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High irradiation and increased temperature induce different strategies for competent photosynthesis in young and mature fig leaves

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ABSTRACT

To achieve and maintain optimal and effective photosynthetic functioning under limiting or excess irradiation, acclimation of photosynthetic apparatus requires coordination of biochemical and physiological processes. Due to these processes, leaves usually display high ability to adjust to alteration of microclimate conditions. Photochemical and biochemical adaptations in young and mature leaves of common fig (*Ficus carica* L.) in response to combination of high irradiation (~1300 μmol m^{-2} s^{-1}) and increased temperature (~35 °C) at midday in the field were investigated. Therefore, photosynthetic performance, accumulation of Rubisco large subunit (LSU), activity of enzymatic antioxidants, and oxidative damage on membrane lipids in the morning and at midday were determined. Photosynthetic efficiency (Fv/Fm) in young leaves at midday significantly decreased going along with lower amount of accumulated Rubisco LSU. High irradiation and increased temperature caused significant increase of catalase and peroxidases activities in young leaves, leading to unchanged level of lipid peroxidation. Mature leaves decreased their total chlorophyll content at midday which was accompanied with steady photosynthetic efficiency, shown as constant maximum quantum efficiency and unchanged amount of Rubisco LSU. The level of lipid peroxidation increased in mature leaves, suggesting that increased activities of superoxide dismutase and catalase were not sufficient to prevent oxidative damage. Photoprotective strategies in young leaves enabled them to minimize oxidative damage due to competent antioxidative system and downregulated photosynthetic activity while mature leaves maintained their photosynthetic functionality although they did not have completely efficient antioxidative system.

1. Introduction

In their natural environment, plants are exposed to various stressors that act together, and intense irradiations combined with increased temperatures are the most frequently experienced stresses under field conditions. When changes in environmental conditions exceed plant capacity for acclimation, photoinhibition occurs (Nishiyama et al., 2011). As a result, plants display decreased quantum yield of photosystem II (PSII) and disturbed photochemistry (Takahashi and Murata, 2008; Tyystjarvi, 2008). Primary sites of high irradiance stress alone are reaction centers of PSII (Lichtenthaler and Burkart, 1999). In conditions when light is in excess, part of absorbed energy cannot be efficiently used for photosynthesis and it is dissipated as a heat or as fluorescence (Müller et al., 2001; Nishiyama et al., 2011). On the other hand, plants exposed to increased temperature display inhibition of oxygen evolving center (OEC) and reaction centers of PSII. The OEC is the most temperature-sensitive component of photosynthetic apparatus and even the slightly elevated temperature causes its deactivation (Allakhverdiev et al., 2008). Moderate heat stress was shown to inhibit repair of damaged PSII which accelerates photoinhibition (Takahashi and Murata, 2008). Also, increased temperature impairs the biosynthesis of total chlorophylls and accelerates their degradation (Ashraf and Harris, 2013).

Increased light intensity and elevated temperature in combination revealed differential damage of photosynthetic pigments, proteins, and thylakoid membranes depending on the exposure time. While short-term exposure caused slower and reversible damage occurrence, long-term exposure induced irreversible damage. Damage repair after

Abbreviations: APX, ascorbate peroxidase; BSA, bovine serum albumin; Car, carotenoids; CAT, catalase; Chl, chlorophyll; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; Chl *a*+*b*, total chlorophyll content; Chl *a*/*b*, the ratio of Chl *a* and Chl *b*; Chl *a*/*b*/Car, the ratio of total chlorophyll content and carotenoids; DTT, dithiothreitol; ECL, enhanced chemiluminescence; Fv/Fm, maximum quantum yield of PSII; GPOX, guaiacol peroxidase; HRP, horseradish peroxidase; K-P buffer, potassium phosphate buffer; LHC, light harvesting complex; ML, mature leaves; NBT, nitroblue tetrazolium; OEC, oxygen evolving center; PI_{ABS}, performance index; PPFD, photosynthetic photon flux density; PSII, photosystem II; PVP, polyvinyl pyrrolidine; Q_A, primary electron acceptor of the PSII; ROS, reactive oxygen species; Rubisco LSU, 1,5-bisphosphate carboxylase/oxygenase large subunit; SDS-PAGE, sodium dodecil sulfate polyacrylamide gel electrophoresis; SOD, superoxide dismutase; T, temperature; TBARS, thiobarbituric acid reactive substances; YL, young leaves.

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longer exposure was inhibited due to formation of reactive oxygen species (ROS) (Larcher, 1994; Hewezi et al., 2008). ROS can directly damage photosynthetic apparatus or inhibit protein synthesis that is necessary for effective repair after photoinhibition. Formation of superoxide radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\cdot}$) is usually related to the overexcited acceptor side of PSII and enzymatic antioxidants are the most efficient in their removal (Pospisil, 2012). In order to reduce ROS formation to the minimum, efficient and balanced activity of antioxidant system is necessary. Enzymatic antioxidant mechanisms involve activity of several enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) (Foyer and Noctor, 2000). Harmful consequences of excess irradiation together with increased temperature are evident as damaged membrane lipids, protein, and nucleic acids (Gill and Tuteja, 2010) as well as the degradation of large subunit of Rubisco (Rubisco LSU) (Desimone et al., 1996).

When young leaves develop on top of canopy, they are exposed the most to the challenging environmental pressure. Therefore, they usually encounter levels of irradiance that exceed their photosynthetic capacity which makes them more susceptible to photoinhibition than fully expanded leaves. Although young leaves usually possess adaptation mechanisms that help them to prevent oxidative damage, they are still very sensitive due to ongoing formation of photosynthetic apparatus (Juvany et al., 2013). There are several possible adaptation mechanisms that take place during young leaves' development that enable their increased tolerance to stressful conditions in comparison to mature leaves. Young leaves typically show lower levels of photosynthetic pigments, lower maximum quantum yield of PSII (Fv/Fm), and increased antioxidative enzyme activities (Jiang et al., 2006a; Maayan et al., 2008; Lepeduš et al., 2011).

In this study, we used young (YL) and mature (ML) leaves of common fig (*Ficus carica* L., Moraceae), a Mediterranean deciduous tree characterized by remarkable vegetative growth. Leaf development begins in early spring and production of young leaves continues until midsummer. Our aim was to evaluate photochemical and biochemical adaptations of two distinct developmental leaf stages, YL and ML, as response to the combination of high irradiation and increased temperature in order to determine the significant pathway used in the protection of YL in such conditions. According to the recognized adaptation strategies of YL (Jiang et al., 2006a; Lepeduš et al., 2011; Juvany et al., 2013), we hypothesized that high irradiation and increased temperature in the field would induce different responses in YL leaves. Since ML usually show decrease in total chlorophyll content, excess of absorbed light directed into photochemical reactions diminishes, enabling adequate photosynthetic functionality. Increased efficiency of antioxidative system and downregulation of photosynthetic activity, accompanied by lower amount of Rubisco LSU, should be achieved in fig YL to reduce oxidative damage. To determine physiological performance of investigated leaf types in the field, photosynthetic performance, Rubisco LSU accumulation as well as activity of enzymatic antioxidants, and the extent of oxidative damage on membrane lipids were measured.

2. Material and methods

2.1. Plant material

Common fig (*Ficus carica* L.) trees, cultivar Zamorčica, were sampled in Osijek, Croatia (45°33′29.4″S, 18°43′2.7″I). The identification and determination of cultivar were made using specific plant descriptors (the shape of canopy, leaves, and fruits) as well as characterization of the planting site and environmental characteristics developed by the International Plant Genetic Resources Institute for common fig (IPGRI and CIHEAM, 2003). Since common fig is a Mediterranean tree known for its polymorphism, possible morphological differences due to growth in continental climate were taken into account so the additional descriptor for Croatian cultivars was used for determination of the cultivar Zamorčica (Badelj Mavsar et al., 2008). Investigation was performed on two fig trees of one clone to exclude possible variations due to different genotype growing on the same soil type plot. Two types of leaves were used: young (YL, 5–6 cm long) and mature (ML, 20–25 cm long) ones. All measurements were performed in three repetitions during June 2011. Based on atmospheric conditions, we selected days when light intensity and atmospheric temperature were alike. During the day, two sampling times were chosen: morning, at 7 am, and midday, at 1 pm. In the morning, light intensity (photosynthetic photon flux density, PPFD) was in range 150 ± 20 μmol m^{-2} s^{-1} and temperature (T) was 17 ± 1 °C, while at midday, light intensity varied 1300 ± 100 μmol m^{-2} s^{-1} and temperature was 35 ± 2 °C. Measurements of light intensity and temperature around the entire canopy did not differ between positions of sampled YL and ML. For measurements of light intensity and atmospheric temperature in the field, Quantitherm QRT1 light meter (Hansatech, UK) was used.

For photosynthetic pigments and biochemical analysis as well as for SDS extraction, the composed sample was made for each leaf type. Each composed sample was made of five randomly selected leaves, and three replicates were taken for each analysis. For all determinations, leaf tissue was used after the main veins were removed. Plant material was homogenized using liquid nitrogen into a fine powder and then used for further procedures.

2.2. Photosynthetic pigment determination

Powdered plant material was extracted with the cold absolute acetone and then reextracted several times until it was completely uncolored. The concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (Car) were determined spectrophotometrically (Specord 40, Analytik Jena, Germany) at 470, 661.6, and 644.8 nm according to Lichtenthaler (1987). The chlorophyll *a* and *b* ratio (Chl *a*/*b*) and the Chl *a* + *b* to Car ratio (Chl *a* + *b*/Car) as well as the Chl *a* + *b* concentration was calculated.

2.3. Fast chlorophyll a fluorescence kinetics

Changes in maximum yield of primary photochemistry (Fv/Fm) and performance index (PI_{ABS}) were measured on ten randomly selected leaves of each type using Handy Plant Efficiency Analyzer (Handy-PEA, Hansatech, UK). Measurements were performed in field, on fully dark-adapted leaves using lightweight leaf clips with shutter plate. After the dark adaptation (30 min), the Chl *a* fluorescence transients were induced with the pulse of saturating red light (3200 μmol m^{-2} s^{-1}, peak at 650 nm). Recorded data were used in JIP-test in order to calculate Fv/Fm and PI_{ABS} parameters (Strasser et al., 2004).

2.4. SDS-PAGE and immunodetection

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was made according to Laemmli (1970). Plant material was extracted with hot (80 °C) sodium dodecyl sulpfate (SDS) buffer (0.13 M Tris/HCl (pH = 6.8), 4.6% (w/v) SDS, 16% (v/v) glycerol, and 0.59% (v/v) mM DTT) for 10 min at 80 °C, centrifuged and then reextracted (Lepeduš et al., 2005b). Protein content was determined using the bovine serum albumine (BSA) as a standard (Bradford, 1976). Each loaded homogenate, containing 20 μg of total cell proteins, was separated by SDS-PAGE and transferred to a nitrocellulose membrane according to Towbin et al. (1979). The membranes were incubated in primary Rubisco LSU antibody anti-RbcL (Agrisera, dilution 1:5000) and then in HRP anti-rabbit IgG secondary antibody (Santa Cruz, dilution 1:10000). Finally, the membranes were incubated using Lumi-Light Western Blotting substrate (Roche) and protein bands detected on ECL films (AGFA) according to standard procedure. The ImageJ software was used for protein bands quantification.