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BRIEF COMMUNICATION

In vivo evaluation of the high-irradiance effects on PSII activity in photosynthetic stems of *Hexinia polydichotoma*

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Abstract

Green photosynthetic stems are often responsible for photosynthesis due to the reduction of leaves in arid and hot climates. We studied the response of PSII activity to high irradiance in the photosynthetic stems of *Hexinia polydichotoma* in the Taklimakan Desert by analysis of the fast fluorescence transients (OJIP). Leaf clips of a chlorophyll fluorometer were used in conjunction with a sponge with a 4-mm-width groove to prevent light leakage for precise *in vivo* measurements. High irradiance reduced performance indices, illustrating the photoinhibition of PSII to some extent. However, the decrease in active reaction centers (RC) per PSII absorption area and maximum quantum yield indicated a partial inactivation of RCs and an increase in excitation energy dissipation, resulting in downregulation of photosynthetic excitation pressure. In addition, the increased efficiency of electron transport to PSI acceptors alleviated overexcitation energy pressure on PSII. These mechanisms protected the PSII apparatus as well as PSI against damages from excessive excitation energy. We suggested that *H. polydichotoma* exhibited rather photoadaptation than photodamage when exposed to high irradiance during the summer in the Taklimakan Desert. The experiment also demonstrated that the modified leaf clip can be used for studying dark adaptation in a photosynthetic stem.

Additional key words: cylindrical photosynthetic stems; dark adaptation; thermal dissipation.

Leaves are very important organs for performing carbon fixation and transpiration. In angiosperms, a leaf shape is associated with environmental factors and exhibits variations as a consequence of natural selection (Nicotra *et al*. 2011). In arid and hot environments, plants often reduce a leaf area severely to lower water loss and achieve water balance. In these species, the green photosynthetic stems are responsible for photosynthesis and transpiration *in lieu* of full-sized leaves.

The fast fluorescence transient analysis (OJIP) is a fast and nondestructive method to measure the response of PSII activity to environmental changes (Tsimilli-Michael and Strasser 2008) and has been used widely for characterizing the photochemical quantum yield of PSII photochemistry and electron transport activity (Stirbet and Govindjee 2011). Full dark adaptation in leaves

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Abbreviations: ABS/RC – average absorbed photon flux per PSII reaction center; Chl – chlorophyll; DI_o/RC – dissipated energy flux per PSII; ET_o/RC – electron transport flux from Q_A to Q_B; HI – high irradiance; LI – low irradiance; PI_{abs} – performance index for energy conservation from photons absorbed by PSII antenna to the reduction of Q_B; PI_{total} – performance index for energy conservation from photons absorbed by PSII antenna to the reduction of PSI acceptors; RC – reaction center; RE_o/RC – electron transport flux until PSI acceptors per PSII; TR_o/RC – maximum trapped excitation flux per PSII; φ_{Do} – dissipated energy flux; φ_{Eo} – quantum yield of the electron transport flux from Q_A to Q_B; φ_{Po} – maximum quantum yield of primary PSII photochemistry; φ_{Ro} – quantum yield of the electron transport flux until the PSI electron acceptors; δ_{Ro} – efficiency with which an electron from Q_B is transferred until PSI acceptors.
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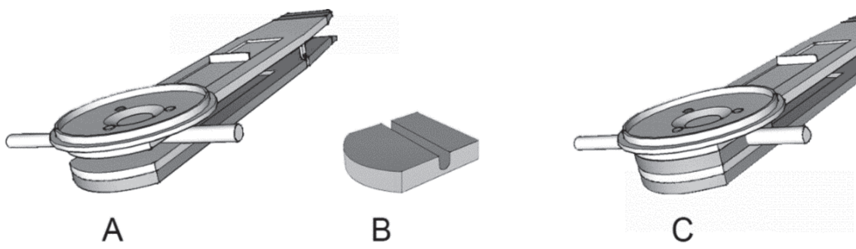

Fig. 1. Schematic representation of modified leaf clip. The leaf clip provided by instrument manufacturer (*A*). It shows that cylindrical stem hinders the closure of leaf clip. The sponge with a 4-mm-width groove (*B*). The modified leaf clip in conjunction with a sponge with a 4-mm-width groove (*C*). It shows that the leaf clip is closed completely.

is necessary for measuring the fast chlorophyll (Chl) fluorescence rise (Strasser and Strasser 1995). A Chl fluorimeter generally provides a special leaf clip to achieve the dark adaptation. Unlike broad leaves, however, cylindrical photosynthetic stems often hinder the closure of the leaf clip, leading to incomplete dark adaptation (Fig. 1*A*).

Hexinia polydichotoma, a perennial and drought-tolerant herb, grows in gullies of deserts and is distributed widely in the Tarim Basin, which is located in the center of the Eurasian continent, possessing a hot and dry climate (Su *et al*. 2012). In *H. polydichotoma*, photosynthetic stems are the major organs for photosynthesis due to severely reduced leaves. We exploited the responses of PSII activity to high irradiance in the photosynthetic stems of *H. polydichotoma* in the Taklimakan Desert through OJIP analysis. The leaf clips of the Chl fluorimeter were slightly modified for *in vivo* measurements of OJIP in the photosynthetic stem.

The study site was located in the moving desert, near the upstream portion of the Hetian River at the southern fringe of the Tarim Basin in China (80°34'15"E, 38°11'25"N). The Tarim Basin shows characteristics typical for a continental climate. Annual precipitation in the desert is only 10.7 mm and evaporation exceeds 3,800 mm (Yang 1987). The experimental site has sparsely distributed *Populus euphratica* with the ground water table in the range of 2.5–3 m.

We chose cloudy and clear days for low and high irradiance treatments, respectively. Cloudy days (low irradiance, LI) showed PAR between 400 and 500 μmol(photon) m^{-2} s^{-1} and clear days (high irradiance, HI) exhibited PAR of > 1,500 μmol(photon) m^{-2} s^{-1}. Light intensity was measured above the plants by PAR sensor of a *PAM 2500* (*2060-M*, *Heinz Walz GmbH*, Effeltrich, Germany). Measurements were done in July 2012. Five individuals were selected, three or four photosynthetic stems from each plant were tagged for measuring Chl *a* fluorescence.

Chl *a* fluorescence induction kinetics was measured by a pocket *PEA* fluorimeter (*Hansatech*, Norfolk, UK). The measurements were performed after the photosynthetic stems were dark-adapted for 30 min using leaf clips provided by the instrument manufacturer. Each treatment included 18 replicates. A light intensity of 3,500 μmol m^{-2} s^{-1} at 660 nm was provided to a 4-mm-diameter area of the leaf to generate maximal fluorescence for all measurements. Light leakage during dark adaptation often occurred due to the cylindrical photosynthetic stem, thus we added a sponge with a 4-mm-width groove in the leaf clip to achieve full dark adaptation (Fig. 1*B*,*C*). The fast fluorescence rise kinetics was recorded from 10 μs to 1 s. The fluorescence intensity at 20 μs, 300 μs, 2 ms, and 30 ms and the maximum fluorescence were collected. The relative parameters were obtained from JIP-test analysis to qualify PSII behavior (Strasser *et al*. 2004, Jiang *et al*. 2008, Stirbet and Govindjee 2011, Gomes *et al*. 2012). Mean comparisons were performed with an independent-samples *T* -test using *SPSS* (*SPSS 13.0*) at 5% level.

Fig. 2 illustrates the behavior of 15 biophysical parameters of PSII in the photosynthetic stems of *H. polydichotoma*. For each parameter, the values were standardized in relation to those under LI. HI decreased the parameters related to yields (φ_{Po} and φ_{Eo}) and both of the performance indices of PI_{abs} and PI_{total}, indicating that photoihibition occurred in the photosynthetic stems of *H. polydichotoma* when exposed to HI.

HI increased all specific fluxes (ABS/RC, TR_o/RC, ET_o/RC, RE_o/RC, and DI_o/RC). However, a significant decrease in RC/ABS indicated a decreased number of Q_A-reducing RCs per PSII antenna Chl, as a result of a partial inactivity of RCs (Stirbet and Govindjee 2011). This suggested that some active RCs were converted into heat sinks, enhancing energy dissipation (Strasser *et al*. 2004). The inactivity of some RCs led to the increased DI_o/RC, which represents the effective dissipation of active RC. In general, high rates of energy dissipation were confirmed by an increase in φ_{Do}. Thus, the heat dissipation of excessive excitation energy slowed the overreduction of the photosynthetic electron transfer chain and minimized the potential photooxidative damage (van Heerden *et al*. 2007). Similar results were obtained for *Graptophyllum* species (Thach *et al*. 2007) and bayberry (*Myrica rubra*) (Guo *et al*. 2006). In addition, lowered φ_{Po} showed a negative effect on PSII activity, but it was considered as a positive adaptation for the downregulation of photosynthetic excitation pressure (Raven 2011).

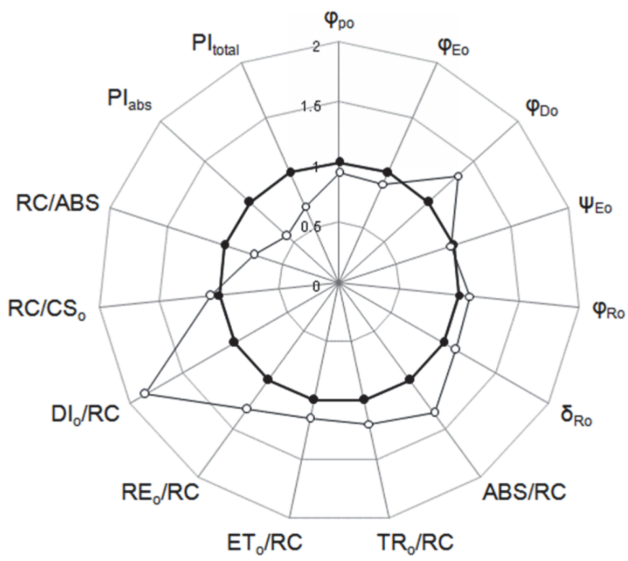

Fig. 2. Radar plot of 15 parameters derived from OJIP transients through JIP-test analysis. The effects of high irradiance (*open circle*) on fluorescence parameters of photosynthetic stems in *Hexinia polydichotoma* were expressed relative to those under low irradiance (*solid circle*) (set as 1). Each parameter was derived from the corresponding mean transient. ABS/RC – average absorbed photon flux per PSII reaction center; DI_o/RC – dissipated energy flux per PSII; RC – reaction center; ET_o/RC – electron transport flux from Q_A to Q_B; RE_o/RC – electron transport flux until PSI acceptors per PSII; PI_{abs} – performance index for energy conservation from photons absorbed by PSII antenna to the reduction of Q_B; PI_{total} – performance index for energy conservation from photons absorbed by PSII antenna to the reduction of PSI acceptors; TR_o/RC – maximum trapped excitation flux per PSII; φ_{Do} – dissipated energy flux; φ_{Eo} – quantum yield of the electron transport flux from Q_A to Q_B; φ_{Po} – maximum quantum yield of primary PSII photochemistry; φ_{Ro} – quantum yield of the electron transport flux until the PSI electron acceptors; δ_{Ro} – efficiency with which an electron from Q_B is transferred until PSI acceptors.

In addition, we observed that φ_{Ro} and δ_{Ro} increased when exposed to HI. The increased φ_{Ro} and δ_{Ro} suggested an improvement in the efficiency of electron transport from trapped electrons or from plastoquinol (PQH2) *via* cytochrome b_6/f to the PSI end electron acceptors (Yan *et al*. 2013). More proportion of energy is transferred into the PSI end electron acceptors (Zivcak *et al*. 2014). Thus, overexcitation energy pressure in PSII can be alleviated objectively in spite of not increasing sum of energy flux into PSI acceptors. Moreover, the enhanced efficiency of the electron transport to PSI may be associated with increased cyclic electron flow, which can alleviate over-excitation energy pressure to protect the PSII apparatus from photooxidative damage (Takahashi *et al*. 2009). The result was consistent with those of Zivcak *et al*. (2014) in barley seedlings. Studies have shown that drought (Campos *et al*. 2014), salinity (Lu and Vonshak 2002), and heat (Zushi *et al*. 2012) enhanced the electron transport to the PSI acceptor and shifted the distribution of excitation energy in favor of PSI. It suggests that the electron transport pattern related to PSI can play an important role in photoprotection of PSII in adverse conditions.

Light drives photosynthesis, but excessive light also damages the photosynthetic apparatus. PSII is one of the major targets for light damage (Takahashi and Badger 2011), but PSI, which accepts electrons from PSII, becomes irreversibly photodamaged if the capacity of PSI electron acceptors becomes exceeded (Tikkanen *et al*. 2014). Therefore, the photoinhibition of PSII induced by HI in *H. polydichotoma* reduced the energy flux into PSI and played an important role in protecting PSI apparatus from photodamage (Yang *et al*. 2014).

In *H. polydichotoma*, HI decreased the performance indices and inhibited the activity of PSII. However, the increase in the number of inactive RCs induced by HI slowed the reduction of Q_A. Electron transport beyond Q_A decreased, resulting in an increase in excitation energy dissipation. In addition, the enhanced efficiency of the electron transport to PSI acceptors alleviated overexcitation energy pressure. These mechanisms protected the PSII apparatus as well as PSI from damage from excess excitation energy. The results suggested that *H. polydichotoma* seemed to exhibit a photoadaptation rather than the photodamage when exposed to HI during the summer in the Taklimakan Desert. Moreover, the experiment demonstrated the use of the modified leaf clip for achieving dark adaptation. In future studies, we can use the leaf clip for measuring fluorescence parameters of xerophytes with photosynthetic stems or halophytes with succulent leaves or stems.

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