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Photosystem inhibition and protection in tomato leaves under low light

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ABSTRACT

In this study, the effect of low light (LL, 340–360 μmol m^{-2} s^{-1}) on thylakoid membrane activity, photosystem I and II (PSI and PSII) activities, transient quantum yields, reactive oxygen species (ROS), cyclic electron flow (CEF) and proton motive force of tomato leaves was investigated. Results indicated that LL treatment led to low integrity of the thylakoid membrane, ATPase activity, and photoinhibition of PSII and PSI. The treatment also yielded low electron transport rate [ETR(II) and ETR(I)], high PSI donor side limitation [Y(ND)] and efficient electron transfer between the intermediate carriers to the final acceptors of PSI (δRo). Hence, the possible inhibition sites include Q_A-Q_B and PSI-F_d. Moreover, LL increased the excitation pressure, ROS scavenge enzyme activities, CEF/Y(II) radio, formation of proton gradient and decreased chlorophyll a/b ratio in the thylakoid membrane, thereby alleviating inhibition of PSII and PSI to a certain extent.

1. Introduction

Light is one of the main factors that substantially affect plant growth. Plants in greenhouses in Northern China is readily subjected to damage under low light (LL), which inhibits plant photosynthesis and induces variations in the thylakoid membrane (Dai et al., 2009; Fan et al., 2013; Shao et al., 2014). Tomato (*Lycopersicon esculentum* Mill) is, a light crop, widely cultivated in northeastern China. Therefore, the effects of LL on tomato photosystem inhibition must be determined through systematic research.

Light energy is the driving force for photosynthesis; therefore, inhibition inevitably generates in photosynthetic organisms under LL (Anderson and Chow, 2002; Murata et al., 2007; Tyystjärvi and Aro, 1996). Several mechanisms underlying photosystem inhibition have been proposed (Goh et al., 2011; Noam et al., 2003; Ohnishi et al., 2005). Studies have focused on photosystem under multiple stresses. Generally, photosystem II (PSII) is speculated to be the inhibition site. PSII is generally considered more sensitive than PSI and is easily damaged under temperature and light stresses (Zhang et al., 2014). Exposure of leaves to moderate light and chilling temperature led to selective damage to PSII in tropical trees; the damage to PSII activity could be quickly repaired under low light in several hours, whereas PSI activity was minimally affected during stress and recovery treatments (Huang et al., 2010). However, several studies have shown that the damage to PSII is negligible under LL and chilling temperature (< 100 μmol m^{-2} s^{-1})in contrast to the serious damage to PSI (Havaux and Davaud, 1994). Terashima et al. (1994) found that PSI was more easily inhibited in the leaves of *Cucumis sativus* compared with PSII, which showed almost no damage; hence, PSII is believed to be the main site of inhibition (Sonoike, 1996b, 1998). Li et al. (2004) also found that PSI inhibition was the main factor that limits subsequent recovery under irradiation after chilling treatment. LL stress significantly affects PSII and PSI; but the effects likely to be masked by temperature in short period of time. Therefore, the effect of LL alone on inhibition of PSII and PSI must be investigated.

Under fluctuating light, plants can generally adapt their photosynthetic characteristics to the light condition in the environment by changing their photosynthetic apparatus for several hours to a week (Kono and Terashima, 2014). Mechanisms, including excitation pressure, PSII/PSI ratio, anti-oxidative scavenging system, Mehler's reaction and cyclic electron flow (CEF) around PSI may

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protect photosystems (Joliot and Johnson, 2011) by eliminating surplus electrons and ROS. ROS are eliminated by conversion to water and heat (Miyake, 2010; Takahashi and Badger, 2011). Non-photochemical quenching (NPQ) is necessary for dissipating excess energy in the energy imbalance between PSI and PSII; the fastest responses were observed when qE was linked to the xanthophyll cycle (XC) (Roach and Krieger-Liszkay, 2014). PSII/PSI ratio and CEF can also change the redox state of PSI (Shikanai, 2014; Tikkanen et al., 2014).

Our previous study indicated that the net photosynthesis in tomato was reduced under LL treatment (Meng et al., 2012). In the present study, we investigated the effect of low light on PSII and PSI. This work aims to (1) determine the inhibition site (whether PSII, PSI, or others) and (2) identify the mechanism by which photosystems are regulated under LL conditions.

2. Materials and methods

2.1. Plant materials and growth conditions

A popular tomato variety 'W' was used in the experiments. 'W' produced high-quality fruits, and the tomato seedlings exhibited good growth performance from 5 April to 28 May 2013 in a greenhouse at the experimental farm of Shenyang Agricultural University (41° 82′ N, 123° 56′ E), which is located at the southern boundary of the temperate zone. Six-leaf-old seedlings grown in the matrix were cultivated in a chamber under sunlight climate. The chamber was built by Kooland and had an average relative humidity of 60%. The highest photosynthetic photon flux density (PPFD) at midday reached 1450 $\mu mol\ m^{-2}\ s^{-1}$.

2.2. LL treatment and subsequent inhibition

In previous studies in our lab (Yang et al., 2007), the intensity of LL (75, 50 and 25% of NL) which significantly reducing photosynthesis were optimized, using the same tomato cultivar as the present study. It found that LL (25% of NL) had remarkable effect on seedling growth and photosynthesis. Therefore, LL treatment (25% of NL) was established in our study to clarify the mechanism of photosynthesis inhibition. The LL experiment and physiological measurements were conducted from 18 May to 28 May in 2013. During this period, the day and night temperatures in the sunlight −climate chamber were 25 °C (from 6:00 a.m. to 5:30 p.m.) and 15 °C (from 6:00 p.m. to 5:30 a.m.), respectively. Seedlings with similar vigor were planted in a nutrient substance in a same room and divided into two groups with 46 pots in each group. One group was placed under natural light (NL) with maximum PPFD in the range 1380–1450 $\mu mol\ m^{-2}\ s^{-1}$ at noon (sunshine conditions), and the other group was placed under LL with maximum PPFD in the range 340–360 $\mu mol\ m^{-2}\ s^{-1}$ for 11 days. The means of PPFD under NL and LL throughout the experimental days were shown in Supplementary Fig. S1 in the online version at DOI: http://dx.doi.org/10.1016/j.scienta.2017.01.039. Insufficient light was compensated by an efficient automatic plant growth sodium lamp under both treatments. Moreover, a set of 18 tomato seedlings from each group was used to separately determine the fast and slow chlorophyll a fluorescence parameters, and 10 tomato seedlings from each group were tested for the physiological indices. The sixth leaf from each plant was used for subsequent experiments. All experiments were conducted using the sixth fully expanded leaves and were repeated three times with three replicates for each analysis.

2.3. P515 relaxation kinetics and transients of 550–515 nm signal

The dual-beam 550 nm to 515 nm difference signal was monitored simultaneously by using the P515/535 module of the Dual-PAM-100 and the automated routines provided by the DUAL-PAM software with minor modifications. After 1 h of dark adaptation, rapid P515 changes induced by saturating single turnover flashes were recorded to evaluate the integrity of the thylakoid membrane and activity in ATPase. Slow dark–light–dark induction transients of the 550 nm to 515 nm signals reflect changes in both the membrane potential (electrochromic pigment absorbance shift) and the zeaxanthin content. The transients were measured when actinic light (AL; 531 $\mu mol\ m^{-2}\ s^{-1}$) was turned on after 30 s and off after 330 s. Before this measurement, the leaf was kept for 2 h in darkness, resulting in low zeaxanthin content. Determination of zeaxanthin content, transmembrane potential and proton gradient using the dark-light-dark induction transients was done as described previously by Schreiber and Klughammer (2008). All measurements were performed at a CO_2 concentration of $400 \pm 10\ \mu mol\ mol^{-1}$.

2.4. Chlorophyll fluorescence and P700 measurement

The Chlorophyll fluorescence of PSII and the redox state of P700 were simultaneously measured at room temperature with the automated induction and recovery program Dual-PAM-100 fluorometer (Walz, Effeltrich, Germany) and Dual-PAM software. The sixth leaves under NL and LL were dark-adapted for 20 min before measurement. The fluorescence and $P700^+$ signals were recorded with a saturation pulse (300 ms) of saturating light (10,000 $\mu mol\ m^{-2}\ s^{-1}$) to determine the minimum fluorescence of the dark-adapted state (F_o), the maximum fluorescence (F_m) in the dark-adapted state and the maximum $P700^+$ (P_m). The fluorescence parameters included the effective quantum yield of PSII [Y(II)], the quantum yield of non-regulated energy dissipation [Y(NO)], and the quantum yield of regulated energy dissipation [Y(NPQ)]. They were calculated as follows: $F_v/F_m = (F_m - F_o)/F_m$, $Y(II) = (F_m - F_s)/F_m$, $Y(NO) = F_s/F_m$, $Y(NPQ) = 1 - Y(II) - Y(NO)$. F_m' represents maximum fluorescence values upon illumination in the light-adapted state and F_s is steady-state fluorescence in light. F_v/F_m reflects the maximum quantum yield of PSII (Kramer et al., 2004; Lei et al., 2014). Y(NPQ) and Y(NO) reflect the ability of a plant to self-protection.

The maximal P700 changes (P_m) were measured with a dual wavelength (830/875 nm) unit (Huang et al., 2012; Klughammer and Schreiber, 2008) by Dual-PAM-100, which was recorded by applying a saturation pulse after pre-illumination with far-red light and explained the level at P700 fully oxidised the amount of efficient PSI complex. P_m' was determined similarly to P_m, but without far-red pre-illumination. The parameters included PSI donor side limitation [Y(ND)], PSI acceptor side limitation [Y(NA)], the effective quantum yield of PSI [Y(I)]. They were calculated as follows: $Y(ND) = 1 - P700\ red$, $Y(NA) = (P_m - P_m')/P_m$, $Y(I) = 1 - Y(ND) - Y(NA)$. Y(ND) is the fraction of overall P700 that is oxidised due to a lack of donors. While Y(NA) represents the fraction that cannot be oxidised because of a lack of acceptors. And Y(I) accounted for the fraction of overall P700 in a given state, which is reduced and not limited by the acceptor side. The parameters related to CEF were calculated as follows: $Y(CEF) = Y(I) - Y(II)$, $Y(CEF)/Y(II) = [Y(I) - Y(II)]/Y(II)$.

Ten sixth leaves in different tomato seedlings were dark adapted for 20 min in a leaf clip prior to Chlorophyll a fluorescence transient measurement with a Plant Efficiency Analyzer fluorometer (PEA, Hansatech Instruments Ltd, King's Lynn, Norfolk, UK), according to methods (Jiang et al., 2008; Strasser et al., 2004; Yordanov et al., 2008). The fast chlorophyll a fluorescence of dark-adapted leaves was induced by an array of six red (peak at 650 nm) LEDs of 3000 $mmol\ m^{-2}\ s^{-1}$ for 2 s. Many biophysical parameters derived from cardinal points in the fluorescence versus time curve were used to calculate the following parameters according to the JIP-test. They reflect photosynthetic efficiencies at the onset of illumination,