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

S. Mlinarić [a], J. Antunović Dunić [a], I. Štolfa [a], V. Cesar [a,*], H. Lepeduš [b]

[a] Department of Biology, Josip Juraj Strossmayer University of Osijek, Ulica cara Hadrijana 8/A, HR-31000 Osijek, Croatia
[b] Faculty of Humanities and Social Sciences, Josip Juraj Strossmayer University of Osijek, Lorenza Jägera 9, HR-31000 Osijek, Croatia

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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.

* Corresponding author. Tel.: +385 31399938; fax: +385 31399939.
E-mail addresses: selma.mlinaric@biologija.unios.hr (S. Mlinarić), jasenka.antunovic@biologija.unios.hr (J. Antunović Dunić), ivna.stolfa@biologija.unios.hr (I. Štolfa), vcesarus@yahoo.com (V. Cesar), hlepedus@yahoo.com (H. Lepeduš).

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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.

2.5. Determination of lipid peroxidation level

Lipid peroxidation was determined as described by Verma and Dubey (2003) and expressed as the amount of thiobarbituric acid reactive substances (TBARS). Plant material was extracted with 0.1% trichloroacetic acid. The absorbance was measured at 532 nm and the value for nonspecific absorption at 600 nm was subtracted. The concentration of TBARS was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.6. Antioxidant enzyme analyses

For the catalase (CAT; EC 1.11.1.6) and superoxide dismutase (SOD; EC 1.15.1.1) assay, plant material was extracted in ice-cold 100 mM K-P buffer (pH = 7.5) with addition of polyvinyl pyrrolidine (PVP). The CAT activity was determined spectrophotometrically by following the consumption of H_2O_2 at 240 nm for 2 min (Aebi, 1984). The reaction mixture for CAT activity contained 50 mM potassium phosphate buffer (pH = 7.5) and 30% (w/v) H_2O_2 and reactions were initiated by adding 10–20 μL of enzyme extract to the total volume of 2 mL. The activity of SOD was assayed by determining its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) as described by Giannopolitis and Ries (1977). The reaction mixture for SOD activity consisted of 50 mM K-P buffer (pH = 7.5), 13 mM methionine, 75 μM NBT, 0.1 mM EDTA, 2 μM riboflavin, and 1–20 μL of enzyme extract to the total volume of 1 mL. The mixture was illuminated at ~120 μmol m^{-2} s^{-1} 1 for 10 min and the absorbance was read at 560 nm. The same mixture, with no extract added, but without illumination was used as blank. One unit (U) of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of NBT reduction, compared to the same illuminated sample with no added enzyme.

For the ascorbate peroxidase (APX; EC 1.11.1.11) assay, plant material was extracted in ice-cold 100 mM potassium phosphate (K-P) buffer (pH = 7.0) with 5 mM Na-ascorbate and 1 mM EDTA with addition of PVP. The APX activity was determined spectrophotometrically by observing the decrease in absorbance at 290 nm (Nakano and Asada, 1981). The reaction mixture contained 50 mM potassium phosphate buffer (pH = 7.0) with 0.1 mM EDTA, 50 mM ascorbic acid, and 100 μL of enzyme extract to the total volume of 1 mL. The reaction was started with the addition 30% (w/v) H_2O_2.

For the guaiacol peroxidase (GPOX; EC 1.11.1.7) assay, plant material was extracted in ice-cold 100 mM Tris-HCl buffer (pH 8.0) containing polyvinylpyrrolidone (PVP). The GPOX activity was determined spectrophotometrically by observing the increase in absorbance at 470 nm (Siegel and Galston, 1967). Reaction mixture contained 200 mM KH_2PO_4, 200 mM $Na_2HPO_4 \times 12\ H_2O$, 5 mM guaiacol, and the reaction was started by adding 100 μL of enzyme extract to the total volume of 1 mL.

2.7. Data analysis

The differences between data measured for ML and YL at two different sampling times (morning and midday) were analyzed by one-way analysis of variance (ANOVA) using Statistica 8.0 software (StatSoft, Inc. 2007). The mean values from all experiment repetitions were compared by Fisher's least significant difference (LSD) *post hoc* test and the differences were considered significant at $p < 0.05$.

3. Results

The Chl *a* fluorescence parameters (Fv/Fm and PI_{ABS}) in ML and YL of common fig measured in the morning (~150 μmol m^{-2} s^{-1}; ~17 °C) and at midday (~1300 μmol m^{-2} s^{-1}; ~35 °C) are shown in Fig. 1. High irradiance and increased temperature at midday decreased Fv/Fm value in both leaf types (Fig. 1a), although YL showed stronger inhibition (25.5%) than ML (14.0%) compared to the morning values.

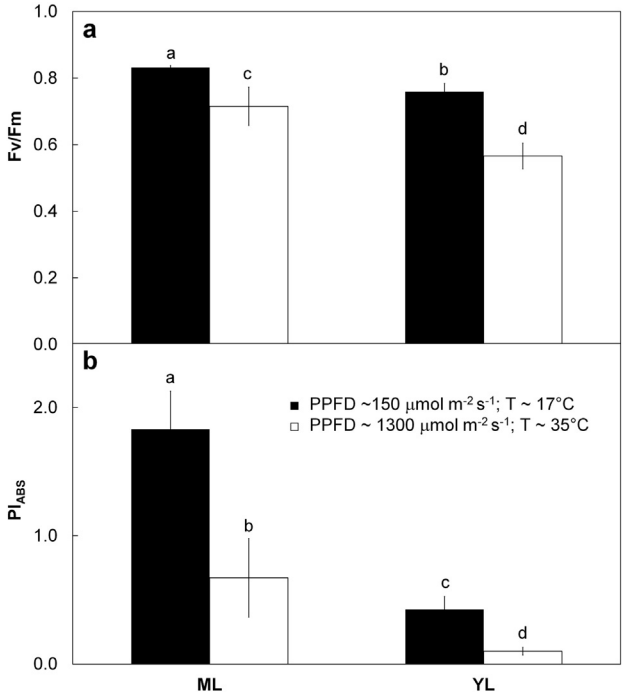

Fig. 1. Maximum yield of primary photochemistry (Fv/Fm, a) and performance index (PI_{ABS}, b), measured in the morning (PPFD = 150 ± 20 μmol m^{-2} s^{-1}; T = 17±1 °C) and at midday (PPFD = 1300 ± 100 μmol m^{-2} s^{-1}; T = 35 ± 2 °C) in young (YL) and mature (ML) leaves of common fig (*Ficus carica* L.). Vertical bars represent mean values ± SD. The vertical bars with different lower-case letters are significantly different from each other at $p < 0.05$ according to Fisher's least significant difference (LSD) test.

There were significantly higher values measured in ML in the morning and at midday compared to YL. The PI_{ABS} values measured at midday decreased 76.4% in YL and 63.3% ML compared to the morning values (Fig. 1b). Further, YL revealed significantly lower values compared to ML measured at both sampling times.

The concentrations of photosynthetic pigments and ratios (Chl $a + b$, Chl a/b, Car and Chl $a + b$/Car) are shown in Fig. 2. High irradiance and increased temperature caused 18.0% decrease in Chl $a + b$ content in ML, while YL displayed 54.3% increase compared to the values measured in the morning (Fig. 2a). The Chl $a + b$ measured at both sampling times revealed significantly lower values in ML compared to YL. Chl a/b ratio observed in both leaf types was not influenced by high light and increased temperature, although YL revealed significantly higher values compared to ML at both sampling times (Fig. 2b). The Car concentration measured at midday showed 32.9% decrease in ML and 61.4% increase in YL compared to morning values (Fig. 2c). Significantly lower Car concentrations were observed in YL, compared to ML, at both sampling times. High irradiation and increased temperature at midday caused 20.1% increase in Chl $a + b$/Car ratio in ML, while in YL, no significant change was observed (Fig. 2d). Measurements of Chl $a + b$/Car ratio at both sampling times revealed higher values in ML compared to YL.

Relative abundances of Rubisco LSU detected in ML and YL in the morning and at midday are shown in Fig. 3. No visible changes were observed in ML between accumulated protein in the morning and at midday. In YL, high irradiance and increased temperature caused reduction in LSU accumulation. Generally, higher Rubisco LSU abundance was detected in ML compared to YL.

The level of lipid peroxidation, expressed as TBARS content, and activities of antioxidant enzymes (SOD; CAT, APX, GPOX) are shown in Fig. 4. The content of TBARS at midday in ML increased 13.3%, while in YL, there was no significant difference, compared to morning values (Fig. 4a). Further, significantly higher TBARS content was observed in ML at both sampling times, compared to YL. High irradiation and increased temperature enhanced SOD activity in ML, while in YL, there

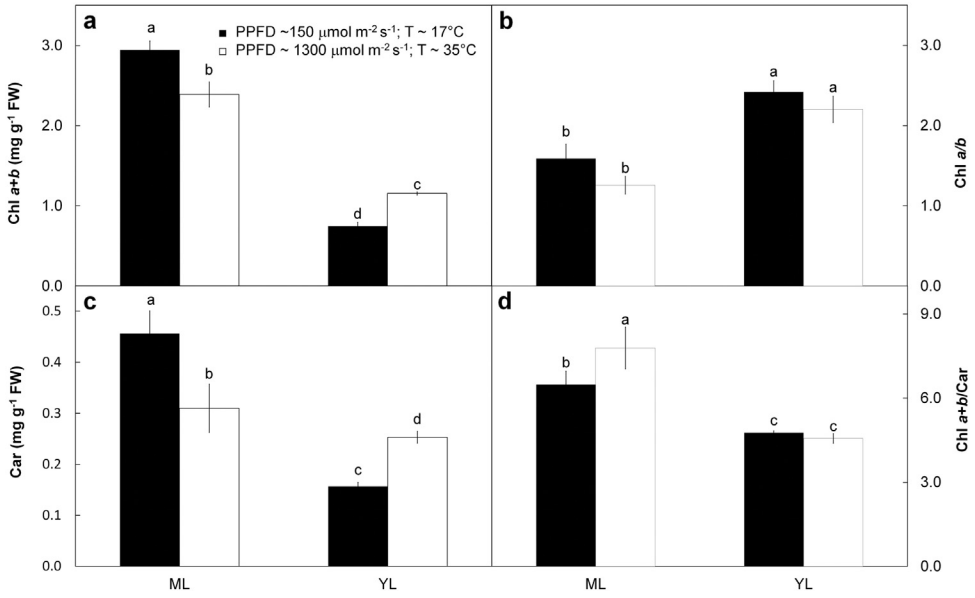

Fig. 2. Total chlorophyll content (Chl $a + b$; mg/g FW, a), carotenoids (Car; mg/g FW, b), chlorophyll a/b ratio (Chl a/b, c), and chlorophyll $a + b$ to carotenoid ratio (Chl $a + b$/Car, d) measured in young (YL) and mature (ML) leaves of common fig (*Ficus carica* L.) in the morning (PPFD = 150 ± 20 μmol m^{-2} s^{-1}; T = 17 ± 1 °C) and at midday (PPFD = 1300 ± 100 μmol m^{-2} s^{-1}; T = 35±2 °C). Vertical bars represent mean values ± SD. The vertical bars with different lower-case letters are significantly different from each other at $p < 0.05$ according to Fisher's least significant difference (LSD) test.

was no significant difference observed compared to the morning values (Fig. 4b). The SOD activity in ML and YL in the morning showed no significant difference, while SOD activity measured at midday in ML showed higher value compared to YL. High irradiation and increased temperature caused increase in CAT activities in both ML (28.5%) and YL (26.4%) compared to morning measurements (Fig. 4c). There were significantly higher values measured in ML in the morning and at midday compared to YL. The APX activity in ML showed no significant difference influenced by high irradiation and increased temperature, while in YL, 36.7% increase was observed at midday in comparison to morning values. The APX activity in ML and YL in the morning showed no significant difference, while APX activity measured at midday in ML showed lower value compared to YL. The GPOX activity in ML showed no significant difference at midday compared to morning values. High irradiation and increased temperature in YL caused 23.1% increase in activity compared to morning values. Higher GPOX activities were measured in YL at both sampling times, compared to ML.

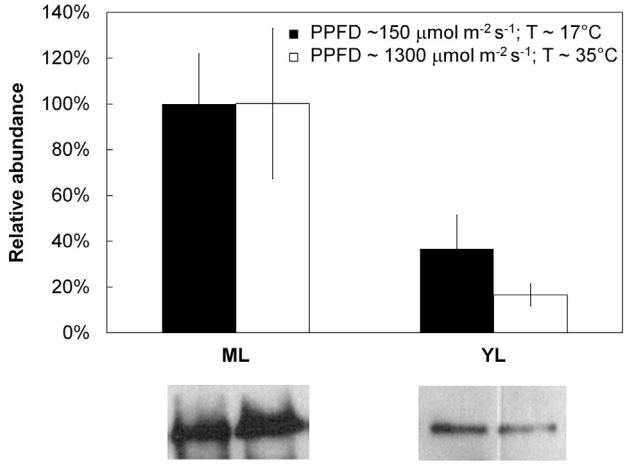

Fig. 3. Relative abundances of Rubisco LSU in mature (ML) and young (YL) leaves of common fig. Relative abundances were measured in the morning (PPFD = 150 ± 20 μmol m^{-2} s^{-1}; T = 17 ± 1 °C) and at midday (PPFD = 1300 ± 100 μmol m^{-2} s^{-1}; T = 35 ± 2 °C). Vertical bars represent mean values ± SD. Values are displayed as % relative to values measured in the morning in ML (100%).

4. Discussion

The maximum quantum yield of PSII, given as Fv/Fm ratio, is often used as reliable indicator for the photosynthetic apparatus functionality evaluation. Both leaf types measured in the morning (~150 μmol m^{-2} s^{-1}; ~17 °C) revealed Fv/Fm values higher than 0.75 (Fig. 1a) which means that they possess completely functional PSII (Bolhar-Nordenkampf et al., 1989). Although both leaf types revealed significant decrease of PSII efficiency at midday (~[illegible] μmol m^{-2} s^{-1}; ~35 °C), ML were able to maintain their functionality even upon exposure to high irradiation and elevated temperature. Also, the performance index (PI_{ABS}) (Fig. 1b) was measured. This parameter was recognized as far more sensitive indicator of plant vitality and physiology status in response to environmental stresses than Fv/Fm (Strauss et al., 2006). It combines three independent steps of primary photochemistry, such as absorption and trapping of excitation energy, electron transport further than primary plastoquinone (Q_A), as well as the non-photochemical dissipation of excess excitation energy (Tsimilli-Michael et al., 2000; Silvestre et al., 2014). In order to reduce imbalance between light absorption and its utilization in stressful conditions, coordinated downregulation of overall photosynthetic processes occurs. Recovery in non-stresful conditions can rapidly increase photosynthetic performance, indicating that PSII was not damaged, but rather downregulated (van Heerden et al., 2007). Both leaf types demonstrated significant reduction of photosynthetic efficiency at midday (Fig. 1b), although at both sampling times, noticeably higher PI_{ABS} values were observed in ML compared to YL. Better performance in fig ML indicated more efficient use of absorbed light energy in photochemical processes.

Light harvesting complexes (LHC) are composed of both Chl *a* and Chl *b*, and any change in Chl *a*/*b* ratio reflects alterations in antenna size (Oguchi et al., 2003). Maxwell et al. (1999) reported that stability of Chl *a*/*b* in epiphytic bromeliad *Guzmania monostachia* suggests that this species has great potential for acclimation to high and low irradiation due to formation of fewer, but more competent photosynthetic units. Total Chl *a*/*b* ratio was lower in ML compared to YL, but at midday in both fig leaf types, the ratio was unchanged (Fig. 2b), suggesting that

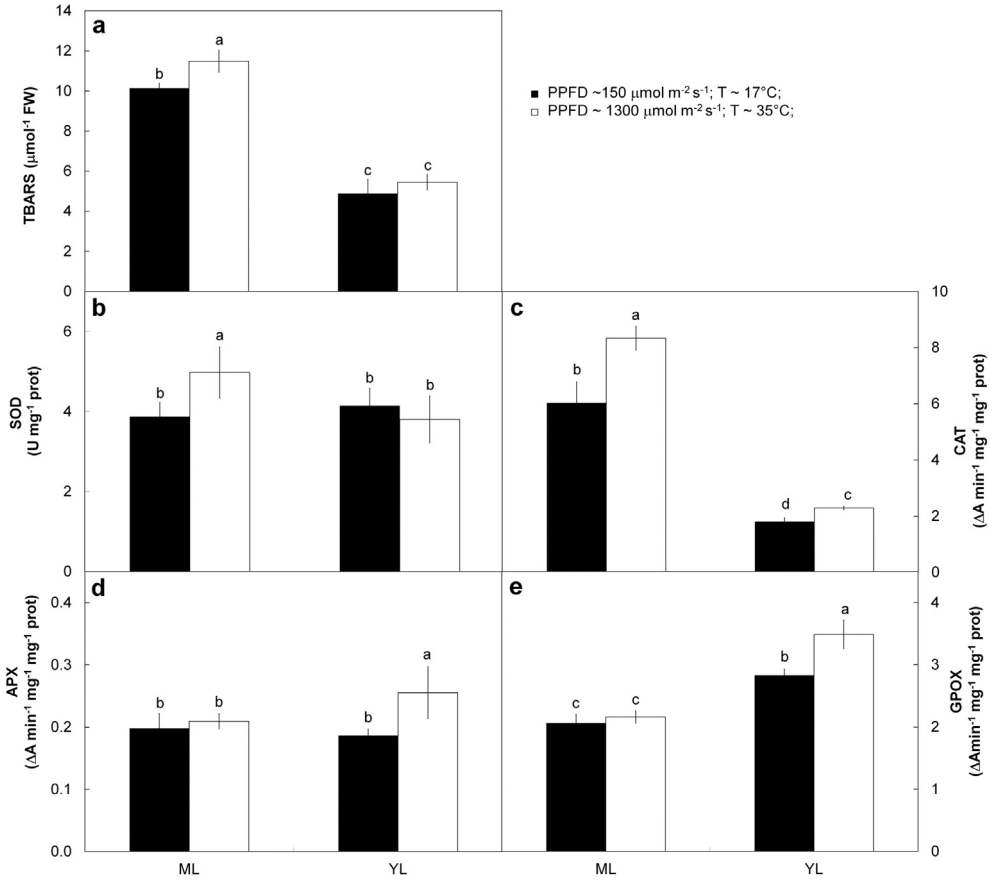

Fig. 4. Changes in concentration of TBARS (μmol g^{-1} FW, a) and specific activities of superoxide dismutase (SOD; U mg^{-1} proteins, b), catalase (CAT; ΔA min^{-1} mg^{-1} protein, c) and ascorbate peroxidase (APX; ΔA min^{-1} mg^{-1} protein, d) and guaiacol peroxidase (GPOX; ΔA min^{-1} mg^{-1} protein, e) in mature (ML) and young (YL) common, fig (*Ficus carica* L.) leaves measured in the morning (PPFD = 150 ± 20 μmol m^{-2} s^{-1}; T = 17 ± 1 °C) and at midday (PPFD = 1300 ± 100 μmol m^{-2} s^{-1}; T = 35 ± 2 °C). Vertical bars represent mean values ± SD. The vertical bars with different lower-case letters are significantly different from each other at $p < 0.05$ according to Fisher's least significant difference (LSD) test.

adjustment in antenna size was not that important for acclimation to high irradiation. It seems that changes in Chl $a + b$ content contributed more to adaptation to high irradiation conditions than balance in Chl*a* and hl *b*. Determination of Chl $a + b$ (Fig. 2a) content showed generally higher Chl $a + b$ values in ML compared to YL, which is the consequence of developmental processes in leaves (Jiang et al., 2006b; Lepeduš et al., 2011). In our study, results showed that Chl $a + b$ values were changing depending on the irradiation intensity and temperature. High irradiation and increased temperature induced decrease in ML while Chl $a + b$ values in YL increased (Fig. 2a). It was suggested that ongoing development of YL could stimulate synthesis of photosynthetic pigments despite changes in environmental conditions (Maayan et al., 2008) which could be the case for the fig YL. On the other hand, lower Chl $a + b$ content in ML indicated that they were able to adjust photosynthetic apparatus to decrease light absorption and in that way to reduce the pressure on the electron transport chain to avoid photoinhibition which is in agreement with previous investigations (Lichtenthaler and Burkart, 1999; Jung, 2004).

Carotenoids are crucial in protection against photooxidative damage since they are involved in direct quenching of ROS and play important role in thermal dissipation of excess energy (Demmig-Adams and Adams, 1996; Behera and Choudhury, 2003). Despite the lower amounts of Car in YL compared to ML, high irradiance and increased temperature increased Car at midday in YL (Fig. 2c), indicating that stressful conditions caused photoprotective response to avoid photooxidation. On the other hand, degradation of Car in ML suggested increased susceptibility to high irradiation and elevated temperature. This is further confirmed by increase in Chl $a + b$/Car ratio (Fig. 2c) in ML at midday. Stressful conditions accelerated Car degradation and disturbed their protective function in ML (Lichtenthaler et al., 2007) which is additionally supported by the result of enhancement in TBARS content (Fig. 4a).

Further, our results revealed lower accumulation of Rubisco LSU in YL than in ML as it was reported earlier for some species like maple (*Acer platanoides* L.) (Lepeduš et al., 2011), oak (*Quercus rubra* L.) (Premkumar et al., 2001), and spruce (*Picea abies* (L.) Karsten) (Lepeduš et al., 2005a). Rubisco LSU accumulation was unchanged in ML at midday compared to accumulation detected in the morning, while YL revealed somewhat lower abundance at high irradiance and increased temperature (Fig. 3). It was reported recently that fully expanded leaves of woody plants undergo slower turnover of Rubisco protein since maintenance of photosynthetic activity requires high energy consumption (Suzuki et al., 2010) which was in concordance with our results for ML. On the other hand, changes in environmental conditions that cause downregulation of photosynthetic performance often decrease level of Rubisco accumulation. During the midday, when the changes in light intensity and temperature were the greatest, decrease in internal CO_2 concentration due to closure of stomata induced the inhibition of photosynthesis. This consequently changes specific saccharide contents that influence Rubisco accumulation (Hrstka et al., 2007). It was reported by Desimone et al. (1996) that higher light intensities increased the amount of active oxygen that stimulated the degradation of Rubisco LSU. Moreover, Jagtap et al. (1998) reported that decrease in Fv/Fm followed by lower Rubisco content in some *Sorghum* varieties was acclimation response to high irradiation since high temperature showed no direct influence on Rubisco content. Furthermore, Irihimovitch and Shapira (2000) suggested that ROS formation induced by high light intensity could

result with downregulation of Rubisco synthesis. Therefore, decreased Rubisco LSU accumulation in fig YL was the most probable response to high irradiation rather than elevated temperature.

Elevated temperature, whether as single stress or combined with some other stress like high irradiation or drought, often induces lipid damage (Lu et al., 2007; Chen et al., 2008). The environmental conditions at midday caused significant damage of lipids (higher TBARS level) in ML compared to data revealed in the morning (Fig. 4a). Since it is known that peroxidation of lipids is one of the indicators of oxidative stress, it was suggested that increased TBARS level might be a sign of reduced efficiency of antioxidant system, unable to cope with boost in ROS generation (Chen et al., 2008; Gill and Tuteja, 2010) which might be the case in fig ML. However, the amount of TBARS in YL at midday was not changed compared to the amount measured in the morning. Additionally, YL revealed lower TBARS content compared to ML at both sampling times. Considering that, it can be assumed that fig YL possess more efficient antioxidant system due to more efficient activity of antioxidative enzymes that prevented YL from oxidative damage which was reported earlier for several species (Cai et al., 2005; Jiang et al., 2005; Lepeduš et al., 2011). Efficient antioxidant system reduces formation of ROS or removes already produced ROS. Activation of SOD is considered as a primary line of defense in plants. The H_2O_2 produced by SOD is further scavenged by CAT, APX, and GPOX to lower levels (Pospisil, 2012). In fig ML, activity of SOD as response to high irradiation and increased temperature significantly increased while activity in YL has not been changed (Fig. 4b) compared to activities in the morning. Further, both fig leaf types revealed significantly higher CAT activities at midday (Fig. 4c) compared to the activities measured in the morning. Although increased CAT activity plays noticeable role in oxidative stress protection, our results showed that measured CAT activity in ML was significantly higher compared to YL, but it was not sufficient to prevent lipid damage in ML. Besides increased SOD and CAT activities, APX and GPOX activity in ML at midday did not change in comparison to activities in the morning. Unchanged peroxidase activity in ML indicates reduced capacity for H_2O_2 removal which is often the case when leaves are exposed to high irradiance which corresponds with previous investigations (Foyer and Noctor, 2000; Lu et al., 2007). On the contrary, YL showed significantly higher activity of both, APX and GPOX at midday (Fig. 4d). Higher peroxidase activity plays an important role in the elimination of ROS (Yu et al., 2013), which indicates better antioxidative response in fig YL. Since increased light and temperature at midday did not influence the APX and GPOX activity in ML which was followed with increased TBARS level, CAT, APX, and GPOX increased activity in YL were more successful in ROS scavenging and thus enabling YL to diminish membrane damage.

5. Conclusion

Based on the obtained results, it can be concluded that in young fig leaves, high irradiation and increased temperature induced photoprotective strategies characterized by competent antioxidative system, downregulated photosynthetic activity accompanied with increased accumulation of total Chl, and sustained degradation of Rubisco LSU which enabled them to minimize oxidative damage. As expected in fully developed fig leaves (ML), acclimation to high irradiation and increased temperature involved lowering of total Chl thus excess of photon flux ongoing into photochemical reactions was diminished which resulted with steady PSII functionality and stable level of Rubisco LSU. However, lipid peroxidation occurred as antioxidative system did not show enough capacity to cope with evolved ROS.

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References

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121–126.
Allakhverdiev, S.I., Kreslavski, V.D., Klimov, V.V., Los, D.A., Carpentier, R., Mohanty, P., 2008. Heat stress: an overview of molecular responses in photosynthesis. Photosynth. Res. 98, 541–550.
Ashraf, M., Harris, P.J.C., 2013. Photosynthesis under stressful environments: an overview. Photosynthetica 51, 163–190.
Badelj Mavsar, D., Bohanec, B., Bučar Miklavčič, M., Butinar, B., Javornik, B., Jakše, J., Podgornik, M., Prgomet, Ž., Skrt, A., Tomažić, I., Vrhovnik, I., Valenčić, V., 2008. The common fig (*Ficus carica* L.) in Istria: morfological, molecular and some chemical characteristics. Univerza na Primorskem, Znanstveno.raziskovalno središče, Založba Annales, Koper.
Behera, R.K., Choudhury, N.K., 2003. High irradiance-induced changes in carotenoid composition and increase in non-photochemical quenching of Chl *a* fluorescence in primary wheat leaves. J. Plant Physiol. 160, 1141–1146.
Bolhar-Nordenkampf, H.R., Long, S.P., Baker, N.R., Oquist, G., Schreiber, U., Lechner, E.G., 1989. Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. Funct. Ecol. 3, 497–514.
Bradford, M.M., 1976. Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Cai, Z.-Q., Slot, M., Fan, Z.-X., 2005. Leaf development and photosynthetic properties of three tropical tree species with delayed greening. Photosynthetica 43, 91–98.
Chen, L.S., Li, P., Cheng, L., 2008. Effects of high temperature coupled with high light on the balance between photooxidation and photoprotection in the sun-exposed peel of apple. Planta 228, 745–756.
Demmig-Adams, B., Adams, W.W., 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends Plant Sci. 1, 21–26.
Desimone, M., Henke, A., Wagner, E., 1996. Oxidative stress induces partial degradation of the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase in isolated chloroplasts of barley. Plant Physiol. 111, 789–796.
Foyer, C.H., Noctor, G., 2000. Oxygen processing in photosynthesis: regulation and signalling. New Phytol. 146, 359–388.
Giannopolitis, C.N., Ries, S.K., 1977. Superoxide dismutases. 1. Occurrence in higher plants. Plant Physiol. 59, 309–314.
Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48, 909–930.
Hewezi, T., Leger, M., Gentzbittel, L., 2008. A comprehensive analysis of the combined effects of high light and high temperature stresses on gene expression in sunflower. Ann. Bot. 102, 127–140.
Hrstka, M., Urban, O., Petriu, E., Babak, L., 2007. Diurnal regulation of ribulose-1,5-bisphosphate carboxylase/oxygenase activity and its content in Norway spruce needles. Photosynthetica 45, 334–339.
IPGRI, CIHEAM, 2003. Descriptors for Fig. International Plant Genetic Resources Institute, Rome, Italy.
Irihimovitch, V., Shapira, M., 2000. Glutathione redox potential modulated by reactive oxygen species regulates translation of Rubisco large subunit in the chloroplast. J. Biol. Chem. 275, 16289–16295.
Jagtap, V., Bhargava, S., Streb, P., Feierabend, J., 1998. Comparative effect of water, heat and light stresses on photosynthetic reactions in *Sorghum bicolor* (L.) Moench. J. Exp. Bot. 49, 1715–1721.
Jiang, C.-D., Li, P.-M., H.-Y., G., Zou, Q., Jiang, G.-M., Li, L.-H., 2005. Enhanced photoprotection at the early stages of leaf expansion in field-grown soybean plants. Plant Sci. 168, 911–919.
Jiang, C.-D., Jiang, G.-M., Wang, X., Li, L.-H., Biswas, D.K., Li, Y.-G., 2006a. Increased photosynthetic activities and thermostability of photosystem II with leaf development of elm seedlings (*Ulmus pumila*) probed by the fast fluorescence rise OJIP. Environ. Exp. Bot. 58, 261–268.
Jiang, C.-D., Shi, L., Gao, H.-Y., Schansker, G., Toth, S.Z., Strasser, R.J., 2006b. Development of photosystems 2 and 1 during leaf growth in grapevine seedlings probed by chlorophyll *a* fluorescence transient and 820 nm transmission in vivo. Photosynthetica 44, 454–463.
Jung, S., 2004. Effect of chlorophyll reduction in *Arabidopsis thaliana* by methyl jasmonate or norflurazon on antioxidant systems. Plant Physiol. Biochem. 42, 225–231.
Juvany, M., Muller, M., Munne-Bosch, S., 2013. Photo-oxidative stress in emerging and senescing leaves: a mirror image? J. Exp. Bot. 64, 3087–3098.
Laemmli, U.K., 1970. Cleavage of structural proteins during assembly of head of bacteriophage T4. Nature 227, 680–685.
Larcher, W., 1994. Photosynthesis as a tool for indicating temperature stress events. In: Schulze, E.D., Caldwell, M.M. (Eds.), Ecophysiology of Photosynthesis: Ecological Studies. Springer Verlag, Berlin, pp. 261–278.
Lepeduš, H., Gaća, V., Cesar, V., 2005a. Guaiacol peroxidases and photosynthetic pigments during maturation of spruce needles. Croat. Chem. Acta 78, 355–360.
Lepeduš, H., Schlensog, M., Muller, L., Krupinska, K., 2005b. Function and molecular organisation of photosystem II in vegetative buds and mature needles of Norway spruce during the dormancy. Biologia 60, 89–92.
Lepeduš, H., Gaća, V., Viljevac, M., Kovač, S., Fulgosi, H., Šimić, D., Jurković, V., Cesar, V., 2011. Changes in photosynthetic performance and antioxidative strategies during maturation of Norway maple (*Acer platanoides* L.) leaves. Plant Physiol. Biochem. 49, 368–376.
Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol. 148, 350–382.

Lichtenthaler, H.K., Burkart, S., 1999. Photosynthesis and high light stress. Bulg. J. Plant Physiol. 3–16.
Lichtenthaler, H.K., Ac, A., Marek, M.V., Kalina, J., Urban, O., 2007. Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. Plant Physiol. Biochem. 45, 577–588.
Lu, P., Sang, W.-G., Ma, K.-P., 2007. Activity of stress-related antioxidative enzymes in the invasive plant crofton weed (*Eupatorium adenophorum*). J. Integr. Plant Biol. 49, 1555–1564.
Maayan, I., Shaya, F., Ratner, K., Mani, Y., Lavee, S., Avidan, B., Shahak, Y., Ostersetzer-Biran, O., 2008. Photosynthetic activity during olive (*Olea europaea*) leaf development correlates with plastid biogenesis and Rubisco levels. Physiol. Plant. 134, 547–558.
Maxwell, K., Marrison, J.L., Leech, R.M., Griffiths, H., Horton, P., 1999. Chloroplast acclimation in leaves of *Guzmania monostachia* in response to high light. Plant Physiol. 121, 89–95.
Müller, P., Li, X.P., Niyogi, K.K., 2001. Non-photochemical quenching. A response to excess light energy. Plant Physiol. 125, 1558–1566.
Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 867–880.
Nishiyama, Y., Allakhverdiev, S.I., Murata, N., 2011. Protein synthesis is the primary target of reactive oxygen species in the photoinhibition of photosystem II. Physiol. Plant. 142, 35–46.
Oguchi, R., Hikosaka, K., Hirose, T., 2003. Does the photosynthetic light-acclimation need change in leaf anatomy? Plant Cell Environ. 26, 505–512.
Pospisil, P., 2012. Molecular mechanisms of production and scavenging of reactive oxygen species by photosystem II. Biochim. Biophys. Acta 1817, 218–231.
Premkumar, A., Mercado, J.A., Quesada, M.A., 2001. Effects of in vitro tissue culture conditions and acclimatization on the contents of Rubisco, leaf soluble proteins, photosynthetic pigments, and C/N ratio. J. Plant Physiol. 158, 835–840.
Siegel, B.Z., Galston, W., 1967. The peroxidase of *Pisum sativum*. Plant Physiol. 42, 221–226.
Silvestre, S., Araujo, S.D., Patto, M.C.V., da Silva, J.M., 2014. Performance index: an expeditious tool to screen for improved drought resistance in the *Lathyrus* genus. J. Integr. Plant Biol. 56, 610–621.
Strasser, R.J., Tsimilli-Michael, M., Srivastava, A., 2004. Analysis of the chlorophyll *a* fluorescence transient. Chlorophyll Fluorescence 19, 321–362.
Strauss, A.J., Kruger, G.H.J., Strasser, R.J., Van Heerden, P.D.R., 2006. Ranking of dark chilling tolerance in soybean genotypes probed by the chlorophyll a fluorescence transient O-J-I.-P. Environ. Exp. Bot. 56, 147–157.
Suzuki, Y., Kihara-Doi, T., Kawazu, T., Miyake, C., Makino, A., 2010. Differences in Rubisco content and its synthesis in leaves at different positions in *Eucalyptus globulus* seedlings. Plant Cell Environ. 33, 2231.
Takahashi, S., Murata, N., 2008. How do environmental stresses accelerate photoinhibition? Trends Plant Sci. 13, 178–182.
Towbin, H., Staehelin, T., Gordon, J., 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354.
Tsimilli-Michael, M., Eggenberg, P., Biro, B., Köves-Pechy, K., Vörös, I., Strasser, R.J., 2000. Synergistic and antagonistic effects of arbuscular mycorrhizal fungi and *Azospirillum* and *Rhizobium* nitrogen-fixers on the photosynthetic activity of alfalfa, probed by the polyphasic chlorophyll a fluorescence transient O-J-I-P. Appl. Soil Ecol. 15, 169–182.
Tyystjarvi, E., 2008. Photoinhibition of photosystem II and photodamage of the oxygen evolving manganese cluster. Coord. Chem. Rev. 252, 361–376.
van Heerden, P.D.R., Swanepoel, J.W., Kruger, G.H.J., 2007. Modulation of photosynthesis by drought in two desert scrub species exhibiting C3-mode CO2 assimilation. Environ. Exp. Bot. 61, 124–136.
Verma, S., Dubey, R.S., 2003. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Sci. 164, 645–655.
Yu, C., Huang, S.J., Hu, X.M., Deng, W., Xiong, C., Ye, C.H., Li, Y., Peng, B., 2013. Changes in photosynthesis, chlorophyll fluorescence, and antioxidant enzymes of mulberry (*Morus* ssp.) in response to salinity and high-temperature stress. Biologia 68, 404–413.