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Thylakoid membrane reorganization, induced by growth light intensity, affects the plants susceptibility to drought stress

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Abstract

One of the major questions in photosynthesis research is related to the role of the ultrastructure of thylakoid membranes in higher plants for their functional performance at optimal and stress environmental conditions. In this work, we compared the drought stress response of pea plants grown at moderate and low light conditions, based on our recent findings that they differ in the extent of grana stacking and in the thermal stability of the major light-harvesting complex of PSII that plays important regulatory role for plant survival. Our data demonstrate that low light-adapted plants are structurally and functionally less affected by the water deficit, strongly suggesting a major role of thylakoid structural organization for drought stress response.

Additional key words: circular dichroism; differential scanning calorimetry; JIP-test; rehydration.

Introduction

It has been estimated that since 1950s over 70% of the agricultural lands on a planetary scale have experienced increasing tendency to more frequent drought episodes (Wang *et al.* 2014). Regions suffering from chronic drought include North America, central South America, and large areas of Europe and Asia (Wang *et al.* 2014). Europe, being among the most strongly affected continents, experiences the large hydroclimate fluctuations with the highest extremes recorded in a period of 1,000 years (Markonis *et al.* 2018). It is expected that global

warming (either by 1.5°C as proposed by the 2015 Paris Agreement, or by 2°C) would involve increase of the duration of severe drought episodes and larger number of people (including urban population) would be affected (Liu *et al.* 2018). Most of the direct and indirect social and economic impacts of drought, *e.g.*, famine, human migration, livestock mortality, increase of disease in wildlife, *etc.*, are due to drought-stress-induced decrease in crop productivity. Photosynthesis – the central metabolic pathway in plant primary metabolism, is inevitably affected by drought stress and thus chlorophyll (Chl) *a* fluorescence techniques such as JIP-test are suitable to

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Abbreviations: CD – circular dichroism; c_p^{ex} – excess heat capacity; Chl – chlorophyll; DSC – differential scanning calorimetry; DM – dry mass; F_0 – minimal fluorescence intensity in dark-adapted state; F_m – maximal fluorescence intensity in dark-adapted state; FM – fresh mass; LL – low light intensity; LLd – drought stress at low light intensity; LLr – rehydration at low light intensity; N – turnover number of Q_A reduction and reoxidation acts; MC540 – merocyanine 540; MDA – malondialdehyde; ML – moderate light intensity; MLd – drought stress at moderate light intensity; MLr – rehydration at moderate light intensity; PI_{ABS} – performance index; PI_{total} – overall functional activity of PSII, PSI, and the intersystem electron carriers; PEG – polyethylene glycol; RWC – relative water content; TBA – thiobarbituric acid; T_m – denaturation temperature; TR_0/RC – excitation energy trapped by an active PSII reaction center immediately after illumination; TM – turgid mass; δ_{R_0} – the probability that an electron residing in the intersystem electron transport chain would reach the terminal electron acceptors at the acceptor side of PSI; γ_{RC} – the probability that a chlorophyll *a* molecule located in PSII serves as a reaction center; ϕ_{P_0} – maximum quantum yield of the primary photochemical reaction in PSII; ψ_{E_0} – the probability that an exciton trapped by PSII reaction center would drive electron transport beyond Q_A .

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be applied for phenotyping of plants experiencing water deficit (Oukarroum *et al.* 2007, Strasser *et al.* 2010, Goltsev *et al.* 2012, Kalaji *et al.* 2016, 2018). Stomatal closure, which modulates water loss due to transpiration at the expense of lowering of the CO₂ supply needed for the Calvin cycle (Benešová *et al.* 2012, Tombesi *et al.* 2015, Martin-StPaul *et al.* 2017), leads to hampered NADP⁺ regeneration and eventually to overreduction of photosynthetic electron transport chain and production of reactive oxygen species (Wang *et al.* 2018, Zhou *et al.* 2019). Furthermore, cytoplasm shrinkage upon water deficiency brings about increase in molecular crowding which compromises protein stability, provokes protein aggregation (Hall 2006, Furuki *et al.* 2012, Kuznetsova *et al.* 2014, Sarkar and Pielak 2014), alters the physical properties of the lipid bilayers, and promotes membrane fusion (reviewed in Crowe *et al.* 2011, Morandi *et al.* 2018). These effects concern not only the cytoplasmic structures but also the architecture of the inner membrane system of the chloroplasts – the thylakoids. At optimal growth conditions, the thylakoid membranes of higher plants possess specific architecture combining regions of stacked adjacent membrane patches – grana, interconnected with unstacked membrane regions – stroma lamellae (Austin and Staehelin 2011). Besides the spatial segregation, the stacked and unstacked membrane regions also differ in their protein composition – while grana are enriched in PSII complexes, the stroma regions are enriched in PSI (Anderson 2002, Danielsson *et al.* 2004), which is believed to be crucial for the optimal distribution of the excitation energy between the two photosystems (van der Weij-de Wit *et al.* 2007, Petrova *et al.* 2018).

In drought-stressed plants, however, thylakoid membranes appear deformed and disorganized, and depending on the severity of the dehydration and susceptibility of species/cultivars, it may lead to impairment of grana formation (Zhang *et al.* 2015, Filek *et al.* 2016, Shao *et al.* 2016). Furthermore, it was reported that oligomerization state of the photosynthetic complexes in the thylakoid membranes, their specific phospho- and galactolipid species content, and fatty acid saturation are among the factors modulating drought stress effects (Quartacci *et al.* 1995, Lauriano *et al.* 2000, Torres-Franklin *et al.* 2007). The structural integrity of the photosynthetic complexes is widely affected by their microenvironment. Interaction between PSII core complex and its major light-harvesting complex (LHCII), as well as their oligomerization state and stability, strongly depend on and are mediated by the surrounding lipids (Seiwert *et al.* 2017, Kotakis *et al.* 2018, Sheng *et al.* 2018). Moreover, light harvesting and light transfer by LHCII towards the two photosystems is largely determined by the architecture of thylakoid membranes (Petrova *et al.* 2018). Thus, it seems that the functionality of the photosynthetic apparatus under conditions of drought stress is a result of the interplay between the tendency of avoidance of overreduction of the photosynthetic electron transport chain and the dehydration-driven changes in the physico-chemical properties of the thylakoid membranes.

Light quality and quantity is also known to moderate the size and composition of the photosystems' antennae,

i.e., low light adaptation triggers enlargement of PSII antennae (mostly due to increase in the Lhcb1 and Lhbc2 components of the LHCII complex), while high light acclimation leads to the opposite effect (Anderson 1986, Lindahl *et al.*, 1995, Ballottari *et al.* 2007). On the other hand, no changes in the composition of the light-harvesting antennae were observed for PSI complex under varying light conditions and the association of LHCII molecules plays vital role for the expansion of the PSI absorption cross section under low light (Ballottari *et al.* 2007).

In agreement with numerous works focused on plants adaptation to low light (Lichtenthaler *et al.* 1982, Anderson *et al.* 1988, Bailey *et al.* 2001, Ballottari *et al.* 2007, Kirchhoff *et al.* 2007, Kouřil *et al.* 2013), our recent work revealed that adaptation of *Pisum sativum* cv. RAN1 plants to low light (LL) intensity leads to decrease in Chl *a/b* ratio by 5.5 % and higher propensity for grana formation (Petrova *et al.* 2019). In addition, a significant decrease in the thermodynamic stability of the lateral macroorganization of the photosynthetic complexes (by *ca.* 3°C), LHCII trimers and monomers (by 4–6°C) was found under LL conditions, as compared to plants grown at moderate light (ML) intensity. These changes were associated with partial deactivation of PSII reaction centers and suppressed electron transport to PSI end acceptors under LL (Petrova *et al.* 2019). Therefore, it is of high interest to probe whether growth light-induced structural reorganization of the photosynthetic apparatus might be beneficial for the response to drought and other types of stresses. Recent report shows that higher plant response to drought stress in terms of photosynthetic activity is manifested in transformation of active PSII reaction centers into dissipating traps and in decreased electron flux per PSII reaction center (Kalaji *et al.* 2018). On the other hand, Yamakawa *et al.* (2018) pointed out that the mechanism of the response of mosses towards drought stress is highly dependent on the growth light intensity.

In the present work, we compared the response of ML and LL pea plants to drought stress (MLd and LLd, respectively) and consequent rehydration (MLr and LLr, respectively). Applying JIP-test along with techniques providing information on the molecular organization and thermal stability of the thylakoid membranes, we seek to establish correlation between the patterns of response towards water stress and the structural characteristics of the photosynthetic apparatus (in particular, thermodynamic stability of the photosynthetic complexes and their lateral and vertical macroorganization).

Materials and methods

Plant material: Pea plants (*Pisum sativum* L., cv. RAN1) were grown hydroponically in tap water in a room under controlled conditions: ambient temperature of 23°C, 16-h dark/8-h light period, and moderate [200 μmol(photon) m⁻² s⁻¹, ML] or low [20 μmol(photon) m⁻² s⁻¹, LL] light intensity. For induction of drought stress, 10-d-old pea plants were grown for another 4 d in cultivation medium supplemented with 30% polyethylene glycol (PEG). The level of dehydration was determined by calculating

the relative water content (RWC) of detached pea leaves according to the following equation: $RWC [\%] = (FM - DM)/(TM - DM)$, where FM is the fresh mass, DM – dry mass, TM – turgid mass obtained after soaking the leaves in distilled H₂O for 12 h. For achieving rehydration of the desiccated plants, the PEG cultivation solution was replaced with tap water in which the pea plants were grown for another 4 d.

Thylakoid membranes were isolated according to the procedure described in Petrova *et al.* (2018). Thylakoids from drought-stressed and rehydrated plants were isolated at the end of the desiccation and rehydration periods, respectively. Thylakoids from untreated plants were isolated from 14-d-old plants. Isolated thylakoids were stored at –20°C in 20 mM Tricine (pH 7.6) buffer supplemented with 5 mM MgCl₂, 250 mM sorbitol, and 30% glycerol. Before each experiment, the membranes were washed and resuspended to a desired Chl concentration in the same buffer devoid of glycerol.

Lipid peroxidation of thylakoid membranes: Malondialdehyde (MDA) content in isolated control and stressed thylakoid membranes was regarded as an indicator of the level of drought stress-induced peroxidation of the lipids in the thylakoids. The concentration of MDA in the thylakoids was determined according to the procedure of Mishra and Singhal (1992) with modifications. Thylakoid suspensions with concentration of 50 µg(Chl) ml^{–1} were mixed with equal volume of reaction medium containing 1% thiobarbituric acid (TBA) initially dissolved by heating up to 60°C, and 40% trichloroacetic acid. After that, the samples were incubated for 25 min at 95°C, cooled down on ice, and centrifuged at 250 × g for 1 min. TBA-MDA adduct concentration was spectrophotometrically (*Specord 210 Plus* spectrophotometer, *Analytik Jena*, Germany) determined using absorption intensity at 532 nm and extinction coefficient (ϵ_{532nm}) of 155 mM^{–1} cm^{–1}.

Thylakoid membrane lipid phase fluidity: Thylakoid membrane fluidity was assessed using the ability of the fluorescent probe merocyanine 540 (MC540, *Sigma-Aldrich*) to incorporate into the thylakoid membrane bilayer. For this purpose, thylakoid membranes isolated from nontreated and drought-stressed plants with concentration of 15 µg(Chl) ml^{–1} were incubated with 0.2 µM MC540 for 30 min at room temperature (Krumova *et al.* 2008, Petrova *et al.* 2018). Excitation spectra of thylakoid suspensions incubated with MC540 were detected in the range 400–580 nm at emission wavelength 595 nm. The ratio of the fluorescence intensities at 560 and 528 nm, $F_{560/528}$, was taken as an indicator of the propensity of MC540 to penetrate the thylakoid membrane lipid phase (*LS-50B* luminescence spectrometer, *Perkin Elmer*, Waltham, MA, USA) (Krumova *et al.* 2008).

Thermodynamic stability of thylakoid membranes: Differential scanning calorimetry (DSC) was applied to reveal alterations in the thermodynamic stability of the thylakoid membranes related to desiccation and rehydration. Thermograms of thylakoid suspensions with Chl

concentration of 1.3 mg(Chl) ml^{–1} were recorded with *DASM-4* microcalorimeter (*Privalov*, Pushchino, Russian Federation) with scanning rate of 0.5°C in the temperature range 30–100°C. The thermograms were processed with the aid of *Origin 8.1* software. After subtraction of buffer-buffer scan and correction with sigmoidal baseline, the thermograms were normalized to the Chl concentration of the samples. The temperatures of denaturation (T_m) and the excess heat capacity (c_p^{ex}) values were determined from the position of the peaks of the calorimetric transitions and their respective amplitudes.

Macroorganization of the photosynthetic complexes:

The overall arrangement and the changes in the conformation of the pigment-protein complexes in control (nontreated), water-stressed, and rehydrated thylakoids were examined by circular dichroism (CD). CD spectra of isolated thylakoids with OD₆₈₀ = 1 were recorded at room temperature with *Jasco J-815 CD* spectrometer (*Easton*, MD, USA) in the range of 400–800 nm and applying 0.5-nm data pitch, 3-nm band width, and 100 nm min^{–1} scanning speed. The intensity of the following CD bands was calculated by subtracting a reference value at defined wavelength or by subtracting the intensities of two adjacent CD bands with opposite signs for doublet CD signals, *i.e.*, (+)694 nm (CD_{694nm} – CD_{800nm}), (–)679 nm (CD_{800nm} – CD_{679nm}), (+)483 nm/(–)473 nm (CD_{483nm} – CD_{473nm}), (+)444 nm/(–)436 nm (CD_{444nm} – CD_{436nm}), (+)506 nm (CD_{506nm} – CD_{600nm}), (–)657 nm (CD_{630nm} – CD_{657nm}).

JIP-test: The effect of drought stress on the photosynthetic apparatus functionality and its reversibility was investigated by the JIP-test. Chl fluorescence induction curves of intact fully developed leaves of control, drought-stressed, and rehydrated plants were registered with *Multifunctional Plant Efficiency Analyzer (M-PEA, Hansatech Instruments Ltd., King's Lynn, UK)* after a 30-min dark adaptation. Selected JIP parameters (*see text table below*) were evaluated and their assignment and implication was based on Strasser *et al.* (2004). Fluorescence intensity was recorded for 1 s during excitation with high intensity beam [PAR of 4,000 µmol(photon) m^{–2} s^{–1}].

Statistics: All the tested parameters are represented as mean values and their standard errors ($\pm$ SE). JIP parameters were determined independently for three experimental repetitions, each including five plants with two leaf pairs measured on both sides of the central vein ($n = 15$). Spectroscopic and calorimetric data were obtained for four individual isolations of thylakoid membranes ($n = 4$). Significant differences of the means were detected with the aid of *Welch's t-test* (Gaetano 2019) with level of significance 0.05. Data averaging and statistical analyses were performed with the aid of *Origin 8.1* software.

Results

Hydration level of drought-stressed and rehydrated plants: The RWC of the leaves of untreated ML and LL

JIP parameter	Assignment and implication
F_0	Minimal fluorescence intensity in dark-adapted state
F_m	Maximal fluorescence intensity in dark-adapted state
N	The turnover number of Q_A reduction and reoxidation acts taking place until fluorescence maximum is reached. N gives relative view on the number of electrons needed to reduce all the electron carriers in the photosynthetic electron transport chain beyond Q_A
γ_{RC}	The probability that a Chl a molecule located in PSII serves as a reaction center. γ_{RC} is reversely related to the amount of light energy absorbed by a PSII reaction center, <i>i.e.</i> , the size of the effective PSII antennae
TR_0/RC	Excitation energy trapped by an active PSII reaction center immediately after illumination
ϕ_{P_0}	Maximum quantum yield of the primary photochemical reaction in PSII. ϕ_{P_0} indicates what part of the absorbed light energy can be trapped by a PSII reaction center and potentially utilized for photosynthetic electron transport
ψ_{E_0}	The probability that an exciton trapped by PSII reaction center would drive electron transport beyond Q_A , <i>i.e.</i> , efficiency of electron transport after Q_A
δ_{R_0}	The probability that an electron residing in the intersystem electron transport chain would reach the terminal electron acceptors at the acceptor side of PSI
PI_{ABS}	Indicator of the performance of PSII expressed as its ability to utilize exciton energy for reduction of intersystem electron carriers normalized to the amount of absorbed energy (performance index)
PI_{total}	Overall functional activity of PSII, PSI, and the intersystem electron carriers viewed as the potential of light energy absorbed by PSII to be used for reduction of the terminal acceptors in PSI

plants was $97.0 \pm 2.6\%$ and $97.4 \pm 3.6\%$, respectively. Treatment with 30% PEG for 4 d provoked striking wilting of the leaves and significant desiccation of both MLd ($58.8 \pm 2.4\%$) and LLd ($60.6 \pm 4.5\%$) leaves. At the end of the rehydration period, the RWC of both MLd and LLd samples (denoted hereafter MLr and LLr) recovered to $90.5 \pm 2.0\%$ and $93.4 \pm 4.4\%$, respectively.

Peroxidation of thylakoid lipids: The concentrations of the TBA-MDA adduct was $1.45 \pm 0.16 \times 10^{-4}$ mM in ML and $1.27 \pm 0.20 \times 10^{-4}$ mM in LL thylakoids. For MLd samples, a double increase in the TBA-MDA content was observed up to $2.89 \pm 0.4 \times 10^{-4}$ mM and this value was insignificantly changed to $2.80 \pm 0.3 \times 10^{-4}$ mM upon the consequent rehydration, thus it did not affect the extent of peroxidation of thylakoid lipids. For LL ($1.27 \pm 0.1 \times 10^{-4}$ mM), LLd ($1.24 \pm 0.2 \times 10^{-4}$ mM), and LLr ($1.03 \pm 0.2 \times 10^{-4}$ mM) thylakoids, no significant variations of TBA-MDA content were found.

Lipid phase fluidity of thylakoid membranes: The values of $F_{560/528}$ parameter determined for control ML and LL thylakoids were very close, 1.40 ± 0.10 and 1.30 ± 0.10 , respectively. Desiccation of pea plants led to about 30% decrease in $F_{560/528}$ in both MLd (0.80 ± 0.08) and LLd (0.81 ± 0.09) samples. Rehydration of plants resulted in substantial recovery of the values of $F_{560/528}$ parameter (1.20 ± 0.08 for MLd and 1.21 ± 0.08 for LLd), back to that characteristic for untreated ML and LL thylakoids.

Thermodynamic stability of thylakoid membranes: Similarly to our previous results (Petrova *et al.* 2019), both ML and LL control thylakoids exhibited eight endothermic calorimetric transitions located at 56, 59, 62, 65, 68, 71–72, 82, and 88°C (Fig. 1). As discussed in our previous papers, the first thermal transition, observed only as a shoulder at *ca.* 56°C in ML and LL samples, is related to the heat induced impairment of the lateral and vertical

arrangement of the photosynthetic complexes (Dobrikova *et al.* 2003, Petrova *et al.* 2018, 2019), while the transition that reflects LHClI denaturation occurred at 73°C for ML and at 72°C for LL thylakoids (Petrova *et al.* 2019). The thermodynamic profiles of the drought-stressed samples were characterized with the appearance of a clear endothermic event at around 40°C that was missing in the untreated thylakoids (Fig. 1). It was most probably a result of the down-shift of the thermodynamic transition occurring at around 56°C in untreated thylakoids; the amplitude (*i.e.*, c_p^{ex}) of this transition was twice more pronounced for MLd (Fig. 1A) than that for LLd (Fig. 1B) thylakoids. The last thermal transition occurred at 2°C lower temperature in MLd than that in ML samples, while it did not shift significantly for LL thylakoids subjected to drought stress (Fig. 1).

The MLr thermograms exhibited similar features as thylakoids isolated from control ML plants with the exception of significant increase in c_p^{ex} in the temperature range between 60 and 70°C; the last thermal transition also did not revert to its original position (Fig. 1A). The thermodynamic profile of LLr thylakoids did not differ from the untreated control between 55 and 75°C, but exhibited lower c_p^{ex} at 56°C and down-shifted last thermal transition (Fig. 1B). In similarity to the characteristic difference in the transition temperatures of the LHClI assigned transition in untreated ML and LL plants (Petrova *et al.* 2019), the drought-stressed and rehydrated LL samples always exhibited LHClI denaturation temperature lower by *ca.* 1°C than the respective ML samples (Fig. 1).

Circular dichroism spectra of thylakoids: The averaged CD spectra of untreated, drought-stressed, and rehydrated ML and LL thylakoids are presented in Fig. 2. The intensities of the CD bands (+)690 nm and (+)506 nm in MLd sample significantly increased (by 56 and 67%, respectively; Table 1) in comparison to the values observed for ML thylakoids. The excitonic band (+)483 nm/(−)473 nm was

significantly higher for MLd (by 46%; Fig. 2A, Table 1) as compared to ML sample. The CD bands located at (–)657 nm, (–)679 nm, and the band pair (+)444/(–)436 nm were not affected by drought stress under ML conditions (Fig. 2A). None of the CD bands were significantly affected by dehydration of LL-adapted plants (Fig. 2B, Table 1).

Fig. 1. Averaged differential scanning calorimetry profiles of thylakoids isolated from moderate (ML) (A) and low (LL) (B) light-grown pea plants; untreated controls (solid lines; ML, LL), drought-stressed (dash dotted lines; MLd, LLd), and rehydrated (dotted lines; MLr, LLr) samples. SE are plotted for selected excess heat capacity (c_p^{ex}) values.

Rehydration of both MLd and LLd plants was associated with only insignificant variations in the intensity of the excitonic and ψ -type CD bands in MLr and LLr variants with respect to the untreated ones (Fig. 2, Table 1).

JIP-test: The original OJIP curves of ML and LL leaves are compared in Fig. 1S (supplement) and the normalized curves are presented in Fig. 3. The two drought-stressed variants showed a significant increase in F_0 and decrease in F_m as compared to the respective untreated controls, while the rehydrated samples demonstrated the significant overall increase in the fluorescence intensity (Table 2, Fig. 1S).

Whilst the shape of the fluorescence induction curves of MLd and LLd had only slight alterations (Fig. 3), as compared to the respective controls, the ones of MLr and LLr variants differed prominently from their controls demonstrating a higher rate of fluorescence increase in the time course of reaching the I and P points, respectively (Fig. 3).

In order to obtain further insight into the efficiency of photosynthetic energy transduction and electron transport in the drought-stressed and rehydrated plants, we selected JIP parameters (see Materials and methods) that illustrate how the stress applied affected the photosynthetic function of the pea plants at different levels starting from the ability of PSII to absorb photosynthetically active light, exploring the probability of trapping of light energy, and finally probing its potential utilization for electron transport. The evaluated JIP parameters for the two light conditions are compared in Fig. 4. MLd plants were characterized with significant decrease in Φ_{P_0} , Ψ_{E_0} , γ_{RC} , PI_{ABS} , and PI_{total} parameters which was accompanied by significantly higher values for N and TR_0/RC in comparison to the control ML leaves (Fig. 4, Table 3). Similar tendencies were observed for the LLd leaves as compared to LL ones (with the exception of Ψ_{E_0} and TR_0/RC parameters which did not differ significantly for LL and LLd samples); although significant, the effects were less pronounced than that in

Fig. 2. Averaged circular dichroism spectra of thylakoids isolated from moderate (ML) (A) and low (LL) (B) light grown pea plants; untreated controls (solid line; ML, LL), drought-stressed (dash dotted line; MLd, LLd), and rehydrated (dotted line; MLr, LLr) samples. Spectra are recorded at 25°C after 5-min incubation. SE are plotted for intensity values at selected wavelengths.

Table 1. Intensity of selected circular dichroism bands recorded for thylakoid membranes isolated from untreated (ML, LL), dehydrated (MLd, LLd), and rehydrated (MLr, LLr) plants acclimated to moderate (ML) and low light (LL) intensity, respectively. * – significant difference at the level of $\alpha=0.05$ according to *Welch's t*-test. Values are means and (SE), $n = 4$.

Sample	(+)694 nm	(-)679 nm	(+)506 nm	(+)483/(-)473 nm	(+)444/(-)436 nm	(-)657 nm
ML	120.3 (5.89)	37.94 (1.87)	38.00 (3.26)	19.92 (1.07)	21.03 (1.07)	18.32 (1.69)
MLd	187.59* (4.58)	42.16 (4.90)	63.30* (4.39)	29.11* (1.47)	27.09 (4.62)	20.95 (0.67)
MLr	143.55 (7.38)	29.09 (5.42)	45.58 (2.22)	26.10 (1.62)	25.67 (0.92)	15.86 (0.44)
LL	147.45 (26.62)	29.96 (3.82)	49.60 (9.86)	25.88 (5.12)	25.57 (4.04)	19.37 (2.02)
LLd	159.83 (4.10)	41.57 (10.52)	52.08 (3.57)	25.84 (1.81)	26.46 (3.08)	23.09 (1.67)
LLr	157.18 (12.00)	39.97 (12.34)	51.74 (6.27)	29.02 (0.90)	27.93 (1.14)	20.63 (3.27)

Table 2. Selected JIP-test parameters of intact leaves of control plants grown at moderate (ML) and low (LL) light intensity and their dehydrated (MLd, LLd) and rehydrated (MLr, LLr) variants: F_0 – minimal fluorescence intensity in dark-adapted state; F_m – maximal fluorescence intensity in dark-adapted state; N – turnover number of Q_A reduction and reoxidation acts; γ_{RC} – probability that a chlorophyll *a* molecule located in PSII serves as a reaction center; TR_0/RC – excitation energy trapped by an active PSII reaction center immediately after illumination; ϕ_{Po} – maximum quantum yield of the primary photochemical reaction in PSII; ψ_{Eo} – efficiency of electron transport after Q_A ; δ_{Ro} – probability that an electron residing in the intersystem electron transport chain would reach the terminal electron acceptors at the acceptor side of PSI; PI_{ABS} – PSII performance index; PI_{total} – overall functional activity of PSII, PSI, and the intersystem electron carriers. * – significantly different values with respect to control pea plants (ML and LL, respectively) according to *Welch's t*-test, $\alpha=0.05$. Values are means and (SE), $n = 15$.

Sample	F_0	F_m	N	γ_{RC}	TR_0/RC	ϕ_{Po}	ψ_{Eo}	δ_{Ro}	PI_{ABS}	PI_{total}
ML	11,543 (102)	50,887 (333)	35.45 (0.57)	0.33 (0.00)	1.57 (0.02)	0.77 (0.00)	0.59 (0.00)	0.30 (0.00)	2.47 (0.08)	1.15 (0.04)
MLd	14,579* (594)	47,819* (718)	44.72* (0.72)	0.26* (0.01)	2.02* (0.05)	0.70* (0.01)	0.56* (0.01)	0.30 (0.01)	1.17* (0.12)	0.52* (0.06)
MLr	19,362* (553)	58,398* (622)	33.31* (1.00)	0.22* (0.00)	2.41* (0.04)	0.67* (0.01)	0.50* (0.01)	0.30 (0.01)	1.28* (0.11)	0.29* (0.03)
LL	12,292 (171)	48,163 (259)	27.29 (0.33)	0.31 (0.01)	1.80 (0.02)	0.75 (0.00)	0.55 (0.00)	0.28 (0.00)	1.60 (0.06)	0.70 (0.03)
LLd	13,727* (570)	40,505* (659)	35.96* (1.29)	0.27* (0.00)	1.85 (0.05)	0.66* (0.01)	0.54 (0.00)	0.27 (0.01)	0.99* (0.09)	0.45* (0.03)
LLr	19,670* (607)	52,915* (1005)	34.74* (1.05)	0.20* (0.01)	2.26* (0.04)	0.68* (0.00)	0.46* (0.01)	0.22* (0.01)	0.38* (0.05)	0.22* (0.02)

plants grown at ML, δ_{Ro} proved to be insensitive to the applied drought stress for both ML- and LL-adapted plants.

Upon rehydration of the water-stressed ML plants, N parameter reverted to values close and even significantly lower than that of the control ones. The parameters γ_{RC} , TR_0/RC , ϕ_{Po} , ψ_{Eo} , and PI_{total} further deviated from the values determined for ML leaves. PI_{ABS} was not significantly affected by the rehydration of MLd leaves (Fig. 4, Table 2). On the other hand, under LL conditions, N and ϕ_{Po} parameters did not substantially change after rehydration, while the values of γ_{RC} , TR_0/RC , ψ_{Eo} , δ_{Ro} , PI_{ABS} , and PI_{total} further deviated from the control LL plants (Fig. 4, Table 2).

Discussion

This work aimed to evaluate the role of the structural

organization of the photosynthetic apparatus for its reaction towards drought stress and consequent rehydration. For this purpose, we explored the changes in the structural and functional characteristics of the photosynthetic membranes in moderate- and low-light-adapted pea plants subjected to water deficit and their ability to recover upon rehydration.

Drought stress response of thylakoids: As a first step in this study, we showed that lipid peroxidation does not play a major role in the induced drought stress and the consequent structural and functional changes since the concentration of the TBA-MDA adduct in thylakoid suspensions remained very low (at most 3×10^{-4} mM) and was not affected upon subjecting the plants to drought-stress conditions and the following rehydration period.

Our results revealed that both ML- and LL-adapted

Fig. 3. Representative double-normalized JIP curves of intact leaves from moderate (ML) (A) and low (LL) (B) light grown pea plants; untreated controls (*square*; ML, LL), drought-stressed (*circle*; MLd, LLd), and rehydrated (*triangle*; MLr, LLr) samples.

Fig. 4. Spider plots of selected JIP parameters determined for moderate (ML) (A) and low (LL) (B) light grown pea plants; untreated controls (*square*; ML, LL), drought-stressed (*circle*; MLd, LLd), and rehydrated (*triangle*; MLr, LLr) samples. N – turnover number of Q_A reduction and reoxidation acts; γ_{RC} – probability that a chlorophyll *a* molecule located in PSII serves as a reaction center; TR_0/RC – excitation energy trapped by an active PSII reaction center immediately after illumination; ϕ_{P_0} – maximum quantum yield of the primary photochemical reaction in PSII; ψ_{E_0} – efficiency of electron transport after Q_A ; δ_{R_0} – probability that an electron residing in the intersystem electron transport chain would reach the terminal electron acceptors at the acceptor side of PSI; PI_{ABS} – PSII performance index; PI_{total} – overall functional activity of PSII, PSI, and the intersystem electron carriers.

plants react similarly to water stress caused by cultivation in PEG medium. However, the two types of samples exhibited different response towards drought stress in terms of rearrangement of the photosynthetic complexes reflected in specific CD bands (Fig. 2), excess heat capacity of the first calorimetric transition (Fig. 1), and photosynthetic activity probed by JIP-test (Figs. 3, 4) pointing towards better resistance of LL- than ML-grown plants.

The CD spectra of thylakoid membranes are dominated by anomalously shaped ψ -type bands which extend outside the absorbance bands, a feature found to be typical for various plant species (Tóth *et al.* 2016). The variations in the intensity of these bands are typically used to evaluate the 3D macroorganization of the PSII-LHCII supercomplexes in the grana membranes (Tóth *et al.* 2016, Kotakis *et al.* 2018). It has been shown that (+)506 nm and (+)690 nm bands (that are due to Chl *a* and carotenoids in PSII core

complex and LHCII complex, respectively) reflect the arrangement of PSII-LHCII supercomplexes (Tóth *et al.* 2016), while the (–)679 nm band is affected by stacking of the grana (Cseh *et al.* 2000, Dobrikova *et al.* 2003). The excitonic CD bands (+)444 nm/436 nm and (–)657 nm in the thylakoids CD spectra result from short-range pigment interactions within the LHCII monomers and the (+)483 nm/(–)473 nm band is characteristic for the Chl *a* excitonic interactions between LHCII complexes comprising a trimer (Hobe *et al.* 1994, Garab *et al.* 2002, Akhtar *et al.* 2015). Thus, the significant increase of the (+)690 nm and (+)506 nm CD bands and the band pair (+)483 nm/(–)473 nm recorded by us for MLd thylakoids (Fig. 2A) points that dehydration of ML-adapted pea plants brings about rearrangements in the thylakoid membranes affecting both LHCII trimers and PSII-LHCII supercomplexes, leaving the LHCII monomers conformation unaffected.

Our DSC data confirm that LHCII monomer denaturation temperature was not affected by the drought stress, but the lateral order of the membrane was strongly destabilized (reflected in 15°C downshift of the transition temperature of the first endothermic event in MLd thylakoids; Fig. 1A).

In contrast, LLd membranes exhibited nonsignificant changes in the intensity of (+)690 nm, (+)506 nm, and (+)483/(−)473 nm CD bands (Fig. 2B) demonstrating that the macroorganization of the photosynthetic complexes and LHCII conformation in LL-grown plants subjected to drought stress was not dramatically affected under our experimental conditions. In line with this data, the thermal scanning of LLd thylakoids resulted in the much smaller shift of the 57°C shoulder and lower c_p^{ex} of the resulting transition at 40°C than that in MLd samples; again the position of the LHCII assigned transition at 72°C for LL and LLd samples was identical (Fig. 1).

Importantly, in nature, desiccation most often occurs in combination with high air temperatures. Under these conditions, due to restricted transpiration, plants can barely keep temperatures lower than that of the environment (Hirayama *et al.* 2006, Blum 2015, Zhou *et al.* 2015). Depending on the PAR intensity, in combined drought and heat stress, the leaf temperature might reach as high as 39°C (Reynolds-Henne *et al.* 2010). Thus, the reorganization of the 3D thylakoid architecture observed in our DSC experiments of drought-stressed pea plants can be expected to occur *in vivo* in plants exposed to combined heat and water stress in the nature.

Incorporation of the fluorescence probe MC540 into thylakoid membranes markedly decreased within the lipid matrix of both MLd and LLd thylakoids. Navari-Izzo *et al.* (1995, 2000) and Quartacci *et al.* (1995) reported on increased unsaturation of thylakoid membranes fatty acids under drought stress and concluded that membrane fluidity is among the factors that determine the ability of drought-resistant and resurrecting plants to survive drought stress. The effect observed here reports on changes in the lipid packing of the thylakoid lipid matrix that might serve to counteract the reorganization of the photosynthetic complexes occurring upon drought stress in higher plants in order to preserve the membrane integrity. Alternatively, it might be a result of obstructed incorporation of MC540 into the membrane due to changes in the extent of membrane stacking, rearrangement of proteins (as discussed above), exposure or screening of surface charges [as commented in Petrova *et al.* (2018)]. Giardi *et al.* (1996) reported on enhanced PSII core proteins degradation in pea plants under water-deficient conditions. According to Chen *et al.* (2016), dehydration of *Arabidopsis thaliana* provokes reduction of grana membranes stacking, disassembly of the PSII-LHCII supercomplexes and increase in the content of monomeric LHCII which is followed by PSII core disintegration. All these processes are most probably associated with altered physical state of the lipid phase; therefore future in-depth studies on the membrane fluidity under different extent of membrane hydration level would provide valuable information on the relation between the stress response and the lipid phase behavior.

Numerous JIP analyses of drought-stressed plants have

identified the most sensitive parameters that demonstrate the level at which dehydration damages the photosynthetic apparatus and the mechanisms providing the ability of plants to recover/resurrect (reviewed in Kalaji *et al.* 2016). Drought is known to affect the OJIP curve of higher plants by enhancing the K- and L-peaks, the former indicating deactivation or destabilization of the oxygen-evolving complex and the latter pointing to an increased functional connectivity between the PSII reaction centres (Oukarroum *et al.* 2007, Kalaji *et al.* 2016). PI_{ABS} and PI_{total} are integrative JIP parameters which were found to decrease upon drought stress (Oukarroum *et al.* 2007, 2018; Goltsev *et al.* 2012, Kalaji *et al.* 2018). Being a product of γ_{RC} , Φ_{Po} , and Ψ_{Eo} , PI_{ABS} is affected by the absorption per PSII reaction centre, the primary photochemistry in PSII, and the electron transport beyond Q_A^- (Strasser *et al.* 2004). In MLd the double decrease of PI_{ABS} can be explained by drought-provoked changes in γ_{RC} , Φ_{Po} , and Ψ_{Eo} . However, in LLd only γ_{RC} and Φ_{Po} parameters were lower and PI_{ABS} was reduced 1.6 times as compared to LL. These data suggest significantly better preserved photosynthetic electron transport in LL-adapted plants subjected to drought stress with respect to ML-adapted ones. (Table 2). PI_{total} , reflecting the overall activity of PSII and PSI, is the product of PI_{ABS} and δ_{Ro} (Strasser *et al.* 2004). In our experimental conditions, δ_{Ro} , which is parameter sensitive to the efficiency of reduction of the PSI end acceptors, was not affected neither in MLd nor in LLd samples. Oukarroum *et al.* (2018) report that the δ_{Ro} parameter is affected the least in a lichen species subjected to drought. Therefore, the decrease of PI_{total} in both MLd and LLd plants must be due to lowering of PSII efficiency that was more pronounced for MLd than that for LLd leaves. In previous works (Strasser *et al.* 2010, Goltsev *et al.* 2012, Kalaji *et al.* 2018), the decrease in TR_0/RC in drought-stressed plants is explained in terms of partial inactivation of PSII reaction centres. In our experiments, TR_0/RC , however, significantly increased in MLd and was not affected in LLd leaves (Fig. 4, Table 2). We can attribute the putative inactivation of PSII reaction centres in MLd to the rearrangement of photosynthetic complexes accompanied by an altered thylakoid membrane lipid packing. Altogether the JIP data revealed that LLd samples preserved better PSII functionality than that of MLd ones.

Rehydration response: The structural organization and stability of rehydrated ML and LL thylakoids showed significant recovery after the drought stress. While LLr thylakoids resembled closely the LL ones with respect to their CD and DSC features (Figs. 1, 2), the MLr samples differed dramatically from their respective controls in the 60–70°C temperature range (Fig. 1). Therefore, it appears that LL thylakoids are more resistant and thus adaptable to drought stress and consequent rehydration, while ML thylakoids respond to these stress conditions by structural changes that, however, are largely reversible – the rehydrated membranes were able to restore their fluidity, protein arrangement, and stacking under our experimental conditions.

Interestingly, the rehydration did not result in significant

reversibility of the functional JIP parameters (Figs. 3, 4; Table 2), strongly suggesting that the recovery of the functionality of the photosynthetic apparatus requires longer times than that of its structural organization.

Conclusions: The presented data demonstrate that the adjustment of the structural organization of thylakoid membranes and the consequent functional alterations upon growth at low light affects the response of pea plants to drought stress. LL-adapted plants appear to be structurally and functionally more resistant to water deficit than ML ones, most probably because the mechanisms of adaptation to low light intensity provided partial protection of PSII complexes in conditions of dehydration.

References

- Akhtar P., Dorogi M., Pawlak K. *et al.*: Pigment interactions in light-harvesting complex II in different molecular environments. – *J. Biol. Chem.* **290**: 4877-4886, 2015.
- Anderson J.M.: Photoregulation of the composition, function, and structure of thylakoid membranes. – *Ann. Rev. Plant Physiol.* **37**: 93-136, 1986.
- Anderson J.M.: Changing concepts about the distribution of Photosystems I and II between grana-appressed and stroma-exposed thylakoid membranes. – In: Govindjee, Beatty J.T., Gest H., Allen J.F. (ed.): *Discoveries in Photosynthesis. Advances in Photosynthesis and Respiration*. Vol. 20. Pp. 729-736. Springer, Dordrecht 2002.
- Anderson J.M., Chow W.S., Goodchild D.J.: Thylakoid membrane organization in sun/shade acclimation. – *Aust. J. Plant Physiol.* **15**: 11-26, 1988.
- Austin J.R., Staehelin L.A.: Three-dimensional architecture of grana and stroma thylakoids of higher plants as determined by electron tomography. – *Plant Physiol.* **155**: 1601-1611, 2011.
- Bailey S., Walters R.G., Jansson S., Horton P.: Acclimation of *Arabidopsis thaliana* to the light environment: the existence of separate low light and high light responses. – *Planta* **213**: 794-801, 2001.
- Ballottari M., Dall'Osto L., Morosinotto T., Bassi R.: Contrasting behavior of higher plant photosystem I and II antenna systems during acclimation. – *J. Biol. Chem.* **282**: 8947-8958, 2007.
- Benešová M., Holá D., Fischer L. *et al.*: The physiology and proteomics of drought tolerance in maize: early stomatal closure as a cause of lower tolerance to short-term dehydration? – *PLoS ONE* **7**: e38017, 2012.
- Blum A.: Towards a conceptual ABA ideotype in plant breeding for water limited environments. – *Funct. Plant Biol.* **42**: 502-513, 2015.
- Chen Y.E., Liu W.J., Su Y.Q. *et al.*: Different response of photosystem II to short and long-term drought stress in *Arabidopsis thaliana*. – *Physiol. Plantarum* **158**: 225-235, 2016.
- Crowe J.H., Crowe L.M., Carpenter J.E. *et al.*: Anhydrobiosis: Cellular adaptation to extreme dehydration. – *Compr. Physiol.* **30**: 1445-1477, 2011.
- Cseh Z., Rajagopal S., Tsonev T. *et al.*: Thermooptic effect in chloroplast thylakoid membranes. Thermal and light stability of pigment arrays with different levels of structural complexity. – *Biochemistry* **39**: 15250-15257, 2000.
- Danielsson R., Albertsson P.Å., Mamedov F., Styring S.: Quantification of photosystem I and II in different parts of the thylakoid membrane from spinach. – *BBA-Bioenergetics* **1608**: 53-61, 2004.
- Dobrikova A.G., Várkonyi Z., Krumova S.B. *et al.*: Structural rearrangements in chloroplast thylakoid membranes revealed by differential scanning calorimetry and circular dichroism spectroscopy. Thermo-optic effect. – *Biochemistry* **42**: 11272-11280, 2003.
- Filek M., Łabanowska M., Kurdziel M. *et al.*: Structural and biochemical response of chloroplasts in tolerant and sensitive barley genotypes to drought stress. – *J. Plant Physiol.* **207**: 61-72, 2016.
- Furuki T., Shimizu T., Chakrabortee S. *et al.*: Effects of Group 3 LEA protein model peptides on desiccation-induced protein aggregation. – *BBA-Proteins Proteom.* **1824**: 891-897, 2012.
- Gaetano J.: Welch's t-test for comparing two independent groups: An Excel calculator (1.0.1). Available at: https://www.researchgate.net/publication/332217175_Welch's_t-test_for_comparing_two_independent_groups_An_Excel_calculator_101, 2019.
- Garab G., Cseh Z., Kovács L. *et al.*: Light-induced trimer to monomer transition in the main light-harvesting antenna complex of plants: Thermo-optic mechanism. – *Biochemistry* **41**: 15121-15129, 2002.
- Giardi M.T., Cona A., Geiken B. *et al.*: Long-term drought stress induces structural and functional reorganization of photosystem II. – *Planta* **199**: 118-125, 1996.
- Goltsev V., Zaharieva I., Chernev P. *et al.*: Drought-induced modifications of photosynthetic electron transport in intact leaves: Analysis and use of neural networks as a tool for a rapid non-invasive estimation. – *BBA-Bioenergetics* **1817**: 1490-1498, 2012.
- Hall D.: Protein self-association in the cell: A mechanism for fine tuning the level of macromolecular crowding? – *Eur. Biophys. J.* **35**: 276-280, 2006.
- Hirayama M., Wada Y., Nemoto H.: Estimation of drought tolerance based on leaf temperature in upland rice breeding. – *Breeding Sci.* **56**: 47-54, 2006.
- Hobe S., Prytulla S., Kühlbrandt W., Paulsen H.: Trimerization and crystallization of reconstituted light-harvesting chlorophyll *a/b* complex. – *EMBO J.* **13**: 3423-3429, 1994.
- Kalaji H.M., Jajoo A., Oukarroum A. *et al.*: Chlorophyll *a* fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. – *Acta Physiol. Plant.* **38**: 102, 2016.
- Kalaji H.M., Račková L., Paganová V. *et al.*: Can chlorophyll-*a* fluorescence parameters be used as bio-indicators to distinguish between drought and salinity stress in *Tilia cordata* Mill? – *Environ. Exp. Bot.* **152**: 149-157, 2018.
- Kirchhoff H., Haase W., Wegner S. *et al.*: Low-light-induced formation of semicrystalline photosystem II arrays in higher plant chloroplasts. – *Biochemistry* **46**: 11169-11176, 2007.
- Kotakis C., Akhtar P., Zsiros O. *et al.*: Increased thermal stability of photosystem II and the macro-organization of thylakoid membranes, induced by co-solutes, associated with changes in the lipid-phase behaviour of thylakoid membranes. – *Photosynthetica* **56**: 254-264, 2018.
- Kouřil R., Wientjes E., Bultema J.B. *et al.*: High-light vs. low-light: Effect of light acclimation on photosystem II composition and organization in *Arabidopsis thaliana*. – *BBA-Bioenergetics* **1827**: 411-419, 2013.
- Krumova S.B., Koehorst R.B., Bóta A. *et al.*: Temperature dependence of the lipid packing in thylakoid membranes studied by time- and spectrally resolved fluorescence of Merocyanine 540. – *BBA-Biomembranes* **1778**: 2823-2833, 2008.
- Kuznetsova I., Turoverov K., Uversky V.: What macromolecular crowding can do to a protein. – *Int. J. Mol. Sci.* **15**: 23090-

- 23140, 2014.
- Lauriano J.A., Lidon F.C., Carvalho C.A. *et al.*: Drought effects on membrane lipids and photosynthetic activity in different peanut cultivars. – *Photosynthetica* **38**: 7-12, 2000.
- Lichtenthaler H.K., Kuhn G., Prenzel U., Meier D.: Chlorophyll-protein levels and degree of thylakoid stacking in radish chloroplasts from high-light, low-light and bentazon-treated plants. – *Physiol. Plantarum* **56**: 183-188, 1982.
- Lindahl M., Yang D.-H., Andersson B.: Regulatory proteolysis of the major light-harvesting chlorophyll *a/b* protein of photosystem II by a light-induced membrane-associated enzymic system. – *Eur. J. Biochem.* **231**: 503-509, 1995.
- Liu W., Sun F., Lim W.H. *et al.*: Global drought and severe drought-affected populations in 1.5 and 2°C warmer worlds. – *Earth Syst. Dynam.* **9**: 267-283, 2018.
- Markonis Y., Hanel M., Máca P. *et al.*: Persistent multi-scale fluctuations shift European hydroclimate to its millennial boundaries. – *Nat. Commun.* **9**: 1767, 2018.
- Martin-StPaul N., Delzon S., Cochard H.: Plant resistance to drought depends on timely stomatal closure. – *Ecol. Lett.* **20**: 1437-1447, 2017.
- Mishra R.K., Singhal G.S.: Function of photosynthetic apparatus of intact wheat leaves under high light and heat stress and its relationship with peroxidation of thylakoid lipids. – *Plant Physiol.* **98**: 1-6, 1992.
- Morandi M.I., Sommer M., Kluzek M. *et al.*: DPPC bilayers in solutions of high sucrose content. – *Biophys. J.* **114**: 2165-2173, 2018.
- Navari-Izzo F., Ricci F., Vazzana C., Quartacci M.F.: Unusual composition of thylakoid membranes of the resurrection plant *Boea hygroskopica*: Changes in lipids upon dehydration and rehydration. – *Physiol. Plantarum* **94**: 135-142, 1995.
- Navari-Izzo F., Quartacci M.F., Pinzino C. *et al.*: Protein dynamics in thylakoids of the desiccation-tolerant plant *Boea hygroskopica* during dehydration and rehydration. – *Plant Physiol.* **124**: 1427-1436, 2000.
- Oukarroum A., El Madidi S., Schansker G., Strasser R.J.: Probing the responses of barley cultivars (*Hordeum vulgare* L.) by chlorophyll *a* fluorescence OLKJIP under drought stress and re-watering. – *Environ. Exp. Bot.* **60**: 438-446, 2007.
- Oukarroum A., Lebrhi A., El Gharous M. *et al.*: Desiccation-induced changes of photosynthetic transport in *Parmelina tiliacea* (Hoffm.) Ach. analysed by simultaneous measurements of the kinetics of prompt fluorescence, delayed fluorescence and modulated 820 nm reflection. – *J. Lumin.* **198**: 302-308, 2018.
- Petrova N., Stoichev S., Paunov M. *et al.*: Structural organization, thermal stability and excitation energy utilization of pea thylakoid membranes adapted to low light conditions. – *Acta Physiol. Plant.* **41**: 188, 2019.
- Petrova N., Todinova S., Paunov M. *et al.*: Thylakoid membrane unstacking increases LHCII thermal stability and lipid phase fluidity. – *J. Bioenerg. Biomembr.* **50**: 425-435, 2018.
- Quartacci M.F., Pinho C., Sgherri C.L.M., Navari-Izzo F.: Lipid composition and protein dynamics in thylakoids of two wheat cultivars differently sensitive to drought. – *Plant Physiol.* **108**: 191-197, 1995.
- Reynolds-Henne C.E., Langenegger A., Mani J. *et al.*: Interactions between temperature, drought and stomatal opening in legumes. – *Environ. Exp. Bot.* **68**: 37-43, 2010.
- Sarkar M., Pielak G.J.: An osmolyte mitigates the destabilizing effect of protein crowding. – *Protein Sci.* **23**: 1161-1164, 2014.
- Seiwert D., Witt H., Janshoff A., Paulsen H.: The non-bilayer lipid MGDG stabilizes the major light-harvesting complex (LHCII) against unfolding. – *Sci. Rep.-UK* **7**: 5158, 2017.
- Shao R.X., Xin L.F., Zheng H.F. *et al.*: Changes in chloroplast ultrastructure in leaves of drought-stressed maize inbred lines. – *Photosynthetica* **54**: 74-80, 2016.
- Sheng X., Liu X., Cao P. *et al.*: Structural roles of lipid molecules in the assembly of plant PSII-LHCII supercomplex. – *Biophys. Rep.* **4**: 189-203, 2018.
- Strasser R.J., Tsimilli-Michael M., Qiang S., Goltsev V.: Simultaneous *in vivo* recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*. – *BBA-Bioenergetics* **1797**: 1313-1326, 2010.
- Strasser R.J., Tsimilli-Michael M., Srivastava A.: Analysis of the chlorophyll *a* fluorescence transient. – In: Papageorgiou G.C., Govindjee (ed.): *Chlorophyll *a* Fluorescence: A Signature of Photosynthesis*. Advances in Photosynthesis and Respiration. Pp. 321-362. Springer, Dordrecht 2004.
- Tombesi S., Nardini A., Frioni T. *et al.*: Stomatal closure is induced by hydraulic signals and maintained by ABA in drought-stressed grapevine. – *Sci. Rep.-UK* **5**: 12449, 2015.
- Torres-Franklin M.L., Gigon A., De Melo D.F. *et al.*: Drought stress and rehydration affect the balance between MGDG and DGDG synthesis in cowpea leaves. – *Physiol. Plantarum* **131**: 201-210, 2007.
- Tóth T.N., Rai N., Solymosi K. *et al.*: Fingerprinting the macro-organisation of pigment-protein complexes in plant thylakoid membranes *in vivo* by circular-dichroism spectroscopy. – *BBA-Bioenergetics* **1857**: 1479-1489, 2016.
- van der Weij-de Wit C.D., Ihalainen J.A., van Grondelle R., Dekker J.P.: Excitation energy transfer in native and unstacked thylakoid membranes studied by low temperature and ultrafast fluorescence spectroscopy. – *Photosynth. Res.* **93**: 173-182, 2007.
- Wang Q., Wu J., Lei T. *et al.*: Temporal-spatial characteristics of severe drought events and their impact on agriculture on a global scale. – *Quatern. Int.* **349**: 10-21, 2014.
- Wang Z., Li G., Sun H. *et al.*: Effects of drought stress on photosynthesis and photosynthetic electron transport chain in young apple tree leaves. – *Biol. Open* **7**: bio035279, 2018.
- Yamakawa H., van Stokkum I.H., Heber U., Itoh S.: Mechanisms of drought-induced dissipation of excitation energy in sun- and shade-adapted drought-tolerant mosses studied by fluorescence yield change and global and target analysis of fluorescence decay kinetics. – *Photosynth. Res.* **135**: 285-298, 2018.
- Zhang F.J., Zhang K.K., Du C.Z. *et al.*: Effect of drought stress on anatomical structure and chloroplast ultrastructure in leaves of sugarcane. – *Sugar Tech.* **17**: 41-48, 2015.
- Zhou R., Kan X., Chen J. *et al.*: Drought-induced changes in photosynthetic electron transport in maize probed by prompt fluorescence, delayed fluorescence, P700 and cyclic electron flow signals. – *Environ. Exp. Bot.* **158**: 51-62, 2019.
- Zhou R., Yu X., Kjær K.H. *et al.*: Screening and validation of tomato genotypes under heat stress using F_v/F_m to reveal the physiological mechanism of heat tolerance. – *Environ. Exp. Bot.* **118**: 1-11, 2015.