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The mechanism by which NaCl treatment alleviates PSI photoinhibition under chilling-light treatment

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ABSTRACT

The effects of chilling-light stress combined with additional stress on PSI and PSII photoinhibition and their interrelationship have not been known. To explore whether NaCl affects the PSI and PSII photoinhibition and their interrelationship under chilling-light treatment, the PSI and PSII activities were studied under chilling-light with or without NaCl treatment. The results showed that the extent of PSI and PSII photoinhibition both increased under chilling-light, while NaCl aggravated PSII photoinhibition and severely damaged cytochrome b_6/f complex but alleviated PSI photoinhibition. Moreover, DCMU had a similar effect as NaCl in this study, which indicates that NaCl alleviated PSI photoinhibition through reducing electrons transported to PSI. It was also showed that the increased damage to PSII by NaCl did not depend on the inhibition of PSII repair and PSI electron transportation. In conclusion, NaCl alleviated PSI photoinhibition by inhibiting electron transport from PSII under chilling-light conditions. In addition, PSII photoinhibition was not affected by PSI photoinhibition because of a full inhibition of PSII repair by chilling-light treatment. We also speculate that NaCl aggravates PSII photoinhibition by enhancing the damage instead of inhibiting the repair of it under chilling-light conditions.

1. Introduction

Photoinhibition may take place under conditions where the harvested light energy exceeds the requirements to drive photochemical reactions and protective mechanisms become overburdened [1,2]. Photosystem II (PSII) and photosystem I (PSI) are the main target sites of photoinhibition.

PSII has long been considered the primary target of photoinhibition [2,3], as photodamage to PSII occurs under light at any intensity and is unavoidable. Photosynthetic organisms are able to overcome photodamage to PSII by rapid and efficient repair of the damage, which requires the synthesis of D_1 proteins *de novo*. The activity of PSII depends on the balance between the rates of photodamage and repair, and the photoinhibition of PSII becomes apparent when the rate of photodamage exceeds the rate of repair [2,4,5]. Some studies revealed that light can damage PSII directly, and other forms of environmental stress act primarily by inhibiting repair of PS II [6,7].

PSI photoinhibition is rarely observed *in vivo* because PSI is more stable than PSII under most types of abiotic stress, such as high light [8]. PSI photoinhibition was first reported by Terashima and his colleagues [9]. When cucumber leaves were chilled at 4 °C for 5 h under moderate photon flux density (200 μmol m^{-2} s^{-1}), the maximum photosynthetic electron flow through PSI decreased by 70–80%, compared to the controls. In contrast, the maximum electron transport through PSII remained at 80% of that of the controls [9]. Chilling-induced photoinhibition damages the membrane structure and causes degradation of proteins in PSI reaction center, such as the psa A and psa B gene products [10]. Sonoike [11] suggested that the light-induced destruction of the iron-sulfur centers FX, FA, and FB, and possibly phylloquinone and A1, is responsible for photoinhibition of PSI [11]. The recovery of PSI occurs very slowly; it might take several days, even under favorable conditions [12,13], because PSI lacks a quick repair mechanisms like that in PSII.

Plants in northern areas often face chilling conditions. The decrease in photosynthesis induced by a chilling environment is the primary reason limiting the lives and growth of chilling-sensitive plants [14,15]. It has been reported that PSI and PSII photoinhibition are tightly correlated under chilling-light treatment [16,17]. Photoinhibition of PSII protects PSI from photoinhibition while photoinhibition of PSI might enhance the photoinhibition

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of PSII [17]. Under high light, PSI is protected from photoinhibition, because PSII is already damaged and fewer electrons are transported to PSI. However, under chilling-light, when PSII is little damaged, PSI photoinhibition takes place because more electrons are transported to PSI [18], and addition of 3-(3,4-Dichlorophenyl)-1,1-Dimethylurea (DCMU), an inhibitor of PSII, could completely protects PSI from photoinhibition [19]. Recently, it has also been reported that the increase of electrons transported to PSI during the recovery phase significantly inhibits the recovery of PSI [16].

It is well known that chilling-light stress mainly inhibits the activity of PSI [9,10], while other abiotic stress, such as drought and salt, primarily inhibits the activity of PSII instead of PSI [20,21]. We therefore sought to explore whether PSI would be protected from chilling-light stress by other abiotic stresses that enhances PSII damage. To reach this aim, the activities of PSI and PSII were investigated under chilling-light, with and without NaCl stress.

2. Materials and methods

2.1. Plant material

Cucumber (*Cucumis sativus* L. cv. jinchun 4) plants were grown in the field under natural sunlight, at about a 14-h photoperiod (26–32 °C) and 10-h night (22–28 °C). Sufficient nutrients and water were provided to avoid any potential nutrient or drought stress. After growing for about 5 weeks, fully expanding 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 4 °C in a GXZ-5000 light incubator (Jiangnan, China). For photoinhibition treatments, 100 μmol m^{-2} s^{-1} light was used.

2.3. Measurements of chlorophyll fluorescence

Modulated chlorophyll fluorescence was measured with an 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 100 μmol m^{-2} s^{-1} from the FMS-2 light source, steady-state fluorescence (F_s) was recorded after a 2 min illumination, and 0.8 s of saturating light of 8000 μmol m^{-2} s^{-1} was imposed to obtain maximum fluorescence in the light-adapted state (F'_m). The actinic light was then turned off, and the minimum fluorescence in the light-adapted state (F'_o) was determined by a 3 s illumination with far-red light.

The following parameters were then calculated [22]:

(1) Quantum yield of PSII, ΦPSII = $(F'_m - F_s)/F'_m$.
(2) Electron transport rate, ETR = ΦPSII × PFD × 0.5 × 0.84.
(3) Photochemical quenching, qP = $(F'_m - F_s)/(F'_m - F'_o)$.

2.4. Measurements of the chlorophyll a fluorescence transient (OJIP) and the modulated reflected signal of 820 nm (MR)

Induction kinetics of PF and MR were simultaneously recorded using a Multifunctional Plant Efficiency Analyzer, M-PEA (Hansatech Instrument Ltd., UK) as has been described [23–25]. All leaves were dark-adapted under ambient CO_2 conditions at room temperature (25 °C) before the measurements of the induction curves. Measurements were conducted for an induction period of 2 s. Leaves were illuminated by saturating red light of 5000 μmol m^{-2} s^{-1}. The chlorophyll a fluorescence transients were analyzed with the JIP-test [26,27]: Maximum quantum yield of PSII, $F_v/F_m = 1 - (F_o/F_m)$.

The MR signal measured at 820 nm provides information about oxidation of PSI (including PC and P700). The induction curve of MR_{820nm} of the leaves obtained by saturating red light showed a fast oxidation phase and a following reduction phase. The initial slope of the oxidation phase of MR at the beginning of the saturated red light indicates the capability of P700 to get oxidized, which is used to reflect the activity of PSI [24,28,29].

2.5. Histochemical detection of starch

The treated leaves were firstly killed in boiling water for 1 min. Then they were decolorized by immersion in boiling ethanol (96%) for 10 min. The decolorized leaves were immersed in an iodine (I_2) and potassium iodide (KI) solution (1:2) for 1 min. After boiling in ethanol (96%) for 15 s to get rid of the background color, the leaves were extracted at room temperature with fresh ethanol and photographed.

2.6. Chemicals

Sodium chloride (NaCl) was purchased from Kaitong chemical reagent Co. Ltd. (Tianjin, China). 3-(3,4-Dichlorophenyl)-1,1-Dimethylurea (DCMU), chloramphenicol (CM), were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.7. Statistical analysis

LSD (least significant difference) was used to analyze differences between the SHAM treatments, using SPSS 16.

3. Results

3.1. The effect of different concentrations of NaCl together with chilling-light treatment on PSII and PSI photoinhibition

Maximum quantum yield of PSII (F_v/F_m) is a typical indicator of PSII efficiency and is widely used to reflect the photoinhibition of PSII [30,31]. Compared with control leaves (at room temperature in the dark), chilling-light treatment significantly decreased the F_v/F_m and P_{700} oxidation rate (Fig.1). However, the decreased extent of F_v/F_m gradually increased and P_{700} oxidation rate gradually increased with increasing NaCl concentration in the chilling-light treatment (Fig.1). The results suggest that NaCl treatment aggravated the PSII photoinhibition but alleviated the PSI photoinhibition under chilling-light.

3.2. The effect of NaCl on photosynthetic electron transport, excitation pressure, PSI and PSII photoinhibition during chilling-light treatment

F_v/F_m decreased slowly in control leaves with the increase of treatment time under chilling-light, while that in NaCl (0.8 M) treated leaves decreased much faster (Fig. 2A), which indicates that NaCl increased the photoinhibition rate of PSII under chilling-light treatment. The electron transport rate (ETR) is used as a quantitative indicator of the electron transport beyond PSII [22,32]. Photochemical quenching (qP) is used as an indicator of the redox level of the PSII primary electron acceptor, Q_A [22]. The PSII excitation pressure was estimated by 1-qP, which represents closed PSII reaction centers [22]. In the absence of NaCl, ETR gradually decreased during chilling-light treatment, reaching a minimum value after the leaves had been treated for 3 h.