Journal of Plant Physiology 209 (2017) 1–10

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Differential accumulation of photosynthetic proteins regulates diurnal photochemical adjustments of PSII in common fig (*Ficus carica* L.) leaves

Selma Mlinarić[a], Jasenka Antunović Dunić[a], Martina Skendrović Babojelić[b], Vera Cesar[a], Hrvoje Lepeduš[c,*]

[a] Josip Juraj Strossmayer University of Osijek, Department of Biology, Ulica cara Hadrijana 8/A, HR-31000 Osijek, Croatia
[b] Faculty of Agriculture, University of Zagreb, Svetošimunska cesta 25, HR-10000 Zagreb, Croatia
[c] Faculty of Humanities and Social Sciences, Josip Juraj Strossmayer University of Osijek, L. Jägera 9, HR-31000 Osijek, Croatia

ARTICLE INFO

Article history:
Received 25 August 2016
Received in revised form 5 December 2016
Accepted 7 December 2016
Available online 9 December 2016

Keywords:
Photosystem II
OJIP
L-band
Performance index
Photoinhibition

ABSTRACT

Molecular processes involved in photosystem II adaptation of woody species to diurnal changes in light and temperature conditions are still not well understood. Regarding this, here we investigated differences between young and mature leaves of common fig (*Ficus carica* L.) in photosynthetic performance as well as accumulation of the main photosynthetic proteins: light harvesting complex II, D1 protein and Rubisco large subunit. Investigated leaf types revealed different adjustment mechanisms to keep effective photosynthesis. Rather stable diurnal accumulation of light harvesting complex II in mature leaves enabled efficient excitation energy utilization (negative L-band) what triggered faster D1 protein degradation at high light. However, after photoinhibition, greater accumulation of D1 during the night enabled them faster recovery. So, the most photosynthetic parameters, as the maximum quantum yield for primary photochemistry, electron transport and overall photosynthetic efficiency in mature leaves successfully restored to their initial values at 1 a.m. Reduced connectivity of light harvesting complexes II to its reaction centers (positive L-band) in young leaves increased dissipation of excess light causing less pressure to D1 and its slower degradation. Decreased electron transport in young leaves, due to reduced transfer beyond primary acceptor Q_A^- most probably additionally induced degradation of Rubisco large subunit what consequently led to the stronger decrease of overall photosynthetic efficiency in young leaves at noon.

1. Introduction

The combination of increased irradiance and elevated temperature is among the most commonly experienced stresses under field conditions. In such conditions, plants mostly receive more sunlight than can be used for photosynthesis. Photosynthetic apparatus acclimation to diurnal changes in light and temperature conditions includes short-term adjustments to reduce the excess of excitation (Desotgiu et al., 2012). Photosystem II (PSII) was recognized for a long time as one of the major regulatory components of the photosynthetic apparatus. Effect of high light stress on PSII reaction centers (RCs) is often manifested as disturbed photosynthetic electron transport and reduced PSII efficiency known as photoinhibition (Sharma et al., 2015). When photoinhibition occurs, utilization of electrons by light-independent reactions is inadequate, excess excitation leads to plastoquinone (PQ) pool reduction and thus changes the accessibility of electron acceptors (Vass, 2012). Photoprotection and repair of photosynthetic apparatus after photoinhibition occurs at different stages of energy conversion. Light induced damage of PSII in low and moderate light conditions can be repaired fast enough to maintain photo-

Abbreviations: Chl, chlorophyll; DTT, dithiothreitol; ECL, enhanced chemoluminescence; HRP, horseradish peroxidase; LHCII, light harvesting complex II; ML, mature leaves; PEA, plant efficiency analyzer; PPFD, photosynthetic photon flux density; PQ, plastoquinone; PSII, photosystem II; RC, reaction center; Rubisco LSU, 1,5-bisphosphate carboxylase/oxygenase large subunit; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; YL, young leaves.

* Corresponding author.
E-mail addresses: smlinaric@biologija.unios.hr (S. Mlinarić), jasenka.antunovic@biologija.unios.hr (J. Antunović Dunić), mskendrovic@agr.hr (M. Skendrović Babojelić), vcesarus@yahoo.com (V. Cesar), hlepedus@yahoo.com (H. Lepeduš).

http://dx.doi.org/10.1016/j.jplph.2016.12.002
0176-1617/

synthetic activity. Under exposure to high irradiation, extent of photodamage exceeds repair processes (Minagawa and Takahashi, 2004; Tikkanen and Aro, 2012). However, the damage of PSII is generally directed to a RC core subunit, the D1 protein. The D1 is involved in primary charge separation and its Q_B-binding site is crucial for electron transport through PSII (Tomek et al., 2003; Faraloni and Torzillo, 2010). When exposed to light, D1 protein is always subjected to photodamage thus leading to photoinhibition (Aro et al., 1993; Tikkanen and Aro, 2012). Rapid degradation of damaged protein and incorporation of newly synthesized one into PSII is an important step in recovering from photoinhibition. However, light harvesting chlorophyll *a/b* complex (LHCII) of PSII antennae are involved in light absorption processes and in transferring excitation energy to RC. In the condition of excess light, LHCII proteins participate in photoprotection by catalyzing thermal energy dissipation (Ballottari et al., 2012). Both, LHCII and D1 protein content gradually increase during the leaf development (Aro et al., 1993; Takeuchi et al., 2002). Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the crucial enzyme of the Calvin cycle responsible for carbon fixation. Young leaves possess lower levels of this enzyme than mature ones. The increase of photosynthetic activity during leaf development consequently rises Rubisco levels (Premkumar et al., 2001; Maayan et al., 2008; Lepeduš et al., 2011). Rubisco accumulation is usually at the highest levels in the morning (Schaffer et al., 2001). However, the increase of light intensity and temperature stimulate its degradation (Desimone et al., 1996; Hrstka et al., 2007).

Photosynthetic capacity and the composition of photosynthetic pigments is closely related to leaf age. Young leaves generally show lower amounts of photosynthetic pigments and lower efficiency of PSII (Jiang et al., 2006; Lepeduš et al., 2011). In most cases, young leaves have incompletely developed PSII what may limit light harvesting and electron transport processes consequently causing photoinhibition (Cai et al., 2005; Drozak and Romanowska, 2006; Jiang et al., 2006). Growing on top of branches all over the canopy, young leaves develop various adaptations to cope with harsh environmental conditions. Red coloration originating from anthocyanins (Manetas et al., 2002; Liakopoulos et al., 2006) and a higher pool of xanthophyll cycle components in young leaves usually have a photoprotective function (Krause et al., 1995; Thiele et al., 1996). However, the way how lightly green leaves exposed to excess light develop different mechanisms to protect vulnerable photosynthetic apparatus remains to be elucidated.

Therefore, measurement of chlorophyll *a* fluorescence can give the information about the tolerance to light and temperature stress as well as on the utilization of light in photosynthetic apparatus (Müller et al., 2001; Strasser et al., 2004). Under natural conditions, typical diurnal course pattern of photosynthetic performance with two peaks exists. One is in the late morning and another in the late afternoon with the midday depression around noon. Although midday depression is mostly influenced by strong irradiation, photoinhibition is usually intensified when increased irradiance is combined with elevated temperature or some other environmental factor (Guo et al., 2009; Panda, 2011).

Common fig (*Ficus carica* L.) is widely cultivated, Mediterranean deciduous tree, characterized by remarkable vegetative growth and well adapted to different environmental factors and climates. Fig leaf development begins in early spring, and production of young leaves continues until midsummer. The lifespan of common fig leaves is from six to three months depending on sprouting time. Generally, in woody plants young leaves initiate inside the canopy if they are formed in the middle of the summer and are shaded with mature leaves. Contrary, young fig leaves are developing at the top of the branches during the whole summer. Therefore, both leaf types are directly exposed to high irradiation and increased temperature. In this work, we aimed to investigate short-term defense strategies in two distinct developmental fig leaf stages (young and mature) under field conditions and to follow responses of PSII to diurnal changes in light and temperature conditions. It is well known that stressful conditions provoke effective strategies for photoprotection and repair of the photosynthetic apparatus that could be seen at different levels of light conversion along electron transport chain (Melis, 1999; Živcak et al., 2014). Our previous research (Mlinarić et al., 2016) revealed that young fig leaves have fully functional PSII apparatus and that downregulation of photosynthetic activity at midday was an efficient photoprotective strategy that enabled them to cope with unfavorable environmental conditions. Mature leaves, on the other hand, continuously maintained fully functional photosynthetic efficiency at the level of primary PSII photochemistry during the midday. Based on that, we hypothesized that photochemical adaptation of PSII in young and mature leaves depends on their differential diurnal accumulation of the main photosynthetic proteins (D1, LHCII and Rubisco LSU).

To investigate these adaptations, we measured chlorophylls *a* and *b* content, *in vivo* chlorophyll *a* fast fluorescence transients and accumulation of LHCII, D1 protein and Rubisco LSU, during the day course.

2. Materials and methods

2.1. Plant material

Common fig (*Ficus carica* L.) trees were sampled in Osijek, Croatia (45°33′29.4″S, 18°43′2.7″I) during June 2011. The investigation was performed on fig trees of the same clone to exclude possible variations due different genotype growing on the same soil type plot. Two types of leaves were used: young (YL, 5–6 cm long, ~7 days old) and mature (ML, 20–25 cm long, ~30 days old). Each type of leaves was sampled four times during the day course, at 1 a.m., 7 a.m., 1 p.m. and 7 p.m. Each leaf type sample consisted of five randomly selected leaves making the composite sample. After removal of main veins, leaf tissue was macerated into a fine powder in liquid nitrogen.

For measurements of light intensity and atmospheric temperature in the field (Table 1), Quantitherm QRT1 light meter (Hansatech, UK) sensor was used. It can accurately measure across the normal measuring temperature (10–40 °C) and photosynthetic active radiation range (0–5000 $\mu mol\ m^{-2}\ s^{-1}$ as well as extreme saturating light intensities up to 50 000 $\mu mol\ m^{-2}\ s^{-1}$). The sensor was placed on the surface of every leaf used for analyses. Measurements of light intensity and temperature around entire canopy did not differ between positions of sampled YL and ML.

2.1.1. Photosynthetic pigment determination

Powdered plant material was extracted with ice-cold absolute acetone and then re-extracted several times until it was completely uncolored. The concentrations of chlorophylls (Chl *a* and Chl *b*) were determined spectrophotometrically (Specord 40, Analytik Jena, Germany) according to Lichtenthaler (1987).

2.1.2. Fast chlorophyll a fluorescence kinetics

Diurnal changes in photosynthetic activity were measured every 6 h during 24 h, on ten randomly selected leaves of each

Table 1
Diurnal changes of photosynthetic photon flux density (PPFD; $\mu mol\ m^{-2}\ s^{-1}$) and atmospheric temperature (°C) measured on the surface of each leaf used for analyses (n = 30).

	1 a.m.	7 a.m.	1 p.m.	7 p.m.
Temperature (°C)	17.5 ± 0	17 ± 1	35 ± 2	26 ± 0
PPFD ($\mu mol\ m^{-2}\ s^{-1}$)	0	150 ± 20	1300 ± 100	150 ± 20