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Role of thermal dissipation in the photoprotection in cucumber plants after exposure to a chill stress

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Abstract

Experiments were carried out to investigate the changes in CO_2 assimilation, photon allocation, and photosynthetic electron flux in leaves of cucumber (*Cucumis sativus* L.) plants after chilling stress. Chilling significantly decreased CO_2 assimilation, the energy flux *via* linear electron transport (J_{PS2}) and non-constitutive thermal dissipation (J_{NPQ}) but increased fluorescence and constitutive thermal dissipation ($J_{f,D}$) in chilling-sensitive genotype Jinyan No. 4. In contrast, chilling had little effects on J_{NPQ} and $J_{f,D}$ although CO_2 assimilation and J_{PS2} were inhibited in chilling-tolerant genotype Jinchun No. 3. In parallel with the reduction in J_{PS2}, electron flux to oxygenation and carboxylation by ribulose-1,5-bisphosphate carboxylase/oxygenase all significantly decreased while electron flux to O_2 significantly increased, especially in chilling-sensitive genotype. Thermal and fluorescence dissipation were the main energy dissipation pathways whilst water-water cycle was an important electron sink when photosynthetic carbon reduction was suppressed after chilling. Chilling sensitivity of the photosynthetic apparatus was related to the operation of different photoprotection mechanisms.

Additional key words: alternative electron sink; chilling; *Cucumis sativus*; photosynthesis; photosystem 2; .

Introduction

Plants frequently encounter irradiances that exceed their photosynthetic capacity, especially when their capacity of CO_2 fixation is reduced by stress conditions. Consequently, plants have developed several strategies to minimize the harmful effects of excess energy (Ort and Baker 2002). Recent studies have shown that plants initiate processes such as xanthophyll cycle-dependent energy dissipation as heat from antenna in photosystem 2 (PS2), the D1 repair cycle, photorespiration, and the operation of water-water cycle to protect the chloroplast from damage (Schöner and Krause 1990, Schnettger *et al.* 1994, Leipner *et al.* 1997, Osmond *et al*. 1997, Xu *et al*. 1999, Ort and Baker 2002). Under stress such as chilling and drought, CO_2 assimilation is suppressed whilst the excess electronic excitation and reductants may increase, leading to an oxidative stress and photodamage induced by an overproduction of reactive oxygen species (ROS) (Prasad *et al.* 1994).

Quantification of the fate of photon energy absorbed by leaves is important in order to understand the mechanism of photoprotection in chloroplast. Several methods for the quantification of the absorbed energy have been developed (Demmig-Adams et al. 1996, Hendrickson *et al*. 2004, Kramer et al. 2004). Recently, Hendrickson *et al.* (2004) proposed a new calculation model which divides the absorbed energy into the energy flux *via* linear

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Abbreviations: A, antheraxanthin; Chl, chlorophyll; Fm, maximal fluorescence yields; Fm', Fs, maximal and steady-state fluorescence yields in a light-adapted state; *J*a, alternative electron flux in PS2; *J*c, electron flux for photosynthetic carbon reduction; *J*o, electron flux for photorespiratory carbon oxidation; *J*f,D, energy flux *via* fluorescence and constitutive thermal dissipation; *J*NPQ, energy flux *via* ΔpH- and xanthophyll-regulated thermal energy dissipation; *J*PS2, energy flux *via* linear electron transport; *P*Nsat, photon-saturated CO2 assimilation rate; PPFD, photosynthetic photon flux density; PS, photosystem; ROS, reactive oxygen species; RuBPCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; V, violaxanthin; Z, zeaxanthin; Φf,D, quantum efficiency of fluorescence and constitutive thermal dissipation; ΦNPQ, quantum efficiency of ΔpH- and xanthophyll-regulated thermal energy dissipation; ΦPS2, quantum efficiency of PS2 photochemistry.

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electron transport, the energy flux *via* regulated thermal processes, and the energy flux *via* fluorescence and constitutive thermal dissipation. Meanwhile, several studies have also shown that thermal dissipation plays a more important role in the dissipation of excess energy than other processes such as water-water cycle (Hendrickson *et al.* 2004, Hirotsu *et al.* 2004). However, information about the proportion of alternative electron sinks and associated water-water cycle in the photoprotection is still scanty despite numerous reports about the changes in ROS under different biotic and abiotic stresses (Cakmak *et al.* 1992, Shen *et al.* 1999, Garcia-Limones *et al.* 2002, Laloi *et al.* 2004, Mithofer *et al.* 2004).

Recently, we have shown that chill under low irradiance results in the loss or inactivation of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO), together with an increased proportion of electron influx to O_2 and significant increases in the activities of antioxidant enzymes such as superoxide dismutase and ascorbate peroxidase in leaves (Zhou *et al.* 2004b). In this study, we examined the effects of chilling on the partitioning of excitation energy between photochemistry, fluorescence and thermal dissipation and the electron flux in leaves of two genotypes of cucumber with different chilling tolerances of the photosynthetic apparatus.

Materials and methods

Plants: Two cucumber genotypes (*Cucumis sativus* L. cvs. Jinchun No 3 and Jinyan No 4) were grown in a non-heated greenhouse at Zhejiang University, China. Jinchun No 3 is a chilling-tolerant genotype bred for cultivation in unheated greenhouses while Jinyan No. 4 is a chilling-sensitive genotype bred for cultivation in the field (Shen *et al*. 1999). Seeds were sown directly in a growth substrate containing a mixture of soil and perlite (1 : 1, v/v) in 12-cm plastic pots. Average day/night temperatures were 25/17 °C. Plants were watered and fertilized daily with half-strength Enshi nutrient solution (Yu and Matsui 1997). After 2 weeks of pre-culture, groups of six seedlings were transplanted into containers (40×25×15 cm) filled with the same nutrient solution, and then transferred into a growth chamber for 10 d. The environmental conditions were as follows: a 12 h photoperiod, temperature of 25/17 °C (day/night), and photosynthetic photon flux density (PPFD) of 600 μmol (photon) m^{-2} s^{-1}.

Chill and low irradiance treatment: One-half of the plants, at the four-leaf stage, was exposed to 100 μmol(photon) m^{-2} s^{-1} at 9/7 °C and the other half was maintained at 25/17 °C with a PPFD of 600 μmol(photon) m^{-2} s^{-1}. Ten days later, chilled plants were returned to the control growth chamber at 25/17 °C and 600 μmol(photon) m^{-2} s^{-1} PPFD for 2 d. Gas exchange and chlorophyll (Chl) fluorescence quenching analysis were determined after 2 h and 2 d (designated as chilled and recovered, respectively) on return to optimal growth conditions. Meanwhile, leaves were sampled, frozen quickly in liquid nitrogen and stored at −86 °C before being used for the pigment analysis. Throughout the experiment, all measurements were performed randomly on fully expanded leaves, using four replicates.

Leaf gas exchange and energy dissipation analysis: Leaf photon-saturated CO_2 assimilation rate (P_{Nsat}) was measured using a gas exchange system (*CIRAS-1*, *PP System*, Herpenden, Herts., UK) on the second fully developed leaf of each seedling (Zhou *et al*. 2004b). The air temperature, air relative humidity, CO_2 concentration, and PPFD were maintained at 25 °C, 80−90 %, 350 μmol(CO_2) mol^{-1}, and 1 050 μmol(photon) m^{-2} s^{-1}, respectively.

Modulated Chl fluorescence was measured simultaneously with a pulse amplitude fluorimeter (*Hansatech Instruments*, Norfolk, UK) on the same leaves previously used for gas exchange measurements, as described in Zhou *et al*. (2004b). Non-photochemical quenching (NPQ) was calculated as $F_m/F_m' - 1$ according to Demmig-Adams and Adams (1996). The quantum efficiency of photochemical energy dissipation (Φ_{PS2}, $1 - F_s/F_m'$), ΔpH, and xanthophyll-regulated thermal energy dissipation (Φ_{NPQ}, $F_s/F_m' - F_s/F_m$), and fluorescence and constitutive thermal dissipation ($\Phi_{f,D}$, F_s/F_m) were calculated according to Hendrickson *et al.* (2004) with $\Phi_{PS2} + \Phi_{NPQ} + \Phi_{f,D} = 1$. The flux of energy dissipation *via* each process (J_{PS2}; J_{NPQ}; $J_{f,D}$) was calculated by multiplying respective quantum efficiency (Φ) with irradiance and coefficient α, respectively (Harley *et al*. 1992, Hendrickson *et al.* 2004), where α is absorptance × ratio of allocation of excitation energy to photosystem 2 (PS2) and was measured as described by Miyake and Yokota (2000). Utilization of photons absorbed by the PS2 antennae in photosynthetic electron transport and thermal dissipation was then assessed from the quantum efficiency and flux of each process.

Estimation of the flux of alternative electron flow: The flux of electron transport through PS2 (J_{PS2}) was calculated as: $J_{PS2} = \Phi_{PS2} \times \alpha \times PPFD$. The rates of oxygenation by ribulose-1,5-bisphosphate carboxylase/oxygenase, RuBPCO (V_o) and of carboxylation by RuBPCO (V_c) were estimated (Caemmerer and Farquhar 1981). Under atmospheric conditions, the electron fluxes in the two cycles were expressed as $J_c = 4 \times V_c$ and $J_o = 4 \times V_o$, respectively (Krall and Edward 1992). An alternative flux, J_a, caused by electrons that are not used by the carboxylation and/or oxygenation cycles in the total electron flux driven by PS2, was estimated from $J_{PS2} - (J_c + J_o)$ (Miyake and Yokota 2000). O_2-dependent J_a was estimated from the differences between J_a (21 % O_2) and J_a (2 % O_2) (Miyake and Yokota 2000). O_2-independent J_a was then estimated from the difference between J_a and O_2-dependent J_a (Zhou *et al.* 2004b).

Pigment analysis: Pigments for xanthophyll cycle pool (V, violaxanthin; A, antheraxanthin; Z, zeaxanthin) were extracted with 80 % acetone, and were analyzed by HPLC according to Thayer and Björkman (1990). The de-epoxidation form for the xanthophyll cycle was expressed as (A + Z)/(V + A + Z).

Results

Photon-saturated rate of the CO_2 assimilation (P_{Nsat}) for chilling-tolerant genotype (Jinchun No. 3) did not differ from that of chilling-sensitive genotype (Jinyan No. 4) before chilling. Chilling resulted in significant reduction in P_{Nsat} for both genotypes, chilling-tolerant genotype, however, showed significantly higher P_{Nsat} values than chilling-sensitive genotype after exposure to chilling at 7 °C (Fig. 1). After 2 d of recovery, full restoration in P_{Nsat} was observed in chilling-tolerant genotype whilst P_{Nsat} for chilling-sensitive genotype was only 40.6 % of the pre-chill control.

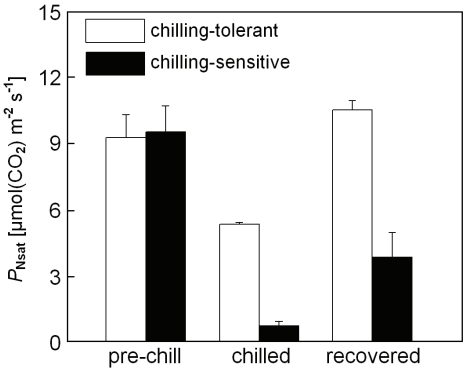

Fig. 1. Effects of chilling on CO_2 assimilation rate under saturating irradiance (P_{Nsat}) in cucumber leaves. Measurements were made after 2 h at 25 °C and 600 μmol(photon) m^{-2} s^{-1}. Values are means±SE of 4 independent measurements.

The allocation of photons absorbed by PS2 antenna to photosynthetic electron transport and thermal dissipation was assessed from the flux *via* linear electron transport (J_{PS2}), ΔpH- and xanthophyll-regulated thermal energy dissipation (J_{NPQ}), and fluorescence and constitutive thermal dissipation ($J_{f,D}$). For pre-chill plants, there were no significant differences in J_{PS2}, J_{NPQ}, and $J_{f,D}$ between the two genotypes (Table 1). J_{PS2} significantly decreased as a result of chilling at 7 °C, but the decrease was less significant in chilling-tolerant genotype. In comparison to the little changes observed in J_{NPQ} and $J_{f,D}$ for chilling-tolerant genotype, J_{NPQ} decreased by 40.0 % and $J_{f,D}$ increased by 85.2 % for chilling-sensitive genotype, respectively. For chilling-sensitive genotype, both J_{NPQ} and $J_{f,D}$ recovered and maintained higher values after 2-d recovery. In comparison, only a slight increase in J_{NPQ} was observed in chilling-tolerant genotype.

Table 1. Changes in the energy flux *via* linear electron transport in PS2 (J_{PS2}), flux for energy loss *via* non-constitutive thermal dissipation (J_{NPQ}), and fluorescence and constitutive thermal dissipation ($J_{f,D}$) as influenced by chilling under low irradiance. Measurements were made at 25 °C and 1 050 μmol(photon) m^{-2} s^{-1}. Means±SE of 4 independent measurements. Values followed by distinct letters indicate significant difference at 5 % level.

Genotype	Stage	Energy flux [μmol(e^-) m^{-2} s^{-1}]		
		J_{PS2}	J_{NPQ}	$J_{f,D}$
Chilling-tolerant	Pre-chill	73.8±6.9 a	124.9±19.0 ab	78.9±16.6 b
	Chilled	48.2±1.2 b	107.6±10.3 b	68.9±7.1 b
	Recovered	78.3±1.2 a	131.7±1.7 ab	75.8±1.8 b
Chilling-sensitive	Pre-chill	77.0±9.8 a	110.5±16.7 b	58.0±7.3 b
	Chilled	21.5±0.4 c	66.4±15.9 c	111.2±20.6 a
	Recovered	45.2±6.6 b	146.7±10.0 a	74.2±14.4 b

Table 2. Changes in energy flux *via* linear electron transport in PS2 (J_{PS2}), including electron flux for photosynthetic carbon reduction (J_c), electron flux for photorespiratory carbon oxidation (J_o), O_2-dependent alternative electron flux (O_2-dependent J_a), and O_2-independent alternative electron flux (O_2-independent J_a) as influenced by chilling under low irradiance. Measurements were made at 25 °C and 1 050 μmol(photon) m^{-2} s^{-1}. Means±SE of 4 independent measurements. Values followed by distinct letters indicate significant difference at 5 % level.

Genotype	Stage	Electron flux [μmol(e^-) m^{-2} s^{-1}]			
		J_c	J_o	O_2-dependent J_a	O_2-independent J_a
Chilling-tolerant	Pre-chill	47.5±5.0 a	20.9±2.2 a	3.6±0.1 c	1.8±0.3 cd
	Chilled	26.9±0.1 b	11.0±0.6 b	8.1±0.7 b	2.3±0.1 bc
	Recovered	53.0±1.5 a	21.7±1.2 a	2.1±1.2 c	1.5±0.2 d
Chilling-sensitive	Pre-chill	49.7±6.5 a	23.1±3.6 a	2.8±0.3 c	1.5±0.4 d
	Chilled	4.2±1.0 c	2.5±0.5 c	11.2±1.3 a	3.6±0.1 a
	Recovered	20.5±6.1 b	10.1±3.3 b	11.6±2.1 a	2.9±0.7 b

The total electron flux in PS2 (J_{PS2}) was further divided into electron flux for photosynthetic carbon reduction (J_c), electron flux for photorespiratory carbon oxidation (J_o), O_2-dependent-alternative electron flux (O_2-dependent J_a), and O_2-independent-alternative electron flux (O_2-independent J_a) (Table 2). For pre-chill plants, there were no significant differences in electron fluxes in the two genotypes, J_c, J_o, and J_a accounted for approximately 64–65, 28–30, and 6–7 % of J_{PS2}, respectively. Chilling significantly decreased J_c and J_o, but increased O_2-dependent J_a in both genotypes, especially in the chilling-sensitive genotype. A reduction in J_c and an increase in O_2-dependent J_a, induced by a 7 °C-chill, were also found in the chilling-sensitive genotype but not in the chilling-tolerant genotype after 2-d recovery under optimal growth conditions. Finally, in comparison with the chilling-sensitive genotype, the chilling-tolerant genotype exhibited significantly higher J_c and J_o and lower O_2-dependent J_a after the chilling treatment. O_2-dependent J_a, for example, increased to 16.7 and 2.6 % after chilling at 7 °C and after chilling followed by 2-d recovery, whilst it constituted 52.1 and 25.7 % of J_{PS2} for the chilling-sensitive genotype, respectively.

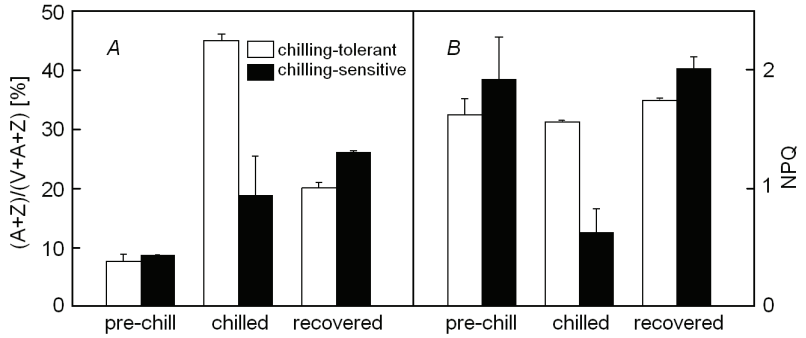

Fig. 2. Effects of chilling on the de-epoxidised form of xanthophyll cycle [(A + Z)/(V + A + Z), *A*], and non-photochemical quenching (NPQ, *B*) in cucumber leaves. Values are means±SE of 4 independent measurements.

We investigated also the changes of conversion state of the xanthophyll cycle pool (A + Z)/(V + A + Z) during the treatment (Fig. 2). Although there were no significant differences in the changes of conversion state for plants grown under optimal growth conditions, chilling, however, resulted in a significant increase in the rate of changes for both genotypes, especially in the chilling-tolerant genotype. This rate sharply increased after chilling and then fell during the recovery for the chilling-tolerant genotype whilst it increased throughout the experiment for the chilling-sensitive genotype. In comparison, significant reduction in the non-photochemical quenching (NPQ) was only found in chilled leaves of the chilling-sensitive genotype and it soon recovered after 2-d recovery under optimal growth conditions.

Discussion

We found large differences in chilling tolerance of the photosynthetic apparatus between two genotypes. Jinchun No. 3, a greenhouse genotype developed for cold season, showed reversible inhibition of photosynthesis and higher CO_2 assimilation rate than Jinyan No. 4, a genotype developed for the warm season (Fig. 1). This is generally in agreement with our early conclusion that genotypes developed under low temperature regime exhibit stronger tolerance of the photosynthetic apparatus to chilling (Yu *et al.* 2002, Zhou *et al.* 2004b). Greenhouse genotype has probably developed a greater ability to acclimate its photosynthetic apparatus to low temperature.

Changes in CO_2 assimilation are associated with those of energy absorption and allocation within the chloroplasts. Interestingly, we found that non-constitutive (J_{NPQ}) and constitutive thermal dissipation and fluorescence energy dissipation ($J_{f,D}$) for the chilling-tolerant genotype were not significantly influenced by chilling although flux *via* linear electron transport (J_{PS2}) was significantly decreased (Table 1). In comparison, J_{NPQ} for the chilling-sensitive genotype significantly decreased whilst $J_{f,D}$ significantly increased after chilling. Therefore, chilling tolerance might be related to its capacity of maintaining thermal dissipation. Probably these two genotypes with different ecological background use different mechanisms in photoprotection as it was found in their different response to dark chilling (Zhou *et al.* 2004a). However, many studies have reported that NPQ increased after chill and drought stresses (Xu *et al.* 1999, Jung 2004). However, we showed that NPQ and J_{NPQ} were slightly changed in the chilling-tolerant genotype or even decreased in the chilling-sensitive genotype after chilling although there was a significant increase in the xanthophyll-cycle capacity (Fig. 2, Table 1). Several reports suggest that non-constitutive thermal dissipation, which is closely associated with the de-epoxidation of the xanthophyll-cycle, is a vital photoprotective process at low temperatures (Leipner *et al.* 1997, Hendrickson *et al.* 2004, Hirotsu *et al.* 2004). Increase in NPQ with increased xanthophyll-cycle capacity was not observed in our

studies, but it was found in other studies (Leipner *et al.* 1997, Xu *et al.* 1999). This discrepancy could be attributed to the differences in experimental conditions since most these studies with increased NPQ were carried out under non-photoinihibition conditions. In our study, however, photoinhibition of PS2 occurred after a long-term chilling (data not shown) and this could have greatly changed the photon allocation within PS2. The long-term chilling in our experiment could have partly inactivated the PS2 core complex which is associated with a Z-independent non-photochemical quenching mechanism (Finazzi *et al.* 2004), resulting in the loss of capacity for non-photochemical quenching in PS2. In fact, NPQ does not include only ΔpH-dependent and xanthophyll-cycle dependent quenching, but also triplet quenching, direct O_2 quenching, and direct oxidized-PQ-pool quenching.

The energy utilized by photochemistry (J_{PS2}) may be eventually consumed by CO_2 assimilation, photorespiration, the water-water cycle, the PS2 cycle flow, *etc*. We found that photorespiration is not a protective mechanism after chilling since the electron flux for photo-respiratory carbon oxidation (J_o) significantly decreased (Table 2). Currently, the role of water-water cycle in photoprotection is a subject of the intensive research (Leipner *et al.* 1997, Makino *et al.* 2003, Rizhsky *et al.* 2003). In some studies, the role of water-water cycle in photoprotection was minimal whilst some authors concluded that water-water cycle was important in photoprotection (Heber *et al.* 2001, Hirotsu *et al.* 2004, Zhou *et al*. 2004b). We found that O_2-dependent J_a, which is believed to be driven mainly by water-water cycle, comprises only 3.6 % of the J_{PS2} and 1.1 % of total photon energy absorbed in pre-chill plants but increased to 51.1 % of J_{PS2} and 5.6 % of the total photon energy absorbed in chilled plants of the chilling-sensitive genotype. The proportion of water-water cycle was even lower in the chilling-tolerant genotype. Apparently, as compared to the thermal dissipation, the role of water-water cycle in the energy dissipation was inconsequential for plants under optimal growth conditions. However, our results showed that water-water cycle is an important alternative electron sink for photosynthetic electrons especially when CO_2 assimilation is suppressed.

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