EBR treatment. Data are the means of five independent measurements with standard errors. Values followed by different letters are significantly different at the 0.05 % level.

Parameters	NT	NTBR	LT	LTBR
F_v/F_m	0.796±0.003 a	0.806±0.002 a	0.422±0.063 c	0.525±0.015 b
Y(II)	0.401±0.020 b	0.476±0.012 a	0.138±0.009 d	0.224±0.006 c
Y(NPQ)	0.342±0.018 a	0.277±0.007 ab	0.207±0.039 b	0.267±0.022 ab
Y(NO)	0.258±0.002c	0.247±0.006 c	0.655±0.048 a	0.509±0.022 b
qP	0.628±0.022 b	0.710±0.017 a	0.377±0.020 d	0.526±0.015 c
NPQ	1.326±0.061 a	1.123±0.022 b	0.334±0.079 d	0.534±0.071 c
P_m	0.223±0.010 ab	0.269±0.022 a	0.179±0.024 b	0.166±0.022 b
Y(I)	0.497±0.028 b	0.590±0.009 a	0.322±0.009 d	0.405±0.013 c
Y(ND)	0.446±0.020 c	0.344±0.008 d	0.646±0.009 a	0.578±0.016 b
Y(NA)	0.058±0.012 a	0.066±0.004 a	0.030±0.006 b	0.017±0.006 b
ETR(I)/ETR(II)	1.239±0.025 c	1.241±0.026 c	2.384±0.222 a	1.813±0.035 b

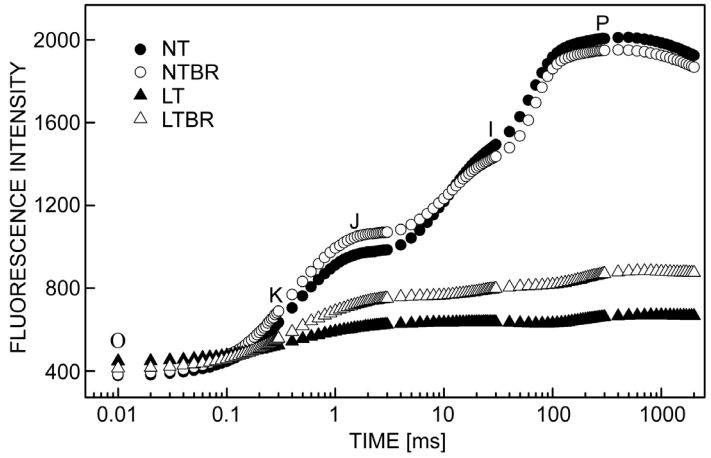

Fig. 2. Effects of 24-epibrassinolide (0.1 μM) and chilling (8 °C) on Chl *a* fluorescence transient (OJIP curve) in leaves of tomato after 4 d of treatment. NT - normal temperature; NTBR - normal temperature with EBR treatment; LT - chilling treatment; LTBR - chilling with EBR treatment. Each curve is the average of five replicates, fluorescence intesity is expressed as [a.u.].

density of RCs (RC/CS). For specific energy fluxes per Q_A^- reducing PS II RC, chilling decreased ET_o/RC**,** however, increased ABS/RC and not influenced TR_o/RC. However, there were no difference in W_K and fraction of OEC between NT and LT. Compared with chilling stress, EBR treatment significantly increased PI_{ABS}, φ_{Po}, φ_{Eo}, ψ_o, RC/CS, ABS/CS, TR_o/CS, and ET_o/CS, and decreased Mo, V_J, and ABS/RC. Under natural temperature, EBR treatment only increased PI_{ABS} and did not influence the other JIP-test parameters (Table 4).

Discussion

Photosynthesis is severely affected by stressful environments (Ashraf and Harris 2013). The process of photosynthesis includes two stages: light-dependent reactions, in which radiation energy absorbed by photosynthetic pigments is converted into ATP and NADPH, and light-independent reactions, in which CO_2 is fixed into saccharides by utilizing the ATP and NADPH produced by light reactions (Taiz and Zeiger 2010, Ashraf and Harris 2013). Our finding that chilling decreased P_N, $P_{N,\,max}$, and AQY (Table 2, Fig 1), suggested that the carbon assimilation and radiation energy utilization were reduced. It is well known that BRs have been shown to increase plant stress tolerance (Krishna 2003, Sasse 2003, Anwar *et al.* 2018). In this study, we observed that EBR increased P_N, $P_{N,\,max}$, AQY, and F_v/F_m in tomato leaves under chilling stress (Table 2, 3, Fig.1). These results suggested that EBR can alleviate the decreases of carbon assimilation and photoinhibition induced by chilling stress. The decrease of photosynthetic rate under stressful conditions was affected by stomatal or nonstomatal limitations (Saibo *et al.* 2009, Ashraf and Harris 2013). In this study, chilling led to the decrease of P_N, g_s, and E, but increased c_i (Table 2), which suggested nonstomatal limitation as the major cause of inhibition of photosynthesis. Compared with chilling

Table 4. Effects of 24-epibrassinolide (0.1 μM) and chilling (8 °C) on JIP-test parameters in leaves of tomato after 4 d of treatment. NT - normal temperature; NTBR - normal temperature with EBR treatment; LT - chilling treatment; LTBR - chilling with EBR treatment. Data are the means of five independent measurements with standard errors. Values followed by different letters are significantly different at the 0.05 % level.

Parameters	NT	NTBR	LT	LTBR
PI_{ABS}	4.276±0.042 b	4.937±0.070 a	0.039±0.007 d	0.195±0.037 c
φ_{Po}	0.824±0.002 a	0.829±0.001 a	0.342±0.020 c	0.539±0.011 b
φ_{Eo}	0.516±0.004 a	0.529±0.002 a	0.085±0.007 c	0.167±0.016 b
ψ_o	0.626±0.006 a	0.637±0.003 a	0.247±0.008 c	0.308±0.025 b
M_o	0.564±0.012 c	0.520±0.007 c	1.188±0.043 a	1.048±0.045 b
V_J	0.366±0.006 b	0.434±0.078 b	0.747±0.009 a	0.687±0.025 a
Fraction of OEC	1.000±0.000 a	0.981±0.028 a	0.949±0.024 a	0.986±0.021 a
W_K	0.453±0.010 ab	0.431±0.009 b	0.473±0.016 a	0.454±0.007 ab
RC/CS	1102 ±47 a	1126 ±27 a	143±2 c	315±14 b
ABS/CS	2013 ±64 a	1948 ±41 a	671±43 c	884±30 b
TR_o/CS	1658 ±49 a	1616 ±35 a	226±10 c	478±25 b
ET_o/CS	1038 ±37 a	1030 ±24 a	56±3 c	148±17 b
ABS/RC	1.83 ±0.04 c	1.73 ±0.04 c	4.67 ±0.26 a	2.81 ±0.06 b
TR_o/RC	1.51 ±0.03 ab	1.44 ±0.03 b	1.58 ±0.05 a	1.51 ±0.02 ab
ET_o/RC	0.94 ±0.03 a	0.92 ±0.02 a	0.39 ±0.02 b	0.47 ±0.03 b

treatment, c_i was significantly decreased in tomato treated by chilling with EBR (Table 2). These results showed that EBR alleviated the decline of photosynthesis under chilling stress partly because it improved the nonstomatal limitation caused by chilling. Cui *et al.* (2017) also observed that EBR increased the stomatal conductance and rates of photosynthesis in tomato cvs. Zhongza9 and Zhongshu4 under chilling conditions, but reduced c_i.

The photosystems are the primary targets for chilling-induced photoinhibition (Bertamini *et al.* 2005), and the susceptibility of PS I and PS II to chilling stress depends on plant species and stressful conditions (Huang *et al.* 2010, Li and Zhang 2015). In this study, the relative fluorescence intensity at point P of the OJIP curve decreased significantly under chilling stress (Fig 2), F_v/F_m and PI_{ABS} were declined by 47.0 and 99.1 % (Table 3, 4), indicating that chilling led to the decrease of photochemical efficiency of PS II, and even photoinhibition. Y(NO) is a good indicator of PS II photodamage (Xiao *et al.* 2019). A high Y(NO) value indicates that both photochemical energy conversion and protective regulatory mechanisms are inefficient. After chilling stress, the values of Y(NO) significantly increased, while the values of Y(II), qP, Y(NPQ), and NPQ strongly decreased (Table 3). These results suggested that excess radiation energy could not be consumed through photochemical quenching and thermal dissipation, which may lead to photoinactivation of PS II, and even photodamage. In fact, we also observed that RC/CS was decreased by 87.0 % in chilled tomato (Table 4), which suggested that chilling stress led to the inactivation of PS II reaction centers and decreased the density of active PS II reaction centers (Zhang *et al.* 2020c). In our present study, there was higher Y(II), qP, Y(NPQ), and NPQ, and lower Y(NO) in LTBR plants than in LT ones (Table 3). These results suggested that the application of EBR protected PS II against photoinhibition by increasing the efficiency of photochemistry and thermal dissipation. In our previous study, we also observed that EBR induced the increases of qP and NPQ in cucumber under chilling treatment (Hu *et al.* 2010). Shu *et al.* (2016) also observed that foliar application of EBR increased F_v/F_m and ΦPS II (actual photochemical efficiency of PS II, same as Y(II) in this study) and qP in tomato leaves under low temperature and weak irradiance by chlorophyll fluorescence imaging. And EBR treatment increased RC/CS in chilled tomato (Table 4), which indicated that the function of PS II was altered by EBR application through the increase in density of active reaction centers. Zhao *et al.* (2019) also observed that exogenous EBR could alleviate the damage caused by chilling to PS II and reduce chilling photoinhibition in *Brassica campestris* L. ssp. *chinensis* var. *rosularis*.

However, we observed that P_m, which can be used to estimate PS I activity (Takagi *et al.* 2017), was not significantly affected by chilling stress (Table 3). In addition, lower values of Y(NA) were also observed (Table 3). The Y(NA) reflects the acceptor side limitation of PS I and could be used as an indicator of PS I photoinhibition (Xiao *et al.* 2019). These results indicated that chilling had less limitation on the acceptor side of PS I, and PS I was relatively insusceptible to chilling stress compared with PS II. PS I photoinhibition is mainly caused by NADPH accumulation leading to overreduction of the PS I acceptor side and the generation of hydroxyl radicals which destroy the PS I complex (Yamori and Shikanai 2016, Xiao *et al.* 2019). It has been shown that CEF can protect PS I against photoinhibition by alleviating overreduction of the PS I acceptor side and by balancing the $NADP^+$/NADPH ratio (Munekage *et al.* 2004, Yamori and Shikanai 2016). The ETR(I)/ETR(II) ratio can serve as an indicator of CEF activation (Yamori *et al.* 2011).

In the present study, we observed that ETR(I)/ETR(II) significantly increased by 92.4 % and Y(NA) maintained at a low level in tomato under chilling stress (Table 3). These results suggested that downregulation of PS II activity and increase of CEF prevented PS I from chilling photoinhibition. However, a lower value of ETR(I)/ETR(II) in LTBR plants was observed (Table 3), which indicated that the protective effect of CEF was relatively smaller in chilled tomato by EBR. We considered that the reason for lower CEF in LTBR was due to the increase of CO_2 assimilation, which would decrease the accumulation of NADPH in chloroplasts.

Since there was greater affection on PS II by chilling stress than PS I, it was necessary to further investigate the inhibition site of PS II. The damages in OEC on the donor side of PS II are always associated with the increase of W_K (Strasser *et al.* 1995, Che *et al.* 2018). W_K did not change in tomato leaves by chilling treatment (Table 4), which indicated that the OEC was not damaged. This was also supported by the data that there were no significant differences in the fraction of OEC (estimation about OEC; Li *et al.* 2010, Chen *et al.* 2014) between LT and NT plants (Table 4). TR_o/RC corresponds to the electron donation from the OEC, unchanged TR_o/RC also indicated that chilling stress did not affect OEC on the donor side of PS II (Chen *et al.* 2014). However, chilling stress inhibited the electron transport at the PS II acceptor side. V_J and M_o increased significantly under chilling stress (Tang *et al.* 2020), which reflected the inhibition of electron transfer from Q_A to Q_B on the PS II acceptor side (Tang *et al.* 2020) and induced accumulation of reduced Q_A (Li *et al.* 2010). Additionally, this was further supported by the significant decrease in φ_{Eo} and Ψ_o (Table 4). The φ_{Eo} refers to the quantum yield of PS II electron transport, and Ψ_o reflects the probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (Chen *et al.* 2014, Guo *et al.* 2021). We also observed ABS/CS, TR_o/CS, and ET_o/CS were lower under chilling stress than in the control (Table 4), which might be attributed to the decrease of PS II active RCs per excited cross section (Jiang *et al.* 2002). In contrast, ABS/RC was higher in plants under chilling stress than in the NT-treated plants (Table 4). Zushi *et al.* (2012) considered that the increase of absorption per active RC was owed to the inactivation of some RCs. However, there was no significant difference in trapping energy flux per RC (TR_o/RC), while the electron transport flux (ET_o/RC) decreased significantly (Table 4), reflecting inhibition in PS II activity (Zushi *et al.* 2012). Compared with chilling treatment, the decreases of V_J and M_o, and the increases of φ_{Eo} and Ψ_o in EBR-treated plants under chilling stress indicated that EBR effectively maintained the stability of the electron transport chain and the activity of PS II (Table 4). EBR treatment alleviated the chill-induced decreases of ABS/CS, TR_o/CS and ET_o/CS, but did not affect TR_o/RC and ET_o/RC (Table 4). These results suggested that EBR promoted the absorption, trapping and electron transport per leaf area in tomato under chilling stress due to the increase of density of active reaction centers, rather than the activity of active RCs.

In conclusion, we found that chilling inhibited CO_2 assimilation and induced photoinhibition of PS II in tomato leaves. And the effect of chilling on PS II was greater than that of PS I, which was due to the protective effect of CEF on the PS I. Chilling stress led to the inactivation of PS II reaction centers and blocked the electron transport at the PS II acceptor side, however, did not affect OEC on the donor side of PS II. EBR alleviated chill-induced inhibition of photosynthesis in tomato leaves partly due to improving nonstomatal limitation caused by chilling. Under chilling stress, exogenous EBR effectively maintained the stability of the electron transport chain and the activity of PS II, and also increased the density of active reaction centers. And, EBR treatment alleviated the chill-induced PS II photoinhibition mainly by the increase of CO_2 assimilation and thermal dissipation of excitation energy in the PS II antennae, while the protective effect of CEF was relatively smaller.

References

Ahammed, G.J., Li, X., Liu, A., Chen, S.: Brassinosteroids in plant tolerance to abiotic stress. - J. Plant Growth Regul. **39**: 1451-1464, 2020.

Allen, D.J., Ort, D.R.: Impacts of chilling temperatures on photosynthesis in warm-climate plants. - Trends Plant Sci. **6**: 36-42, 2001.

Anwar, A., Liu, Y., Dong, R., Bai, L., Yu, X., Li, Y.: The physiological and molecular mechanism of brassinosteroid in response to stress: a review. - Biol. Res. **51**: 46, 2018.

Artuso, A., Guidi, L., Soldatini, G.F., Pardossi, A., Tognoni, F.: The influence of chilling on photosynthesis and activities of some enzymes of sucrose metabolism in *Lycopersicon esculentum* Mill. - Acta Physiol. Plant. **22**: 95-101, 2000.

Ashraf, M., Harris, P.J.C.: Photosynthesis under stressful environments: an overview. - Photosynthetica **51**: 163-190, 2013.

Behnamnia, M., Kalantari, K.M., Rezanejad, F.: Exogenous application of brassinosteroid alleviates drought-induced oxidative stress in *Lycopersicon esculentum* L. - Gen. appl. Plant Physiol. **35**: 22-34, 2009.

Bertamini, M., Muthuchelian, K., Rubinigg, M., Zorer, R., Nedunchezhian, N.: Photoinhibition of photosynthesis in leaves of grapevine (*Vitis vinifera* L. cv. Riesling). Effect of chilling nights. - Photosynthetica **43**: 551-557, 2005.

Brüggemann, W., Klaucke, S., Maas-Kantel, K.: Long-term chilling of young tomato plants under low light. V. kinetic and molecular properties of two key enzymes of the Calvin cycle in *Lycopersicon esculentum* Mill and *L. peruvianum* Mill. - Planta **194**: 160-168, 1994.

Caffagni, A., Pecchioni, N., Francia, E., Pagani, D., Milc, J.: Candidate gene expression profiling in two contrasting tomato cultivars under chilling stress. - Biol. Plant. **58**: 283-295, 2014.

Che, X., Ding, R., Li, Y., Zhang, Z., Gao, H., Wang, W.: Mechanism of long-term toxicity of CuO NPs to microalgae. - Nanotoxicology **12**: 923-939, 2018.

Chen, S., Strasser, R.J., Qiang, S.: *In vivo* assessment of effect of phytotoxin tenuazonic acid on PS II reaction centers. - Plant Physiol. Biochem. **84**: 10-21, 2014.

Cui, L., Cao, K., Zou, Z.: Effects of exogenous 24-epibrassinolide on photosynthesis and ATP synthase B subunit of tomato under low temperature/poor light. - Pak. J. Bot. **49**: 57-62,

2017.
Cui, L., Zou, Z., Zhang, J., Zhao, Y., Yan, F.: 24-epibrassinolide enhances plant tolerance to stress from low temperatures and poor light intensities in tomato (*Lycopersicon esculentum* Mill.). - Funct. integr. Genomics **16**: 29-35, 2016.
Ding, F., Wang, M., Zhang, S., An, X.: Changes in SBPase activity influence photosynthetic capacity, growth, and tolerance to chilling stress in transgenic tomato plants. - Sci. Rep. **6**: 32741, 2016.
Fang, P.P., Yan, M.Y., Chi, C., Wang, M.Q., Zhou, Y.H., Zhou, J., Shi, K., Xia, X.J., Foyer, C.H., Yu, J.Q.: Brassinoteroids act as a positive regulator of photoprotection in response to chilling stress. - Plant Physiol. **180**: 2061-2076, 2019.
Guo, Y., Liu, W., Wang, H., Wang, X., Chen, S.: Action mode of the mycotoxin patulin as a novel natural photosystem II inhibitor. - J. Agr. Food Chem. **69**: 7313-7323, 2021.
Hu, W.H., Song, X.S., Shi, K., Xia, X.J., Zhou, Y.H., Yu, J.Q.: Changes in electron transport, superoxide dismutase and ascorbate peroxidase isoenzymes in chloroplasts and mitochondria of cucumber leaves as influenced by chilling. - Photosynthetica **46**: 581-588, 2008.
Hu, W.H., Wu, Y., Zeng, J.Z., He, L., Zeng, Q.M.: Chill-induced inhibition of photosynthesis was alleviated by 24-epibrassinolide pretreatment in cucumber during chilling and subsequent recovery. - Photosynthetica **48**: 537-544, 2010.
Hu, W.H., Yan, X.H., Xiao, Y.A., Zeng, J.J., Qi, H.J., Ogweno, J.Q.: 24-Epibrassinolide alleviate drought-induced inhibition of photosynthesis in *CaPS Icum annuum*. - Sci. Hort. **150**: 232-237, 2013.
Hu, W.H., Zhou, Y.H., Du, Y.S., Xia, X.J., Yu, J.Q.: Differential response of photosynthesis in greenhouse- and field-ecotypes of tomato to long-term chilling under low light. - J. Plant Physiol. **163**: 1238-1246, 2006.
Huang, W., Yang, Y.J., Hu, H., Zhang, S.B.: Moderate photoinhibition of photosystem II protects photosystem I from photodamage at chilling stress in tobacco leaves. - Front. Plant Sci. **7**: 182, 2016.
Huang, W., Zhang, S.B., Cao, K.F.: The different effects of chilling stress under moderate light intensity on photosystem II compared with photosystem I and subsequent recovery in tropical tree species. - Photosynth. Res. **103**: 175-182, 2010.
Huang, W., Zhang, S.B., Cao, K.F.: Cyclic electron flow plays an important role in photoprotection of tropical trees illuminated at temporal chilling temperature. - Plant Cell Physiol. **52**: 297-305, 2011.
Jiang, D.X., Chu, X., Li, M. Hou, J.J., Chen, G.X.: Exogenous spermidine enhances salt-stressed rice photosynthetic performance by stabilizing structure and function of chloroplast and thylakoid membranes. - Photosynthetica **58**: 61-71, 2002.
Kee, S.C., Martin, B., Ort, D.R.: The effects of chilling in the dark and in the light on photosynthesis of tomato: electron transfer reactions. - Photosynth. Res. **8**: 41-51, 1986.
Kolomeichuk, L.V., Efimova, M.V., Zlobin, I.E., Kreslavski, V.D., Murgan, O.K., Kovtun, I.S., Khripach, V.A., Kuznetsov, V.V., Allakhverdiev, S.I.: 24-epibrassinolide alleviates the toxic effects of NaCl on photosynthetic processes in potato plants. - Photosynth. Res. **146**: 151-163, 2020.
Krishna, P.: Brassinoteroid-mediated stress responses. - J. Plant Growth Regul. **22**: 289-297, 2003.
Lang, J., Barták, M., Hájek, J., Váczi, P., Zikmundová, B.: Chilling effects on primary photosynthetic processes in *Medicago sativa*: acclimatory changes after short- and long-term exposure to low temperatures. - Biologia **75**: 1105-1114, 2020.
Li, J., Yang, P., Gan, Y., Yu, J., Xie, J.: Brassinosteroid alleviates chilling-induced oxidative stress in pepper by enhancing antioxidation systems and maintenance of photosystem II. - Acta Physiol. Plant. **37**: 222, 2015.
Li, J.W., Zhang, S.-B.: Differences in the responses of photosystems I and II in *Cymbidium siense* and *C. tracyanum* to long-term chilling stress. - Front. Plant Sci. **6**: 1097, 2015.
Li, Q., Chen, L.S., Jiang, H.X., Tang, N., Lin, A.H., Yang, G.H.: Effects of manganese-excess on CO_2 assimilation, ribulose-1,5-bisphosphate carboxylase/oxygenase, carbohydrates and photosynthetic electron transport of leaves, and antioxidant systems of leaves and roots in *Citrus grandis* seedlings. - BMC Plant Biol. **10**: 42, 2010.
Mlinarić, S., Cesar, V., Lepeduš, H.: Antioxidative response and photosynthetic regulatory mechanisms in common fig leaves after short-term chilling stress. - Ann. appl. Biol. **178**: 315-327, 2021.
Mumtaz, M.A., Munir, S., Liu, G., Chen, W., Wang, Y., Yu, H., Mahmood, S., Ahiakpa, J.K., Tamim, S.A., Zhang, Y.: *Altered brassinolide sensitivity1* transcriptionally inhibits chlorophyll synthesis and photosynthesis capacity in tomato. - Plant Growth Regul. **92**: 417-426, 2020.
Munekage, Y., Hashimoto, M., Miyake, C., Tomizawa, K.I., Endo, T., Tasaka, M., Shikanai, T.: Cyclic electron flow around photosystem I is essential for photosynthesis. - Nature **429**: 579-582, 2004.
Ogweno, J.O., Song, X.S., Shi, K., Hu, W.H., Mao, W.H., Zhou, Y.H., Yu, J.Q., Nogués, S.: Brassinosteroids alleviate heat-induced inhibition of photosynthesis by increasing carboxylation efficiency and enhancing antioxidant systems in *Lycopersicon esculentum*. - J. Plant Growth Regul. **27**: 49-57, 2007.
Ort, D.R., Baker, N.R.: A photoprotective for O_2 as an alternative electron sink in photosynthesis. - Curr. Opin. Plant Biol. **5**: 193-198, 2002.
Park, E.J., Jeknić, Z., Sakamoto, A., DeNoma, J., Yuwansiri, R., Murata, N., Chen, T.H.H.: Genetic engineering of glycinebetaine synthesis in tomato protects seeds, plants, and flowers from chilling damage. - Plant J. **40**: 474-487, 2004.
Qian, T., Zheng, X., Yang, J., Xu, Y., Lu, S.: Optimal utilization of light energy in semiclosed greenhouse using three-dimensional cucumber model. - Sci. Programming Towards a Smart World **2020**: 8855063, 2020.
Saibo, N.J.M., Lourenço, T., Oliveira, M.M.: Transcription factors and regulation of photosynthetic and related metabolism under environmental stresses. - Ann. Bot. **103**: 609-623, 2009.
Sasse, J.M.: Physiological actions of brassinosteroids: an update. - J. Plant Growth Regul. **22**: 276-288, 2003.
Sharma, I., Ching, E., Saini, S., Bhardwaj, R., Pati, P.K.: Exogenous application of brassinosteroid offers tolerance to salinity by altering stress responses in rice variety Pusa Basmati-1. - Plant Physiol. Biochem. **69**: 17-26, 2013.
Shu, S., Tang, Y., Yuan, Y., Sun, J., Zhong, M., Guo, S.: The role of 24-epibrassinolide in the regulation of photosynthetic characteristics and nitrogen metabolism of tomato seedlings under a combined low temperature and weak light stress. - Plant Physiol. Biochem. **107**: 344-353, 2016.
Strasser, R.J., Tsimilli-Michael, M., Srivastava, A.: Analysis of the chlorophyll *a* fluorescence transient. - In: Pagageorgiou G.C., Govindjee (ed.): Chlorophyll Fluorescence: A Signature of Photosynthesis. Pp. 321-362. Kluwer Academic Publishers, Dordrecht 2004.
Strasser, R.J., Srivastava, A., Govindjee: Polyphasic chlorophyll *a* fluorescence transient in plants and cyanobacteria. - Photochem. Photobiol. **61**: 32-42, 1995.
Sun, Y., He, Y., Irfan, A.R., Liu, X., Yu, Q., Zhang, Q., Yang,

D.: Exogenous brassinolide enhances the growth and cold resistance of maize (*Zea mays* L.) seedlings under chilling stress. - Agronomy **10**: 488, 2020.

Taiz, L., Zeiger, E.: Plant Physiology. 5th Edition. Sinauer Associates, Sunderland 2010.

Takagi, D., Amako, K., Hashiguchi, M., Fukaki, H., Ishizaki, K., Goh, T., Fukao, Y., Sano, R., Kurata, T., Demura, T., Sawa, S., Miyake, C.: Chloroplastic ATP synthase builds up a proton motive force preventing production of reactive oxygen species in photosystem I. - Plant J. **91**: 306-324, 2017.

Takahashi, S., Badger, M.R.: Photoprotection in plants: a new light on photosystem II damage. - Trends Plant Sci. **16**: 1360-1385, 2011.

Tang, X.D., An, B.Y., Cao, D.M., Xu, R., Wang, S.Y., Zhang, Z.D., Liu, X.J., Sun, X.G.: Improving photosynthetic capacity, alleviating photosynthetic inhibition and oxidative stress under low-temperature stress with exogenous hydrogen sulfide in blueberry seedlings. - Front. Plant Sci. **11**: 108, 2020.

Tsimilli-Michael, M.: Revisiting JIP-test: an educative review on concepts, assumptions, approximations, definitions and terminology. - Photosynthetica **57**: 90-107, 2019.

Wu, X.X., Ding, H.D., Chen, J.L., Zhu, Z.W., Zha, D.S.: Amelioration of oxidative damage in *Solanum melongena* seedlings by 24-epibrassinolide during chilling stress and recovery. - Biol. Plant. **59**: 350-356, 2015.

Xia, X.J., Huang, L.F., Zhou, Y.H., Mao, W.H., Shi, K., Wu, J.X., Asami, T., Chen, Z., Yu, J.Q.: Brassinosteroids promote photosynthesis and growth by enhancing activation of Rubisco and expression of photosynthetic genes in *Cucumis sativus*. - Planta **230**: 1185-1196, 2009a.

Xia, X.J., Wang, Y.J., Zhou, Y.H., Tao, Y., Mao, W.H., Shi, K., Asami, T., Chen, Z., Yu, J.Q.: Reactive oxygen species are involved in brassinosteroids-induced stress tolerance in *Cucumis sativus*. - Plant Physiol. **150**: 801-814, 2009b.

Xia, X.J., Zhou, Y., Ding, J., Shi, K., Asami, T., Chen, Z., Yu, J.: Induction of systemic stress tolerance by brassinosteroid in *Cucumis sativus*. - New Phytol. **191**: 706-720, 2011.

Xiao, F., Zhang, Y.L., Yang, Y.L., Zhang, W.F.: Downregulation of PS II activity and increased cyclic electron transport in cotton prevents PS I from photoinhibition due to night chilling. - Photosynthetica **57**: 523-532, 2019.

Yamori, W., Sakata, N., Suzuki, Y., Shikanai, T., Makino, A.: Cyclic electron flow around photosystem I *via* chloroplast NAD(P)H dehydrogenase (NDH) complex performs a significant physiological role during photosynthesis and plant growth at low temperature in rice. - Plant J. **68**: 966-976, 2011.

Yamori, W., Shikanai, T.: Physiological functions of cyclic electron transport around photosystem I in sustaining photosynthesis and plant growth. - Annu. Rev. Plant Biol. **67**: 81-106, 2016.

Ye, Z.P.: A new model for relationship between irradiance and the rate of photosynthesis in *Oryza sativa*. - Photosynthetica **45**: 637-640, 2007.

Yu, J.Q., Huang, L.F., Hu, W.H., Zhou, Y.H., Mao, W.H., Ye, S.F., Nogués, S.: A role for brassinosteroids in the regulation of photosynthesis in *Cucumis sativus*. - J. exp. Bot. **55**: 1135-1143, 2004.

Yu, J.Q., Matsui, Y.: Effects of roots exudates and allelochemicals on ion uptake by cucumber seedlings. - J. chem. Ecol. **23**: 817-827, 1997.

Zhang, F.H., Lu, K., Gu, Y.Y., Zhang, L., Li, W.Y., Li, Z.: Effects of low-temperature stress and brassinolide application on the photosynthesis and leaf structure of tung tree seedlings. - Front. Plant Sci. **10**: 1767, 2020a.

Zhang, J.F., Li, J., Xie, J.M., Yu, J.H., Tang, C.N.: Changes in photosynthesis and carotenoid composition of pepper (*CaPS Icum annuum* L.) in response to low-light and low temperature combined with low-light stress. - Photosynthetica **58**: 125-136, 2020b.

Zhang, L.T., Xu, R., Liu, J.G.: Efficacy of botanical pesticide for rotifer extermination during the cultivation of *NannochloroPS Is oculate* probed by chlorophyll *a* fluorescence transient. - Photosynthetica **58**: 156-162, 2020c.

Zhang, Y.P., Zhu, X.H., Ding, H.D., Yang, S.J., Chen, Y.Y.: Foliar application of 24-epibrassinolide alleviates high-temperature-induced inhibition of photosynthesis in seedlings of two melon cultivars. - Photosynthetica **51**: 341–349, 2013.

Zhao, M., Yang, L., Wang, J., Xie, S., Zheng, Y., Nie, L., Zhu, S., Hou, J., Chen, G., Wang, C.: Transcriptome analysis reveals a positive effect of brassinosteroids on the photosynthetic capacity of wucai under low temperature. - BMC Genomics **20**: 810, 2019.

Zhao, Y., Yu, H., Zhou, J.M., Smith, S.M., Li, J.: Malate circulation: linking chloroplast metabolism to mitochondrial ROS. - Trends Plant Sci. **25**: 446-454, 2020.

Zhou, Y.H., Yu, J.Q., Huang, L.F., Nogués, S.: The relationship between CO_2 assimilation, photosynthetic electron transport and water-water cycle in chill-exposed cucumber leaves under low light and subsequent recovery. - Plant Cell Environ. **27**: 1503-1514, 2004.

Zushi, K., Kajiwara, S., Matsuzoe, N.: Chlorophyll *a* fluorescence OJIP transient as a tool to characterize and evaluate response to heat and chilling stress in tomato leaf and fruit. - Sci. Hort. **148**: 39-46, 2012.