Journal of Plant Physiology 171 (2014) 587–593

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

Physiology

A better energy allocation of absorbed light in photosystem II and less photooxidative damage contribute to acclimation of *Arabidopsis thaliana* young leaves to water deficit

Ilektra Sperdouli, Michael Moustakas *

Department of Botany, School of Biology, Aristotle University of Thessaloniki, University Campus, 54124 Thessaloniki, Greece

ARTICLE INFO

Article history:
Received 30 July 2013
Received in revised form
26 November 2013
Accepted 28 November 2013
Available online 20 March 2014

Keywords:
Drought
Excitation pressure
Leaf developmental stage
Photoprotection
Quantum yield

SUMMARY

Water deficit stress promotes excitation pressure and photooxidative damage due to an imbalance between light capture and energy use. Young leaves (YL) of *Arabidopsis thaliana* plants acclimate better to the onset of water deficit (OnsWD) than do mature leaves (ML). To obtain a better understanding of this differential response, we evaluated whether YL and ML of *A. thaliana* exposed to the OnsWD, mild water deficit (MiWD) and moderate water deficit (MoWD), show differences in their photosynthetic performance, and whether photosynthetic acclimation correlates with leaf developmental stage. Water deficit (WD) resulted in greater photooxidative damage in ML compared to YL, but the latter could not be protected under the OnsWD or MiWD, but only under MoWD. YL of *A. thaliana* with signs of photosynthetic acclimation under MoWD retained higher maximum quantum yield (F_v/F_m) and decreased reactive oxygen species (ROS) formation. YL under MoWD, show a reduced excitation pressure and a better balance between light capture and photochemical energy use, which contributed to their photoprotection, but only under low light intensity (LL, 130 μmol photons m^{-2} s^{-1}) and not under high light (HL, 1200 μmol photons m^{-2} s^{-1}). In conclusion, leaf developmental stage was correlated with photo-oxidative damage and a differential allocation of absorbed light energy in photosystem II (PSII) of *Arabidopsis* leaves under WD.

Introduction

Plant water deficit (WD) occurs when the rate of plant water loss exceeds water uptake, and is a component of several different stresses, including drought, salinity and low temperature (Bray, 1997). To improve crop productivity, it is necessary to understand the mechanism of plant responses to WD with the ultimate goal of improving crop performance (Huang et al., 2008). As the key process of primary metabolism, photosynthesis plays a central role in plant performance under WD (Chaves et al., 2009; Flexas et al., 2009; Lawlor and Tezara, 2009; Pinheiro and Chaves, 2011). Under WD, the balance between light capture and energy use is disturbed (Chaves et al., 2009; Aranjuelo et al., 2011) and the excessive excitation energy in photosystem II (PSII) leads to an impairment of the photosynthetic function and to accumulation of reactive oxygen species (ROS) (Aranjuelo et al., 2011; Wilhelm and Selmar, 2011).

Acclimation of plants to WD is dependent on the plant's ability to avoid and/or endure water stress and is indicated by the accumulation of osmoprotectants and antioxidants to improve plant functioning under WD (Pinheiro et al., 2001; Moustakas et al., 2011; Sperdouli and Moustakas, 2012b). The intensity, duration and rate of progression of the stress will influence plant responses to WD, and dictate whether processes associated with acclimation will or will not occur (Chaves et al., 2009).

Previous work has shown that mild water deficit (MiWD) affected PSII functioning, while under moderate water deficit (MoWD), photosynthetic acclimation was observed, suggesting that PSII activity does not decrease in a drought-dependent way (Sperdouli and Moustakas, 2012a,b). Compared to the increasing knowledge about photosynthetic response to WD revealed by studies using *Arabidopsis* (Woo et al., 2008; Moustakas et al., 2011; Sperdouli and Moustakas, 2012a,b), little attention has been paid to the differential performance of young leaves (YL) and mature leaves (ML) to WD. When YL are exposed to severe WD, they retain their ability to recommence leaf expansion after water is resupplied (Rawson and Turner, 1982; Pantin et al., 2012). Thus, YL tend to be more resistant to WD than ML (Pinheiro and Chaves, 2011).

Chlorophyll fluorescence has proven to be a useful, noninvasive tool for the study of different aspects of photosynthesis, and for the detection of stress in plants (Krause and Weis, 1991). In assessments of WD effects, chlorophyll fluorescence is considered to be a suitable variable for the estimation of inhibition or damage

* Corresponding author. Tel.: +30 2310 998335; fax: +30 2310 998389.
E-mail address: moustak@bio.auth.gr (M. Moustakas).

0176-1617/$ – see front matter © 2014 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.jplph.2013.11.014

in the PSII electron transfer process (Bolhar-Nordenkampf et al., 1989; Calatayud et al., 2006; Li et al., 2008; Massacci et al., 2008; Sperdouli and Moustakas, 2012a,b).

The maximum quantum yield of PSII photochemistry (F_v/F_m) and the quantum efficiency of PSII photochemistry (Φ_{PSII}) in *A. thaliana* leaves under WD decrease less in the proximal (base) than in the distal leaf (tip), the latter representing the older part of the leaf (Sperdouli and Moustakas, 2012a). YL of *A. thaliana* plants were found to acclimate better to the onset of water deficit (OnsWD) by dissipating the excess excitation energy by non-photochemical quenching (NPQ) (Sperdouli and Moustakas, 2012c), and this appeared to be sufficient in scavenging reactive oxygen species (ROS), in order to avoid possible photodamage to PSII under WD (Jung, 2004; Jiang et al., 2005; Sperdouli and Moustakas, 2012c).

In the present study, we evaluated whether YL and ML of *A. thaliana*, in their response to the OnsWD, MiWD and MoWD, show differences in PSII photochemistry, and whether a better balance between light capture and energy use in PSII correlates with less photooxidative damage. The overall hypothesis was that YL of *A. thaliana* would display a better allocation of absorbed light energy in photosystem II and less photooxidative stress than ML, and that YL would thus show photosynthetic acclimation to WD.

Materials and methods

Plant material and growth conditions

Arabidopsis thaliana (L.) Heynh. ecotype Colombia (Col-0) plants were grown in a growth chamber (EF7, Conviron, Montreal, Canada) with controlled environmental conditions under a long day photoperiod 14/10 h, with $40 \pm 5/60 \pm 5\%$ humidity, temperature $22 \pm 1/19 \pm 1\,^{\circ}C$ and light intensity of $120 \pm 20\ \mu mol\ photons\ m^{-2}\ s^{-1}$. WD was imposed on 4-week-old *Arabidopsis* plants by withholding water. The two types of leaves that were examined were fully developed ML and developing YL. YL were assigned the leaves in the center of the leaf rosette with 1.5- to 2-cm length, while the average length of the ML in the rosette was 4.1 ± 0.5 cm.

Soil water status

Soil volumetric water content (SWC), in $m^3\ m^{-3}$, was measured with a 5TE (Decagon Devices, Pullman, Washington, USA) soil moisture sensor, coupled to a ProCheck (Decagon Devices, Pullman, WA, USA) read out device (Sperdouli and Moustakas, 2012b).

Lipid peroxidation measurements

Lipid peroxidation was estimated by measuring malondialdehyde (MDA) accumulation, an indicator of oxidative stress, due to ROS formation. The level of lipid peroxidation in *A. thaliana* YL and ML from each treatment/control, measured as MDA content, was determined by reaction with 2-thiobarbituric acid reactive substances as described by Giannakoula et al. (2008), according to Heath and Packer (1968). Tissue was homogenized in 0.3% TBA in 10% trichloracetic acid at 4 °C. The concentration of MDA was calculated from the difference of the absorbance at 532 and 600 nm spectrophotometrically (PharmaSpec UV-1700; Shimadzu, Tokyo, Japan) using the extinction coefficient of $155\ mmoL^{-1}\ cm^{-1}$ and expressed as nmoL (MDA) g^{-1} fresh weight.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured at room temperature in dark-adapted (20 min) *A. thaliana* YL and ML using an imaging-PAM fluorometer (Walz, Effeltrich, Germany), as described by Sperdouli and Moustakas (2012b). Five areas of interest (AOI) were selected, one in the center of the leaf, two in the outer zone of the front and two in the outer zone of the back of the leaf. First, F_o (minimum chl *a* fluorescence in the dark) and F_m (maximum chl *a* fluorescence in the dark) values were measured with dark-adapted samples, from which F_v/F_m derived, representing the potential (maximum) quantum yield. F_m was obtained with a saturating pulse (SP) of white light ($2400\ \mu mol\ photons\ m^{-2}\ s^{-1}$, 800 ms) followed by application of actinic light (AL) to assess steady-state photosynthesis. A low light intensity of AL $130\ \mu mol\ photons\ m^{-2}\ s^{-1}$ (LL, low light) was selected to match that of the growth light of *A. thaliana* plants, and low enough to avoid photoinhibition, and a high light intensity of AL $1200\ \mu mol\ photons\ m^{-2}\ s^{-1}$ (HL, high light). The illumination time was 2 min with repetitive measurements of F_o' (minimum chl *a* fluorescence in the light) and F_m' (maximum chl *a* fluorescence in the light) values every 20 s, from which, values of other chl fluorescence parameters were calculated automatically by Imaging Win software. The value of F_o' was estimated using the approximation of Oxborough and Baker (1997). The calculated parameters included the effective quantum yield of photochemical energy conversion in PSII (Φ_{PSII}), which estimates the efficiency at which light absorbed by PSII is used for photochemistry $(F_m' - F_s')/F_m'$. The photochemical quenching, q_P,is a measure of the fraction of open PSII reaction centers that is the redox state of quinone A (Q_A), primary acceptor of PSII, and it was calculated as $(F_m' - F_s)/(F_m' - F_o')$ (Genty et al., 1989). The photochemical quenching, q_L which measures the fraction of PSII centers in the open state based on a lake model, was estimated as $(F_m' - F_s/F_m' - F_o')\ (F_o'/F_s')$ (Kramer et al., 2004). The yield of regulated non-photochemical energy loss in PSII (Φ_{NPQ}), the quantum yield for dissipation by down regulation in PSII, was calculated by the equation $\Phi_{NPQ} = 1 - \Phi_{PSII} - 1/[NPQ + 1 + q_L(F_m/F_o - 1)]$, and Φ_{NO}, the quantum yield of non-regulated energy loss in PSII, was calculated as $1/[NPQ + 1 + q_L(F_m/F_o - 1)]$ (Kramer et al., 2004). Excitation pressure, measured as $1 - q_P$, is an estimate of the proportion of closed PSII reaction centers, which reflects the redox state of the electron transport chain (Gray et al., 1996).

Statistical analysis

Each treatment/control was analyzed with six replicates. A standard error (SE) was calculated and data are expressed in mean ± SE of six replicates. Chl fluorescence measurements represent averaged values ($n = 6$) from two independent experiments with three leaf samples (each with 5 AOI) from three different plants, per treatment per experiment. Statistically significant differences between the treatments were analyzed by the Student's *t*-test at $P < 0.05$. A linear regression analysis was also performed (Sperdouli and Moustakas, 2012b,c).

Results

Soil water status

Water deficit was induced gradually by withholding water until SWC decreased to 50–52% of control plants (well-watered). Three categories of WD, in addition to well watered conditions, were characterized (Sperdouli and Moustakas, 2012b): OnsWD (95–96% SWC of control plants), MiWD (66–68% SWC of control plants) and MoWD (50–52% SWC of control plants). Watering of MoWD plants was stopped 10 days before sampling, watering of MiWD plants 6 days before sampling and watering of OnsWD plants 24 h before sampling. Control plants were watered 3 h before measurement.