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# The susceptibility of cucumber and sweet pepper to chilling under low irradiance is related to energy dissipation and water-water cycle

X.-G. LI[*,**], Q.-W. MENG[*,+], G.-Q. JIANG[*], and Q. ZOU[*]

*College of Life Sciences, Shandong Agricultural University, Tai'an, Shandong, 271018, P.R. China*[*]
*Crop Institute, Shandong Academy of Agricultural Sciences, Ji'nan, Shandong, 250100, P.R. China*[**]

## Abstract

The photoprotection of energy dissipation and water-water cycle were investigated by comparing chilling sensitivity of photosystems 2 (PS2) and 1 (PS1) in two chilling-sensitive plants, cucumber and sweet pepper, upon exposure to 4 °C under low irradiance (100 μmol $m^{-2}$ $s^{-1}$) for 6 h. During chilling stress, the maximum photochemical efficiency of PS2 ($F_v/F_m$) decreased only slightly in both plants, but the oxidisable P700 decreased markedly, which indicated that PS1 was more sensitive to chilling treatment under low irradiance than PS2. Sweet pepper leaves had lower $F_v/F_m$, higher non-photochemical quenching (NPQ), and higher oxidisable P700 during chilling stress. Activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX) in cucumber leaves was higher, but APX activity decreased apparently compared to that at room temperature. The productions of active oxygen species ($H_2O_2$, $O_2^{-}\cdot$) increased in both plants, faster in cucumber leaves than in sweet pepper leaves. In sweet pepper leaves, a stronger de-epoxidation of the xanthophyll cycle pigments, a higher NPQ could act as a major protective mechanism to reduce the formation of active oxygen species during stress. Thus sensitivity of both plants to chilling under low irradiance was dominated by the protective mechanisms between PS1 and PS2, especially the energy dissipation and the water-water cycle.

*Additional key words*: ascorbate peroxidase; *Capsicum annuum*; chlorophyll fluorescence; *Cucumis sativus*; electron transport rate; P700; photosystems 1 and 2; superoxide dismutase; xanthophyll cycle pigments.

## Introduction

Low temperature is the major factor limiting the productivity and geographical distribution of chilling-sensitive plant species, including important vegetable crops such as cucumber, tomato, and sweet pepper. The combination of low temperature with irradiance may induce chronic photoinhibition of photosystem 2 (PS2). This is partly because lower temperature generally reduces the rates of biological reactions, particularly carbon dioxide reduction and photorespiration, and therefore limits the sinks for the absorbed excitation energy (Allen and Ort 2001).

In order to remain competitive, photosynthetic organisms seek out the delicate balance between efficient light harvesting under limiting irradiance and regulated dissipation of energy under excess irradiance. Much attention has been focused to the processes of thermal energy dissipation in the light-harvesting antenna complexes of PS2 measured as non-photochemical quenching (NPQ), which protect the photosynthetic apparatus from inactivation and damage caused by excess excited energy (Horton *et al*. 1994). At chilling temperature under low irradiance, NPQ might be an efficient pathway for plants to alleviate PS2 photoinhibition (Xu *et al.* 1999, Liu *et al*. 2001). The xanthophyll cycle-dependent dissipation process contributes to increased stability of the photosynthetic apparatus under excess irradiance at low temperatures (Xu *et al.* 1999).

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[+]Author for correspondence; fax: +86 538 8226399, e-mail: qwmen@sdau.edu.cn
*Abbreviations*: A – antheraxanthin; APX – ascorbate peroxidase; AsA – ascorbic acid; ETR – electron transport rate; $F_0$ – initial fluorescence; $F_m^0$ – maximum yield of fluorescence after dark adaptation for more than 2 h at room temperature prior to treatments; $F_v/F_m$ – maximum photochemical efficiency of PS2 with all reaction centres open; FM – fresh mass; MV – methyl viologen; NBT – nitroblue tetrazolium; NPQ – non-photochemical quenching; PFD – photon flux density; PS – photosystem; SOD – superoxide dismutase; V – violaxanthin; Z – zeaxanthin.
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Another important mechanism is the water-water cycle, which is referred to the reduction of atmospheric $O_2$ into water in PS1 by the electrons generated from water in PS2 and so without a net change of $O_2$. It may be an effective protection mechanism under environmental stress (Asada 1999). The $O_2^{-\cdot}$ photogenerated either directly by the PS1 complex or indirectly by the stromal factor-mediated reaction is disproportional to $H_2O_2$ and $O_2$ catalysed by superoxide dismutase (SOD), and the $H_2O_2$ is reduced to water by ascorbate catalysed with ascorbate peroxidase (APX) in the intact cycle (Asada 1999). However, water-water cycle could be interrupted easily under stress conditions, especially at chilling temperatures (Jakob and Heber 1996, Sonoike 1996, Sonoike *et al*. 1997), thus the accumulation of active oxygen species was induced (Sonoike *et al*. 1997, Terashima *et al.* 1998).

Recently, some reports suggested that PS1 had a greater chilling sensitivity than PS2 upon exposure to chilling under low irradiance, especially in some chilling-sensitive plants, such as cucumber, tomato, *etc*. (Sonoike 1996, 1998). PS1 photoinhibition *in vivo* of higher plants needs several factors (Sonoike 1996): (*1*) chilling temperature (0–10 ºC), (*2*) oxygen, (*3*) relatively weak irradiance (about 100 μmol $m^{-2}$ $s^{-1}$), (*4*) a relatively long time (about 5 h), (*5*) chilling-sensitive plants, and (*6*) the flow of electrons from PS2 proceeding normally. Under such conditions, the damage to PS1 was attributed to the photo-generated active oxygen species (Sonoike 1996, Terashima *et al*. 1998). However, most studies on chilling sensitivity of plants were conducted under violent conditions (Allen and Ort 2001), *e.g*. low temperatures under high irradiance, which usually caused severe photoinhibition of PS2, and thus covered up PS1 responses since PS2 photoinhibition can protect PS1 from further photoinhibition (Tjus *et al.* 1998).

Although cucumber (Terashima *et al*. 1998) and sweet pepper (Liu *et al*. 2001) are sensitive to photoinhibition at chilling temperatures, the xanthophyll cycle-dependent NPQ and water-water cycle were seldom mentioned together in relation to PS1 photoinhibition. The factors causing different chilling-sensitivity of cucumber and sweet pepper are unclear. In the present work, chilling was done for 6 h at 4 ºC under low irradiance (100 μmol $m^{-2}$ $s^{-1}$). The aim of this study was to find differences in chilling-sensitivity between sweet pepper and cucumber by comparing the energy dissipation in PS2, the oxidisable P700, and Mehler reaction upon exposure to chilling stress.

## Materials and methods

**Plants and chilling treatments**: Chilling-sensitive sweet pepper (*Capsicum annuum* L. line 156) and cucumber (*Cucumis sativus* L. cv. Jinchun 14) were used. Seeds were first germinated between moistened filter paper at 25 °C for 3 d. Sprouted burgeons were then planted into plastic pots (one plant per pot) filled with sterilised soil and grown at 25–30/15–20 °C (day/night) under 14-h photoperiod (300–400 μmol $m^{-2}$ $s^{-1}$ PFD) in a greenhouse. Functional leaves from two plants were used in the experiment. To induce chilling stress, with the adaxial side faced-up, the detached leaves with petiole dipped into water were put for 6 h in the growth chamber maintained at 4 °C. 100 μmol $m^{-2}$ $s^{-1}$ PFD was provided by fluorescent lamp. Non-chilling controls were treated at room temperature (25 °C) under 100 μmol $m^{-2}$ $s^{-1}$ PFD.

**Chlorophyll *a* fluorescence measurements**: The photochemical efficiency of PS2 expressed as the ratio of variable fluorescence ($F_v$) to maximum yield of fluorescence ($F_m$) was measured with a portable fluorometer (*FMS2*, *Hansatech*, England) according to the protocol described by van Kooten and Snel (1990). Non-photochemical quenching (NPQ) was calculated according to Schreiber *et al.* (1994). NPQ was estimated as $NPQ = F_m^0 - F_m' - 1$, where $F_m^0$ was measured after dark adaptation for more than 2 h at room temperature prior to chilling treatments. $F_m'$ is the maximum yield of fluorescence in light-acclimated leaves. The electron transport rate (ETR) was calculated as $(1 - F_s/F_m') \times 0.5 \times PFD \times$ leaf absorbance (Xu *et al*. 1999), where $F_s$ is the steady-state fluorescence yield, 0.5 is a factor assuming an equal distribution of absorbed photons between PS2 and PS1, and leaf absorbance was taken as 0.85.

The relative deviation from full balance between the two photosystems, $\beta/\alpha - 1$, was calculated by $(1 - f)/f$ according to Braun and Malkin (1990), where β and α represent the photon activity distribution coefficients of PS2 and PS1, respectively. $\beta = 1/(1 + f)$ and $\alpha = f/(1 + f)$, where $f$ was defined as the degree of openness of PS2 reaction centres, and it was equal to $(F_m - F_s)/(F_m - F_0)$, where $F_0$ is the initial fluorescence.

The oxidisable P700 was measured by the absorbance at 820 nm using a *Walz* modulated detection system (Effeltrich, Germany) consisting of a *PAM 101* control unit and *ED800T* emitter-detector unit (Endo *et al.* 1999). The oxidisable P700 was recorded during red irradiation (>650 nm). The maximum content of the oxidisable P700 was determined using a xenon discharge lamp (50 ms, 1 500 W $m^{-2}$) under a far-red radiation background (720 nm, 0.66 μmol $m^{-2}$ $s^{-1}$).

**Determination of SOD and APX activities**: 0.5 g leaves without midrib was thoroughly ground with a cold mortar and pestle in an ice bath. The grinding medium was 4 $cm^3$ of 0.05 M phosphate buffer (pH 7.8), plus homogenising glass beads. The homogenate was centrifuged at 3 000×*g*

for 15 min at 0–4 °C. The supernatant, hereafter referred to as crude SOD and APX extract, was used for determination.

SOD assay described by Giannopolitis and Ries (1977) was used with some modification. The reaction mixture was composed of 13 mM methionine, 75 μM NBT, 10 μM EDTA-$Na_2$, 2 μM riboflavin, and the appropriate volume of extract [in the blanks, 0.05 M phosphate buffer (pH 7.8) instead of extract]. Distilled $H_2O$ was added to bring the final volume of 3 $cm^3$. The mixtures were irradiated in glass tubes selected for uniform thickness and colour. Identical solutions that were not irradiated served as blanks. The absorbance at 560 nm of the reaction mixture was determined using a UV-visible spectrophotometer (*UV-1601*, *Shimadzu*, Japan).

APX assay described by Dalton *et al*. (1987) was modified. The reaction mixture was composed of 62 $cm^3$ $m^{-3}$ (v : v) of 30 % $H_2O_2$, 0.3 mM AsA, 99.57 μM EDTA-$Na_2$, and the appropriate volume of extract. 0.05 M phosphate buffer (pH 7.8) was added to bring the final volume to 3 $cm^3$. The changes of absorbance at 265 nm were determined using a UV-visible spectrophotometer (*UV-1601*, *Shimadzu*, Japan).

**$O_2^{-\cdot}$ and $H_2O_2$ measurements**: $O_2^{-\cdot}$ was measured according to Wang and Luo (1990). Leaves without midrib were thoroughly ground with a cold mortar and pestle in an ice bath. The grinding medium was 0.05 M phosphate buffer (pH 7.8) with homogenising glass beads. The homogenate was centrifuged at 5 000×*g* for 10 min at 0–4 °C. The supernatant with phosphate buffer (pH 7.8) and 10 mM hydroxylammonium chloride was incubated at 25 °C for 20 min, then 17 mM *p*-aminobenzene sulfonic acid and 7 mM α-naphthylamine were added, and the mixture was incubated at 25 ºC for 20 min. Fi-nally, ethyl ether was added into the mixture, and centrifuged at 1 500×*g* for 5 min, the water phase being used to determine the absorbance at 530 nm.

The assay of $H_2O_2$ was as described by Brennan and Frenkel (1977). Leaves without midrib were thoroughly ground in cold acetone with a cold mortar and pestle in an ice bath. The mixture was placed to sediment at 0–4 ºC and the supernatant was used in the analysis. 20 % $TiCl_2$-HCl (36–38 %) solution and 17 mM ammonia solution were added to the supernatant. The mixture was centrifuged at 3 000×*g* for 10 min. The sedimentation was washed 5 times by acetone. After the sedimentation was dissolved in 2 mM $H_2SO_4$, its absorbance at 410 nm was determined.

**Pigment analysis**: Leaf discs were immersed in liquid $N_2$ immediately after $F_v/F_m$ determination and stored at –80 °C until use. Photosynthetic pigments were extracted from leaf discs with 80 % ice-cold acetone. The extracts were centrifuged at 20 000×*g* for 5 min and supernatants were filtered through a 0.45 μm membrane filter before injection into reversed-phase high performance liquid chromatography (HPLC). Photosynthetic pigments were separated and qualified essentially following the method of Zhao *et al.* (1995).

## Results

**Photoinhibition of two photosystems at chilling temperature under low irradiance**: Photoinhibition of PS2 in leaves of sweet pepper and cucumber was estimated by measuring the maximum photochemical efficiency of PS2 ($F_v/F_m$). $F_v/F_m$ decreased in both plants during 6-h chilling stress relative to non-chilling controls (Fig. 1*A*). At the end of the 6-h stress, $F_v/F_m$ in sweet pepper and cucumber leaves deceased to about 0.5 and 0.6, respectively. Thus the photoinhibitory effect of chilling stress under low irradiance on PS2 was more obvious in sweet pepper. Relative to non-chilling controls, a decrease in initial fluorescence ($F_0$) was observed in both cucumber and sweet pepper leaves during chilling stress (Fig. 1*B*).

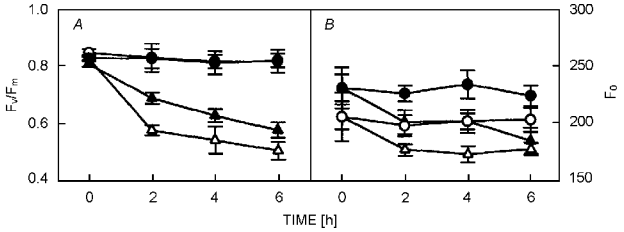


Fig. 1. Changes in maximum photochemical efficiency ($F_v/F_m$) (*A*) and initial fluorescence ($F_0$) of PS2 (*B*) in cucumber (▲) and sweet pepper (Δ) leaves during chilling stress and in non-chilling controls of cucumber (●) and sweet pepper (○). For treated leaves, fluorescence measurements were made after 5-min dark adaptation. Means ± SD of 5 measurements on separate leaves.

To corroborate photodamage of PS1, flash-induced P700 absorbance at 820 nm was investigated (Endo *et al.* 1999, Eichelmann and Laisk 2000). A sharp decrease of the oxidisable P700 was observed in both cucumber and

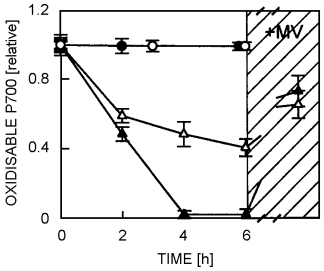


Fig. 2. Changes in the oxidisable P700 in cucumber (▲) and sweet pepper (Δ) leaves during chilling stress and in non-chilling controls of cucumber (●) and sweet pepper (○). Leaves were dark adapted for 15 min prior to measurement. Means ± SD of 3 measurements on separate leaves.

sweet pepper leaves during chilling stress relative to non-chilling controls (Fig. 2). In cucumber leaves, the content of oxidisable P700 decreased sharply to the ground within first 4 h of stress. However, the oxidisable P700 of sweet pepper leaves only decreased for about 59 % after 6 h chilling under low irradiance. This implied that PS1 was more sensitive to chilling under low irradiance than PS2 in cucumber. In contrast, PS1 was relatively stable in sweet pepper. Chilling under irradiance usually causes irreversible damage to PS1 (Kudoh and Sonoike 2002). Nevertheless, after vacuum infiltration by methyl viologen (MV), an electron acceptor, rapid recovery of the oxidisable P700 was observed in both cucumber and sweet pepper leaves, which indicated that the site damaged by chilling was not PS1 reaction centre protein *per se*.

To further elucidate the susceptibility of two photosystems in both plants to chilling stress, the relationship between $F_v/F_m$ and the oxidisable P700 was expressed by linear regression (Fig. 3). The slope of the linear regression in cucumber leaves ($k_1$) was about 4.0317, and that in sweet pepper leaves ($k_2$) was only about 1.8605.

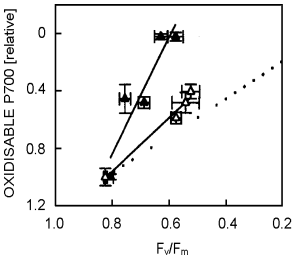


Fig. 3. The relationship between the oxidisable P700 and $F_v/F_m$ in cucumber (▲) ($r^2 = 0.8638$) and sweet pepper (Δ) ($r^2 = 0.9819$) leaves during chilling stress. Values from Figs. 1 and 2. *The dotted line* shows the same sensitivity of photosystems 1 and 2 to chilling under low irradiance and its slope was about 1.26.

**Energy dissipation in PS2** was monitored by measuring the development of NPQ (Fig. 4*A*). NPQ increased when these two plants were exposed to chilling under low irradiance, and it increased apparently faster in sweet pepper leaves than in cucumber leaves (Fig. 4*A*). The xanthophyll cycle reached to maximal de-epoxidation state at the end of chilling stress (Fig. 4*B*). Sweet pepper exhibited substantially faster de-epoxidation kinetics as shown by A+Z formation. In these two plants, the de-epoxidation ratio of xanthophyll cycle pigments (A+Z)/(V+A+Z) rapidly increased during first 2 h. This is consistent with the changes of NPQ during chilling stress. In non-chilling controls, NPQ and (A+Z)/(V+A+Z) did not show much change within 6 h (Fig. 4).

**Energy distribution between PS1 and PS2**: To further investigate the transport of electron through the electron transfer chain, we monitored the changes of the imbalance term, $\beta/\alpha - 1$, during chilling stress under low irradiance. In comparison with the values of non-chilling controls, $\beta/\alpha - 1$ decreased sharply when these two plants were exposed to chilling under low irradiance (Fig. 5). After 2 h of stress, $\beta/\alpha - 1$ was apparently higher in sweet pepper leaves.

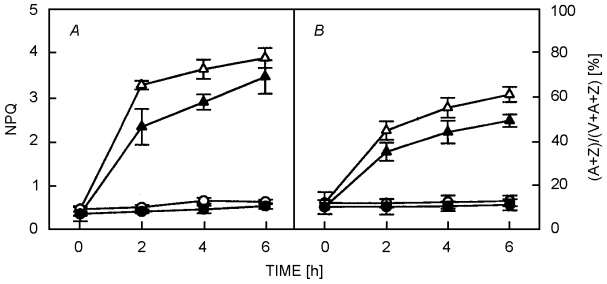


Fig. 4. Development of non-photochemical quenching (NPQ) (*A*) and the de-epoxidation ratio of xanthophyll cycle pigments (A+Z)/(V+A+Z) (*B*) in cucumber (▲) and sweet pepper (Δ) leaves during chilling stress and in non-chilling controls of cucumber (●) and sweet pepper (○) leaves. Means ± S.D. of 5 measurements of separate leaves.

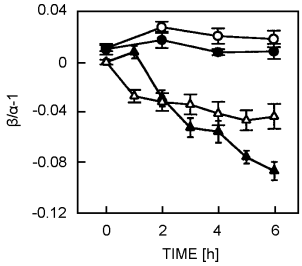


Fig. 5. Changes in the imbalance term, $\beta/\alpha - 1$, in cucumber (▲) and sweet pepper (Δ) leaves during chilling stress and in non-chilling controls of cucumber (●) and sweet pepper (○) leaves. Means ± S.D. of 5 measurements of separate leaves.

**Antioxidant enzymes and production of active oxygen species**: At chilling temperature under low irradiance, SOD activity increased in cucumber leaves (Fig. 6*A*) and was significantly higher than that in sweet pepper leaves. On the contrary, APX activity decreased apparently in cucumber leaves (Fig. 6*B*), though it was still higher than that in sweet pepper leaves. The activity of SOD in sweet pepper leaves decreased (Fig. 6*A*) and activity of APX increased slightly during stress (Fig. 6*B*). The activity of SOD and APX in non-chilling controls of these two plants changed little during the 6-h process (Fig. 6*A*,*B*).

$O_2^{-\cdot}$ and $H_2O_2$ generated faster in cucumber leaves than in sweet pepper leaves under chilling stress (Fig. 6*C, D*). At the end of stress, the production rate of $O_2^{-\cdot}$ was 253 μmol $s^{-1}$ $kg^{-1}$(FM) in cucumber and 111 μmol $s^{-1}$ $kg^{-1}$(FM) in sweet pepper leaves. Accordingly, $H_2O_2$ content was 40.51 mmol $kg^{-1}$(FM) in cucumber leaves and 30.25 mmol $kg^{-1}$(FM) in sweet pepper leaves. $O_2^{-\cdot}$ and $H_2O_2$ in non-chilling controls kept stable during 6 h of the process (Fig. 6*C,D*).

## Discussion

**Photoinhibition of photosystems during chilling stress under low irradiance**: The direction of $F_0$ change depends on the dominant factor between the energy dissipation and the inactivation or damage of PS2 (Xu and Wu 1996). An increase in NPQ leads to a decrease in $F_0$ (Ögren and Öquist 1984) and the inactivation or the damage of PS2 causes the increase of $F_0$ (Xu and Wu 1996). Our results showed that chilling stress did not damage PS2 reaction centres of both plants since $F_0$ decreased during chilling stress (Fig. 1*B*). However, in comparison with cucumber, PS2 in sweet pepper was more sensitive to chilling stress under low irradiance (Fig. 1*A*).

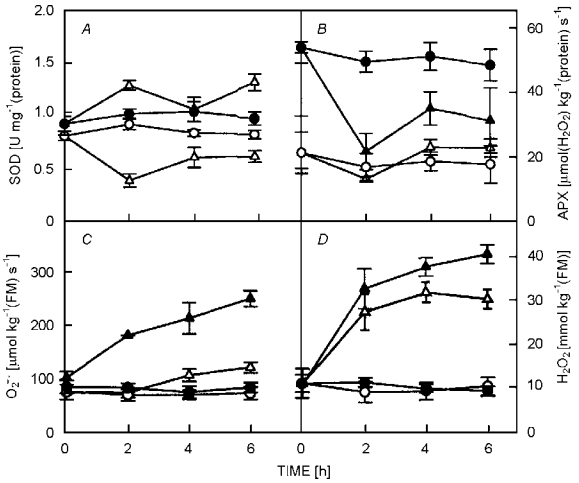


Fig.6. Changes in superoxide dismutase (SOD) (*A*) and ascorbate peroxidase (APX) (*B*) activities, and in superoxide anion radical ($O_2^{\cdot-}$) (C) and $H_2O_2$ contents (*D*) in cucumber (▲) and sweet pepper (Δ) leaves during chilling stress and in non-chilled controls of cucumber (●) and sweet pepper (○). Means ± S.D. of 3 measurements of separate leaves.

PS1 was more sensitive to chilling under low irradiance than PS2 in both sweet pepper and cucumber leaves by comparing the ratio of oxidisable P700 over $F_v/F_m$ (Fig. 3). PS1 in cucumber leaves was more sensitive to chilling stress since $k_1$ was larger than $k_2$. Chilling stress under low irradiance could cause an accumulation of reducing power on the acceptor side of PS1 (Terashima *et al*. 1994, Sonoike 1996) in chilling-sensitive plants. It is obvious that the limited electron acceptors caused the decrease of the oxidisable P700 in both plants (Fig. 2). 100 μmol $m^{-2}$ $s^{-1}$ PFD was excessive to these plants when Calvin cycle was inhibited under low temperature, thus accumulating electrons in the carrier at the PS1 acceptor side. Hence, if the Fe-S centres $F_X$, $F_A$, and $F_B$ are reduced, recombination in the radical pairs $P700^+A_0^-$ and/or $P700^+A_1^-$ can occur when Fe-S centres are not oxidised by an external acceptor within the time of the back-reaction (Barth *et al.* 2001). As Fe-S centres are the primary targets of photo-inactivation of PS1 (Sonoike *et al*. 1995, Sonoike 1996, Tjus *et al*. 1999), increased charge recombination between $P700^+$ and $A_0^-$ as a result of destruction of the three Fe-S centres may occur in cucumber leaves exposed to low irradiance at chilling temperatures (Sonoike *et al*. 1995).

After infiltration of MV, which is an efficient electron acceptor of PS1, charge recombination between $P700^+$ and $A_0^-/A_1^-$ as well as P700 triplet formation were prevented (Fig. 2) (Takahashi and Katoh 1984).

**Energy dissipation and redistribution between PS1 and PS2**: The results showed that the xanthophyll cycle-dependent energy dissipation decreased energy redistribution to PS1 and alleviated photoinhibition of PS1. Chilling stress under low irradiance induced the increase in NPQ and the de-epoxidation ratio of xanthophyll cycle pigments (A+Z)/(V+A+Z) in sweet pepper and cucumber leaves (Fig. 4*A*). When the rate of photochemistry decreased and photon energy was excessive, energy dissipation in PS2 antenna was observed, suggesting that harmless dissipation of excess energy compensated low rates of photochemical utilisation and thus protected the photosynthetic apparatus. More energy was dissipated as heat by antenna in sweet pepper leaves related to their higher xanthophyll cycle conversion state and higher NPQ. Higher $F_v/F_m$ (Fig. 1), lower NPQ (Fig. 4), and lower $\beta/\alpha - 1$ (Fig. 5) in cucumber leaves implied that more electrons might be redistributed to PS1 to form active oxygen species, and causing the over-reduction of P700 in cucumber leaves.

In contrast with cucumber, more severe photoinhibition of PS2 and higher energy dissipation *via* xanthophyll cycle in sweet pepper could decrease energy distribution to PS1 efficiently (Fig. 5) and protect PS1 from further photoinhibition. This offers further evidences to the results on barley (Tjus *et al*. 1998).

**Antioxidant enzymes and active oxygen species involved in the water-water cycle**: When exposed to chilling temperature under low irradiance, the water-water cycle in sweet pepper leaves seemed not to be affected so much as that in cucumber leaves (Fig. 6). At low temperature, electron transport chains tend to form $O_2^{\cdot-}$, which could be dismutated by SOD to form $H_2O_2$ (Fig. 6). This occurs particularly in chloroplasts, where low temperature limits the Calvin cycle reactions, restricting the supply of $NADP^+$ and promoting reduction of $O_2$ by PS1 (Yu *et al*. 2002). Both the production rates of $O_2^{\cdot-}$ and $H_2O_2$ in sweet pepper leaves were lower than in cucumber leaves. But it seems that $H_2O_2$ could not be scavenged efficiently in cucumber leaves owing to the reduced APX activity (Fig. 6*B*).

The thylakoid-bound [4Fe-4S] clusters $F_X$ on psaA and psaB or A/B on psaC of the PS1 complex are the most likely electron donors to $O_2$ (Asada 1994). PS1 photoinhibition is related to the production of active

oxygen species (Terashima *et al.* 1994, 1998, Sonoike 1996). Higher production rate of $O_2^-$, higher activity of SOD, and lower activity of APX could induce higher production of $H_2O_2$ in cucumber leaves (Fig. 6). Our results implied that more severe PS1 photoinhibition in cucumber leaves was related to higher accumulation of active oxygen species under chilling stress.

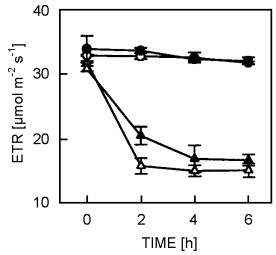


Fig. 7. Changes of electron transport rate (ETR) in cucumber (▲) and sweet pepper (Δ) leaves during chilling stress and in non-chilling controls of cucumber (●) and sweet pepper (○). Means ± S.D. of 3 measurements of separate leaves.

Electron transport rate is controlled by the back-pressure of electrons on the acceptor side and of protons on the donor side of PS1, and both pressures virtually act simultaneously (Laisk *et al*. 1997). Electron back-pressure leads to reduction on the PS1 acceptor side (and also PS2 acceptor side), while proton back-pressure leads to non-photochemical quenching of excitation at PS2 (Bilger *et al*. 1988) and to the photosynthetic control of electron donation to PS1 (Harbinson *et al*. 1990). In the present experiments, the electron transport rate (ETR) calculated by PS2 fluorescence decreased (Fig. 7) during chilling stress. Lower NPQ in cucumber and higher NPQ in sweet pepper implied that ETR was mainly controlled by electron back-pressure in cucumber leaves and by proton back-pressure in sweet pepper leaves. Faster development and a higher NPQ, related to a stronger de-epoxidation of the larger xanthophyll cycle pool in sweet pepper, could act as a major protective mechanism to reduce the formation of active oxygen species during chilling stress.

In addition, the results also showed that cucumber and sweet pepper were different in sensitivity to chilling temperatures. PS2 was sensitive to chilling under low irradiance more in sweet pepper than in cucumber. On the contrary, PS1 was more sensitive in cucumber. Hence the sensitivity of plant to chilling under low irradiance is dominated by the protective mechanisms related both with PS1 and PS2, especially the energy dissipation and the water-water cycle.

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