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The higher sensitivity of PSI to ROS results in lower chilling–light tolerance of photosystems in young leaves of cucumber

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ABSTRACT

The development of PSII tolerance to stress and photoprotection mechanisms during leaf growth has been widely studied, however, knowledge about PSI photoinhibition and interaction between PSI and PSII under stress during leaf growth is still lacking. This study showed that during the chilling–light treatment, the photoinhibitions of PSI and PSII were more severe in young leaves than in fully-expanded leaves of cucumber, but the inhibition of CO_2 assimilation and the accumulation of reactive oxygen species (ROS) were similar in leaves at different development stages. During the chilling–light treatment, PSII photoinhibition was positive correlated to PSI photoinhibition in leaves, however, this correlation no longer existed in leaves pretreated with DCMU, an inhibitor of electron transport from PSII to PSI. Although the photoinhibitions of PSII and PSI in young leaves were more severe, the sensitivity of PSII to excitation pressure was lower in young leaves. The above results demonstrate that, the lower chilling–light tolerance of photosystems in young leaves was due to the higher sensitivity of PSI to ROS and the higher PSII excitation pressure caused by PSI photoinhibition in young leaves, rather than the difference of ROS content and sensitivity of PSII to excitation pressure between the young and fully-expanded leaves.

1. Introduction

At the beginning of leaf ontogenesis, the capacity of carbon assimilation is low until the leaf is fully expanded [1,2]. Thus, when young leaves exposed to high irradiance, only a fraction of absorbed irradiance can be utilized in photochemical reaction via carbon assimilation, which means that much more excessive excited energy is produced. The development of PSII tolerance to stress and PSII photoprotective mechanisms during leaf growth has been widely studied [3–9]. Previous studies showed that the PSII in young leaves is more sensitive to abiotic stress such as high light and high temperature [3–6,8,10], though the photoprotective mechanisms are more enhanced in young leaves than in fully-expanded leaves [3,11,12]. However, PSI tolerance and its contribution to the whole photosynthetic apparatus in leaves at different development stages under stress is neglected.

It has been reported that the activities of PSI and PSII are developed harmoniously during leaf growth, which is important to optimize the photochemical efficiency of whole photosynthetic apparatus [7,13]. However, it has not been known whether the PSI and PSII can cooperate well under stress condition in leaves at different development stages. The different tolerances of PSI and PSII to stress will cause the imbalance between PSI and PSII and interfere with the activity of whole photosynthetic apparatus under stress. Exploring the responses of PSI and PSII to stress in different development stages will help to understand the strategy of plant to adapt changing environments during the development.

PSII is considered as the primary target of photoinhibition [14–16], but inactivation of PSI is rarely observed in vivo because PSI is more stable than PSII under most of abiotic stresses, such as high light and high temperature. PSI photoinhibition was first reported by Terashima and his colleagues [17], PSI was the primary photoinhibition site when cucumber leaves were chilled at 4 °C for 5 h under moderate light (200 μmol m^{-2} s^{-1}). Today, it has been proved that PSI photoinhibition is an universal phenomenon in higher plants under chilling-light treatment [18–23].

Cucumber (*Cucumis sativus* L.) is a typical chilling-sensitive plant. Chilling–light condition often appears in greenhouse during winter. The purpose of this study is to clarify the dynamic tolerance to chilling–light treatment during the development of cucumber leaves, and to find out which factors dominate the chilling–light tolerance during the development of cucumber leaves. To reach this purpose, we investigated the CO_2 gas exchange, chloro-

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phyll a fluorescence transient, 820-nm transmission, fluorescence quenching and changes of ROS in cucumber leaves at different development stages during the chilling–light treatment (6 °C, 400 μmol m^{-2} s^{-1} light).

2. Materials and methods

2.1. Plant material

Cucumber (*C. sativus* L. cv. jinchun 4) plants were grown in field under natural sun light with about 14-h photoperiod (26–32 °C) and 10-h night (22–28 °C). The maximum light intensity was about 1300 μmol m^{-2} s^{-1}. Sufficient nutrients and water were applied to avoid any potential nutrient and drought stresses. After growing for about 5 weeks, expanding leaves with an area of about 20%, 30% 50% area of fully-expanded leaves (abbreviated as 20%A, 30%A, 50%A leaves) and fully-expanded leaves (abbreviated as 100%A leaves) were used in the experiment.

2.2. Chilling–light treatment

The abaxial sides of leaf discs (1 cm^2) were floated on the surface of water at 6 °C. The temperature of water was controlled by RTE-211 water circulator (Thermo, USA). For photoinhibition treatments, 400 μmol m^{-2} s^{-1} light from red and blue (8:1) light emitting diode (LED; Senpro, China) was used.

For reagent treatment, leaves attached with stem were submerged into 70 μM 3-(3,4-dichlorfenyl)-1,1-dimethylkarbonyldiamid (DCMU) or 1 mM chloroamphenicol solution for 3 h under darkness before leaf discs were punched from leaves.

For gas exchange measurements, petioles of excised leaves were dipped into water, leaves were treated by 6 °C and 400 μmol m^{-2} s^{-1} light in GXZ-5000 light incubator (Jiangnan, China). Light was provided by light emitting diodes (LED; Senpro, China), temperature and relative humidity (above 90%) were controlled by GXZ-5000 light incubator. Water content of the excised leaves was constant during 9 h chilling–light treatment.

2.3. Photosynthetic gas exchange measurements

CO_2 gas exchange was measured with a CIRAS-2 portable photosynthesis system (PP Systems, USA) at 25 °C, 65% relative humidity, saturating light intensity of 1200 μmol m^{-2} s^{-1} and different CO_2 concentration (400 or 1200 μmol mol^{-1}). Leaf chamber temperature, relative humidity, light intensity and CO_2 concentration were controlled by automatic control device of the CIRAS-2 portable photosynthetic system.

2.4. Measurements of chlorophyll fluorescence

Modulated chlorophyll fluorescence was measured with a FMS-2 pulse-modulated fluorometer (Hansatech, UK). The light-fluorescence measurement protocol was as follows: the light-adapted leaves were continuously illuminated by actinic light at 400 μmol mol^{-1} from the FMS-2 light source, steady-state fluorescence (Fs) was recorded after 2 min illumination, and a 0.8 s saturating light of 8000 μmol m^{-2} s^{-1} was imposed to obtain the maximum fluorescence in the light-adapted state (Fm′). The actinic light was then turned off, and the minimum fluorescence in the light-adapted state (Fo′) was determined by a 3 s illumination with far-red light.

The following parameters were then calculated [24]

(1) Quantum yield of PSII, ΦPSII = (Fm′ − Fs)/Fm′.
(2) Electron transport rate, ETR = ΦPSII × PFD × 0.5 × 0.84.
(3) Photochemical quenching, qP = (Fm′ − Fs)/(Fm′ − Fo′).
(4) Excitation energy capture by open PSII reaction centers, Fv′/Fm′ = (Fm′ − Fo′)/Fm′.

2.5. Measurements of the chlorophyll a fluorescence transient (OJIP) and 820-nm transmission

The Chl a fluorescence transient was measured using an integral PEA Senior (Hansatech, UK) with dark-adapted leaves under ambient CO_2 conditions at room temperature (25 °C). The saturating red light of 3000 μmol m^{-2} s^{-1} was produced by an array of four light-emitting diodes (LEDs, peak 650 nm).

The chlorophyll a fluorescence transients were obtained by 2 s saturating red light and analyzed with the JIP-test [25,26]:

(1) Maximum quantum yield of PSII, Fv/Fm = 1 − (Fo/Fm).
(2) The exciton efficiency of electron transport beyond Q_A^-, $\Psi_O = 1 - V_j$.
(3) The quantum yield for reduction of end electron acceptors at the PSI acceptor side, $\varphi_{Ro} = (1 - V_i)/(1 - V_j)$.

The 820-nm transmission was measured using an integral PEA Senior (Hansatech, UK). A dark adapted leaf was illuminated with far-red light (250 μmol m^{-2} s^{-1}) at time 0 s, until a steady P700 photooxidation was reached, then the far-red light was closed (at time 10 s). A modulated (33.3 kHz) measuring light at 820 nm was provided simultaneously with the illumination of far-red light. The change in the amplitude of 820-nm transmission ($\Delta I/Io$) was used to compare the relative content of active P700 [23,27].

To calculate $\Delta I/Io$, the following formulas were used [23]: *Io*, the average of the 820-nm transmission signal between 0.4 ms and 10 ms; *Im*, the average of the 820-nm transmission signal between 3 s and 10 s; $\Delta I/Io = (Io - Im)/Io$.

2.6. Photosynthetic electron transport measurements

Five grams of leaf discs were used to isolate thylakoid membranes as described by Peng et al. [28] The photosynthetic electron transport activities of PSI and PSII was measured according to Tjus et al. [19] using an Oxytherm oxygen electrode (Hansatech, UK).

2.7. Histochemical detection of reactive oxygen species (ROS)

In situ detection of superoxide anion radical was performed using the nitroblue tetrazolium (NBT) staining method as described by Kawai-Yamada et al. [29]. Detached leaves were vacuum-infiltrated with 10 mM NaN_3 in 10 mM potassium phosphate buffer (pH 7.8) for 1 min, and incubated in 1 mg ml^{-1} nitroblue tetrazolium (in 10 mM potassium phosphate buffer, pH 7.8) for 20 min in the dark at room temperature. In situ detection of hydrogen peroxide was performed using the 3,3′′-diaminobenzidine (DAB) staining method as described by Thordal-Christensen et al. [30]. Detached leaves were vacuum-infiltrated with 1 mg ml^{-1} DAB solution (pH 3.8) for 1 min and incubated in the dark at room temperature for 6 h. Stained leaves were cleared by boiling in acetic acid/glycerol/ethanol (1:1:3 (v/v/v)) solution before photographs were taken.

2.8. Quantitative determination of hydrogen peroxide and superoxide anion radical contents

The contents of superoxide anion radical and hydrogen peroxide were extracted and determined according to the method of Able et al. [31] and Patterson et al. [32].

0.5 g leaves was extracted with 5 ml 5% (w/v) trichloroacetic acid, and then centrifuged at 16,000*g* for 10 min. The supernatant

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