Journal of Luminescence 198 (2018) 302–308

Contents lists available at ScienceDirect

Journal of Luminescence

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

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

Abdallah Oukarroum[a,*], Ahmed Lebrihi[a], Mohamed El Gharous[b], Vasilij Goltsev[c], Reto J. Strasser[d,e]

[a] AgroBioSciences, University Mohammed VI Polytechnic (UM6P), Lot-660 Hay Moulay Rachid, 43150 Ben Guerir, Morocco
[b] University Mohammed VI Polytechnic (UM6P), Lot-660 Hay Moulay Rachid, 43150 Ben Guerir, Morocco
[c] Department of Biophysics and Radiobiology, Faculty of Biology, St. Kliment Ohridski University of Sofia, Sofia, Bulgaria
[d] Bioenergetics and Microbiology laboratory, University of Geneva, CH-1254 Jussy-Geneva, Switzerland
[e] North West University South Africa, Potchefstroom, North-West Province, South Africa

ARTICLE INFO

Keywords:
Desiccation
JIP-test
Photosystem I
Photosystem II
Poikilohydric organisms
Photoprotection

ABSTRACT

Simultaneous *in vivo* measurements of prompt fluorescence (PF), delayed fluorescence (DF) and modulated reflection (MR) at 820-nm were used to assess effect of short desiccation period (4.5 h) on *Parmelina tiliacea* lichen. The two performance indexes (PI_{ABS} and PI_{total}) as a measure of an overall photosynthetic thalli performance showed a negative effect of desiccation treatment on photosynthetic activity. The maximal intensity of PF and DF recorded during desiccation treatment decreased and at 4.5 h desiccation time *Parmelina tiliacea* thalli loss their variable fluorescence and DF amplitude. This loss of variable fluorescence was due to an increase in inactive reaction centers and a limitation of electron donation on the donor side of photosystem II (PSII) that caused a down-regulation of electron transport chain at the PSII level. However, the efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors was less affect by desiccation treatment. In respect to MR change, re-reduction kinetics of the primary electron donors of photosystems I (P700) and plastocyanin (PC) in desiccated thalli seem to be faster, their amplitudes gradually decrease and a disconnection between the two photosystem (PSII and PSI) were observed. These responses allow to *Parmelina tiliacea* lichen a photoprotection mechanism from the excess light excitation.

1. Introduction

In photosynthesis water molecules are the electron donors for the photosynthetic electron transport chain and water limitation affect negatively the photosynthetic activity. To cope with the scarcity of water, poikilohydric organisms such as few angiosperm plants (resurrection plants) and a large number of mosses and lichens tolerate complete desiccation [1–5]. Tolerant organisms to desiccation were defined by Gaff [6] as organisms able to equilibrate their water content with environmental water in the air and then are able to reach their photosynthetic activity following re-watering. Desiccation induces a series of changes within plant cells such as movement of chloroplasts from a peripheral to a central location [7], loss of their thylakoidal structure and grana stacking [8], immobilisation of the cytoplasm in a stable multicomponent glassy matrix and partitioning of amphiphilic compounds in and around the membranes [9]. Tuba et al. [10] and Georgieva et al. [2] pointed out that desiccation tolerance can be considered as a strategy of drought avoidance in poikilohydric organisms.

In lichen, desiccation-induced loss of light-dependent charge separation and provokes conformational changes of a chlorophyll protein complex are considered as an effective mechanism of photoprotection [11]. The fact that lichens do not possess stomata as in the higher plants, they cannot regulate their evaporation [12]. However, dry state allows to lichen to maintain the overall photosynthetic activity [13,14].

In this work, we studied *Parmelina tiliacea* (Hoffm.) Ach. lichen (*P.*

Abbreviations: ABS, absorption; O-J-I-P-S-M, transient fluorescence induction transient defined by the names of its intermediate steps; P700, the primary electron donor of photosystems I; PS II, photosystem II; Q_A and Q_B, primary and secondary quinone electron acceptors of photosystem II, respectively; RC, reaction center

* Corresponding author.
E-mail addresses: abdallah.oukarroum@um6p.ma, abdallah.oukarroum@gmail.com (A. Oukarroum).

https://doi.org/10.1016/j.jlumin.2018.02.040
Received 24 July 2017; Received in revised form 26 January 2018; Accepted 11 February 2018
Available online 13 February 2018
0022-2313/

Tiliacea) in desiccated state. This lichen is a symbiotic association between the fungus *Parmelina* (heterotrophic mycobiont) and *Trebouxia jamesii* algae a photosynthetic partner (autotrophic photobiont). The thalus of this lichen in dry state has been characterised by a total inactivation of photosynthetic activity and loosed its fluorescence intensity on a dark-to-light transition [14]. However, fully functional photosynthetic photosystem II (PSII) units can be reactivated after a 20–30 s lag-time after addition of a drop of water [14].

The analysis of changes in photosynthesis and photoprotection mechanisms occurred in dry state of *P. Tiliacea* was investigated by simultaneous three signals: prompt fluorescence (PF), delayed fluorescence (DF) and modulated reflection 820-nm (MR) measurements. This multiparametric analysis has developed for studying the energetic behaviour of the photosynthetic apparatus [15–25]. These signals would be recorded as a non-intrusive method to measure photosynthetic electron transport chain and for the monitoring the physiological state of a photosynthetic sample. PF signal was discovered first by Kautsky and Hirsch [26] and following a dark-to-light transition. PF transient indicates three different reduction processes of the electron transport chain and depends on the redox state of the PSII reaction centers (RC) [27–30]. Also and during light-to-dark transition, DF which is discovered first by Strehler and Arnold [31] is detected and reflects the recombination, in the dark, between the reduced primary electron acceptor Q_A^- and the oxidized donor ($P680^+$) of PSII. The third signal, the modulated reflection 820-nm reflects changes in the redox states of the primary electron donors of photosystem I (PSI) and plastocyanin (PC) with a small contribution of ferredoxin [32].

In previous contributions, *P. tiliacea* Thalli were studied to determine responses of the photosynthetic apparatus to rehydrated state. It was observed that following addition of water, the variable fluorescence increased and the photosynthetic PSII units reformed on a short time [14]. Furthermore, *P. tiliacea* PSII in dry state has been shown to be tolerant to liquid nitrogen temperatures [33] and the oxygen evolving complex had remained intact at 50 °C treatment for 24 h [14] compared to wet state. However, the mechanisms occurred in desiccation tolerance of lichens to tolerate these strong stresses are still to be understood. In the present work, which is a continuation of previous works [14,22,23,33], we studied thalli of *P. tiliacea* during their short desiccation (up to 4.5 h) when the reaction center are reversibly active by measuring simultaneously kinetics of PF, DF and MR.

2. Materials and methods

2.1. Plant materials

Thalli of *Parmelina tiliacea* (Hoffm.) Ach. were collected from the south sides of oak trunks, around the laboratory in Lullier-Jussy, Geneva (Switzerland). Before measurements, the collected lichen thalli were air-dried at room temperature for 48 h under low light conditions (~12 μmol photons m^{-2} s^{-1}).

2.2. Simultaneous measurements of the kinetics of prompt fluorescence (PF), delayed fluorescence (DF) and modulated 820 nm reflection (MR)

The measurements of PF, DF and MR on fragments of 4 mm x 4 mm of a single thallus were recorded by using the Multifunctional Plant Efficiency Analyser M-PEA (Hansatech Instruments Ltd, UK). Three emitters are present in the M-PEA sensor unit: (a) 627 ± 10 nm, for the actinic light LED; (b) 820 ± 25 nm, for the modulated light LED, and (c) 735 ± 15 nm, for the far-red light LED. The light of the actinic light LED is focused on the sample surface to provide homogeneous illumination with an intensity of 5000 μmol photons m^{-2} s^{-1}. During the measuring cycle, the PF is measured when the actinic light is on (in the light) and DF is recorded when the light is off (in the dark). The calculated ratio MR/MR_o (MR_o is the value at the onset of the actinic light) is complementary to the fraction $(I_{abs}/I_{inc})_{820\ nm}$ of incident light flux (I_{inc}) that is absorbed (I_{abs}) by the sample at 820 nm. The three signals data acquisition were done in 7 digitalisation ranges (e.g. the first data acquisition is every 0.01 ms in the digitalization range 1 (0.01–0.3 ms) (see [11,14,18] for more details). The first reliable MR time point was recorded at 0.3 ms.

The desiccation treatment was done at room temperature of 20–22 °C. We noted here that wet lichen samples (n = 4–5) were kept in dark for 10 min before the first PF, DF and MR measurements (considered at 0 h time desiccation) and then every 30 min up to 4.5 h. In this work the DF-intensities measured between 10 and 30 μs following lights off was used.

2.3. Performance indexes parameters (PI_{ABS} and PI_{total})

From the PF transient (OJIP curve), several biophysical expressions and fluorescence parameters were calculated by JIP-test [28,30,34–37]. In this study, the performance index PI_{ABS} on an absorption basis (it indicates the overall photosynthetic activity of PSII) PI_{total} (it expresses the overall photosynthetic activity from PSII to PSI) are fluorescence parameters that indicate a quantitative information about the physiological state of plants. The PI_{ABS} has been created out of three independent expressions: the concentration of reaction centers per chlorophyll, an expression related to primary photochemistry $\varphi_{Po}/(1-\varphi_{Po})$ and an expression related to electron transport $\psi_o/(1-\psi_o)$. The expression of PI_{ABS} is:

$$PI=[\gamma_o/(1-\gamma_o)].[\varphi_{Po}/(1-\varphi_{Po})].[\psi_o/(1-\psi_o)]$$

φ_{Po} corresponds to the maximum quantum yield of primary photochemistry [38,39]. The expression $\gamma_o/(1-\gamma_o)$ is proportional to the parameters estimated by JIP-test as equal to the ratio of reaction centers and the absorbance (RC/ABS). ψ_o (=1–V_J) is the fraction of electrons transported beyond Q_A- per exciton trapped by the reaction centers (RC) of PSII. It corresponds to the probability that the energy of a trapped exciton is used for electron transport beyond Q_A.

The peformance index (PI_{total}) is the product of the performance index PI_{ABS} and the probability that an electron can move from the reduced intersystem electron acceptors to the PSI end-electron-acceptors [35]. PI_{total} is defined as:

$$PI_{total} = PI_{ABS}.\delta_{Ro}/(1-\delta_{Ro}) = [\gamma_o/(1-\gamma_o)].[\varphi_{Po}/(1-\varphi_{Po})].[\psi_o/(1-\psi_o)].[\delta_{Ro}/(1-\delta_{Ro})]$$

δ_{Ro} is defined as the efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors. V_t is the relative variable fluorescence and is considered as a measure of the fraction of the primary quinone electron acceptor of PS II in its reduced state [Q_A^- /$Q_{A\ (total)}$]. In Table 1, formulae of all fluorescence parameters used in this study.

3. Results

3.1. Prompt fluorescence and performance index

Fig. 1 showed that at 0 h short desiccation treatment and following a dark-to-light transition, thalli of *P. tiliacea* exhibit a polyphasic fast PF rise and a slow PF rise (PMS). The fast PF rise reveals three different reduction processes of the electron transport chain (O-J, J-I and I-P phases). The O-J phase is called the photochemical phase and is light dependent [40,41]. The J-I phase reflects the reduction of the plastoquinone-pool (PQ) and I-P phase is related to electron flow through PSI [32]. When P-level is reached (~ 0.2–0.3 s) in thalli of *P. tiliacea*, the fluorescence intensity decreases to a dip (S-level) and starts to increase to M peak (~ 2 s). The fluorescence decrease after the P-level was suggested to be due to a fast oxidation of PC and P700 and then a fast activation of the ferredoxin-$NADP^+$-reductase (FNR) compared to in angiosperm plants [42,43]. On the other hand, the S-M phase increase

Table 1
The description of fluorescence parameters (see Strasser et al. [24]).

F_o	Initial Chl *a* fluorescence
F_M	Maximum Chl *a* fluorescence
F_K	Fluorescence intensity at ~0.3 ms
F_J	Fluorescence intensity at ~2-3 ms
F_I	Fluorescence intensity at 30 ms
F_V	F_V, maximum variable Chl fluorescence
$PI_{ABS} = [\gamma_o/(1-\gamma_o)].[\varphi_{Po}/(1-\varphi_{Po})].[\psi_o/(1-\psi_o)]$	Performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors
$PI_{total} = PI_{ABS}.\ \delta_{Ro}/(1-\delta_{Ro})$	Performance index for energy conservation from exciton to the reduction of PSI end acceptors
$\varphi_{Po} = 1-(F_o/F_M) = F_V/F_M$	Maximum quantum yield of primary photochemistry
$\gamma_o = Chl_{RC} / Chl_{total}$	Ratio of reaction centre Chls and the total Chl of PSII
$\psi_o = 1-V_J$	Efficiency with which a trapped exciton can move an electron into the electron transport chain
$\delta_{Ro} = (1-V_I)/(1-V_J)$	Efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors
RC/ABS	Reaction centers per absorption flux
$V_J = (F_J - F_o)/(F_M - F_o)$	Relative variable Chl a fluorescence at the J-step
$V_I = (F_I - F_o)/(F_M - F_o)$	Relative variable Chl a fluorescence at the I-step
$V_t = (F_t - F_o)/(F_M - F_o)$	Relative variable Chl *a* fluorescence at time t

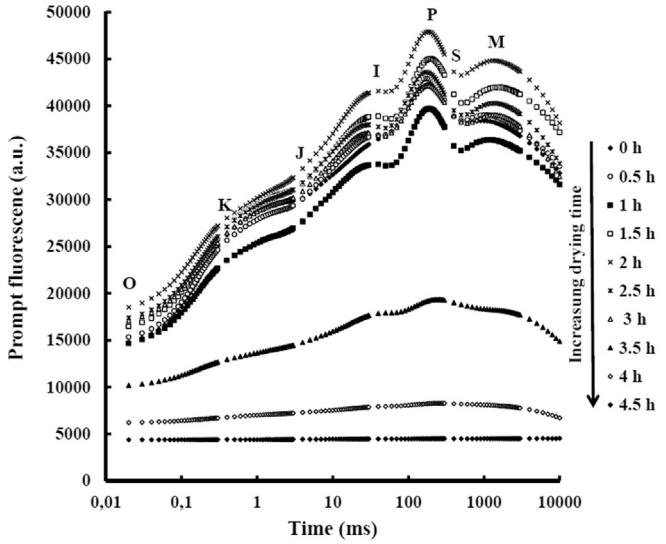

Fig. 1. Prompt fluorescence transients of desiccated *P. tiliacea* thalli kept in dark at room temperature. The transients were recorded during desiccation treatment by red actinic light of 5000 µmol photons m^{-2} s^{-1} and plotted on a logarithmic time scale. The steps O (at 20 µs), J (at 2–3 ms), I (at 30 ms), P (at ~ 300 ms), the dip (S-level) and the M peak (~2 s) are marked.

is associated to an increase in the state transition [44–46]. In thalli of *P. tiliacea*, desiccation treatment induced a progressive decline in the variable PF intensity. The shape of PF recorded during desiccation treatment was changed and a decrease of fluorescence yield compared to PF measured at 0 h was observed. At 4.5 h desiccation, *P. Tiliacea* thalli did not show any increase in their variable fluorescence intensity (straight line).

To probe the effect of short desiccation on thalli of *P. tiliacea*, two parameters quantifying the photosynthetic state were calculated by the JIP-test. The effect of desiccation treatment on the performance indexes (PI_{ABS} and PI_{total}) is shown in Fig. 2. Desiccated *P. Tiliacea* thalli showed a reduction in relative PI_{ABS} and PI_{total} of 86%, 84% at 4 h treatment time respectively. The rate of decrease in the PI_{ABS} and PI_{total} started to fall at 2.5-3 h desiccation treatment time. Looking in details the change in the independent expression of PI_{ABS} and PI_{total} during desiccation treatment, we observed that the parameters $\gamma_o/(1-\gamma_o)$, $\varphi_{Po}/(1-\varphi_{Po})$, and $\delta_{Ro}/(1-\delta_{Ro})$ decreased while the parameter $\psi_o/(1-\psi_o)$ increased. We note here that the desiccation treatment *P. Tiliacea* thalli showed a less effect on the parameter $\delta_{Ro}/(1-\delta_{Ro})$ compared to the three other parameters. Additionally, this result indicates that the maximum quantum yield of primary photochemistry (φ_{Po}), the probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A (ψ_o) and the ratio of reaction centers and the absorbance (RC/ABS) were sensitive to desiccation situation.

3.2. Delayed fluorescence and related parameters

To further understand the effect of short desiccation on *P. Tiliacea* thalli, the recorded DF curves were studied. Fig. 3 showed the DF induction curve recorded in desiccated samples during the dark period after interruptions of the actinic light (at 10–30 ms dark interval). This DF curve measured at 10–30 µs delay-time includes a rise to a peak I_1 (at 7 ms), a decrease intensity to I_2 (at about 100 ms), and finally I_4 peak at 0.5-5 s according to nomenclature of Goltsev and Yordanov [47]. In this study, I_3 was not mentioned because this peak appears in the DF curve at lower actinic light ~1200 µmol photons m^{-2} s^{-1} [15] and in our experiments we used a high actinic light of 5000 µmol photons m^{-2} s^{-1}. I_1 peak appears between the J-step to the I-step of the PF curve, I_2 appeared in the scale of the I–P phase and I_4 appeared at 0.5–0.7 s during the decline of the P-S-M phase. I_1 peak has been reported to be associated to the transmembrane electrical gradient and of the accumulation of RCs with semi-reduced Q_B ($Z^+P680Q_AQ_B^-$) and I_2 was related to an increase of $Z^+P680Q_A^-Q_B^-$ states during PQ pool reduction [48]. I_4 was introduced by Oukarroum et al. [14,22] in *P. Tiliacea* thalli and was suggested to be caused by an increase of a cyclic electron transport flow from the PQ-pool to PSI. It is of our interest to note that an antiparallel relation between J-I-P-S from PF curve and DF curve was observed. In this work we observed that during desiccation treatment in thalli of *P. tiliacea*, DF intensity decreases and up to 4.5 h desiccation, *P. Tiliacea* thalli did not show any change in DF (straight line) such as observed on variable PF.

In Fig. 4, the I_1, I_2 and I_4 peaks, measured from $DF_{10\text{-}30\mu s}$ curves during desiccation treatment were analysed. Upon desiccation treatment to 1.5 h, the $DF_{10\text{-}30\mu s}$ curves increased in amplitude for I_1, I_2 and I_4 and then started to decrease. The effect was more pronounced in I_1 amplitude.

It has been reported that the PF parameter F_K/F_J ratio correlate strongly with the DF parameter I_2/I_1 ratio when pea plants were exposed to high temperature [22] and analysed by simultaneous measurements of PF and DF. The parameter F_K/F_J was introduced by Srivastava and Strasser [49] to indicate the limitation of electron donation on the donor side of PSII. Interestingly in this study also, a strong linear correlation (R^2 = 0.97) between the F_K/F_J and I_2/I_1 ratios was observed (Fig. 5). Here, during desiccation treatment at room temperature of *P. tiliacea* thalli, this parameter (I_2/I_1 ratio) increased as well as the parameter F_K/F_J. However, I_2/I_1 ratio can be also considered as a quantitative measure for the inactivation of the PSII donor side.

3.3. Modulated 820 nm reflection

Kinetic changes at 820 nm in desiccated *P. Tiliacea* thalli induced by red actinic light (5000) that reflect the redox states of P700 and PC was presented in Fig. 6. In respect to the 820 nm measurements at 0 h, a decrease of the 820 nm signal until about 20 ms (to the I-level in PF induction) was linked to an oxidation of PC and P700 (accumulation of $P700^+$ and PC^+), and the increase signal (in parallel to I-P phase in the PF induction) reflects a re-reduction when electrons arrive from PSII [32]. Another oxidation-re-reduction of PC and P700 was detected in MR signal that correspond to the P-S-M transition from the PF induction. During desiccation treatment, re-reduction kinetics of $P700^+$ and PC^+ seem to be faster and their amplitudes gradually decrease. Furthermore, the second re-reduction kinetics of $P700^+$ and PC^+ (paralleled to S-M phase from the PF transition) were partially lost up to 2 h

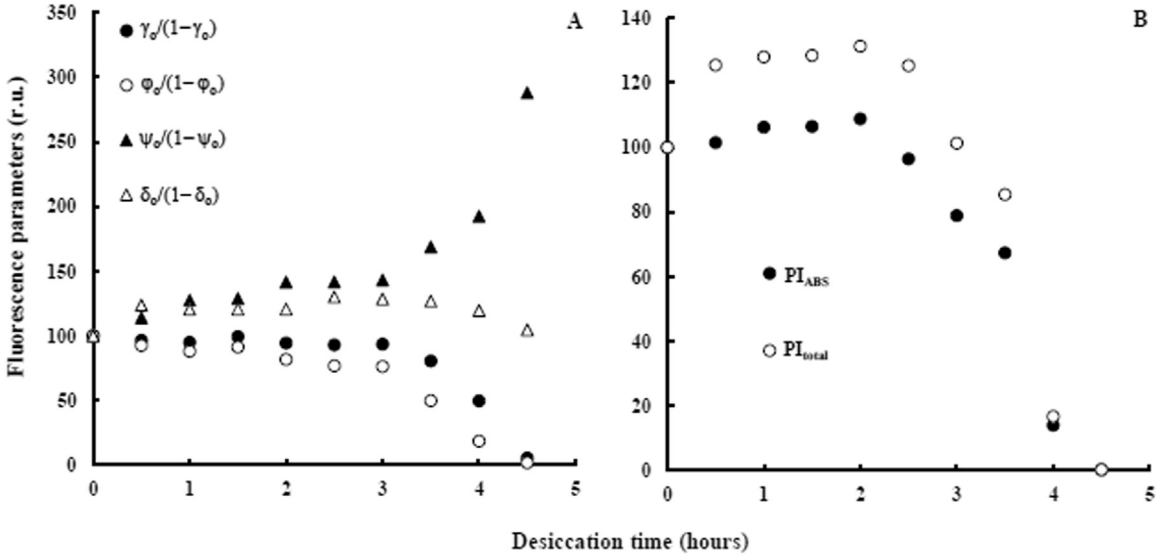

Fig. 2. Performance index PI_{ABS} on an absorption basis (it indicates the overall photosynthetic activity of PSII), PI_{total} (it expresses the overall photosynthetic activity from PSII to PSI) and their independent expression ($\gamma_o/(1-\gamma_o)$, $\varphi_{Po}/(1-\varphi_{Po})$, $\psi_o/(1-\psi_o)$ and $\delta_{Ro}/(1-\delta_{Ro})$ calculated by the JIP-test from the fast rise prompt fluorescence transients and plotted vs. desiccation treatment (for their definition, see Material and Methods section).

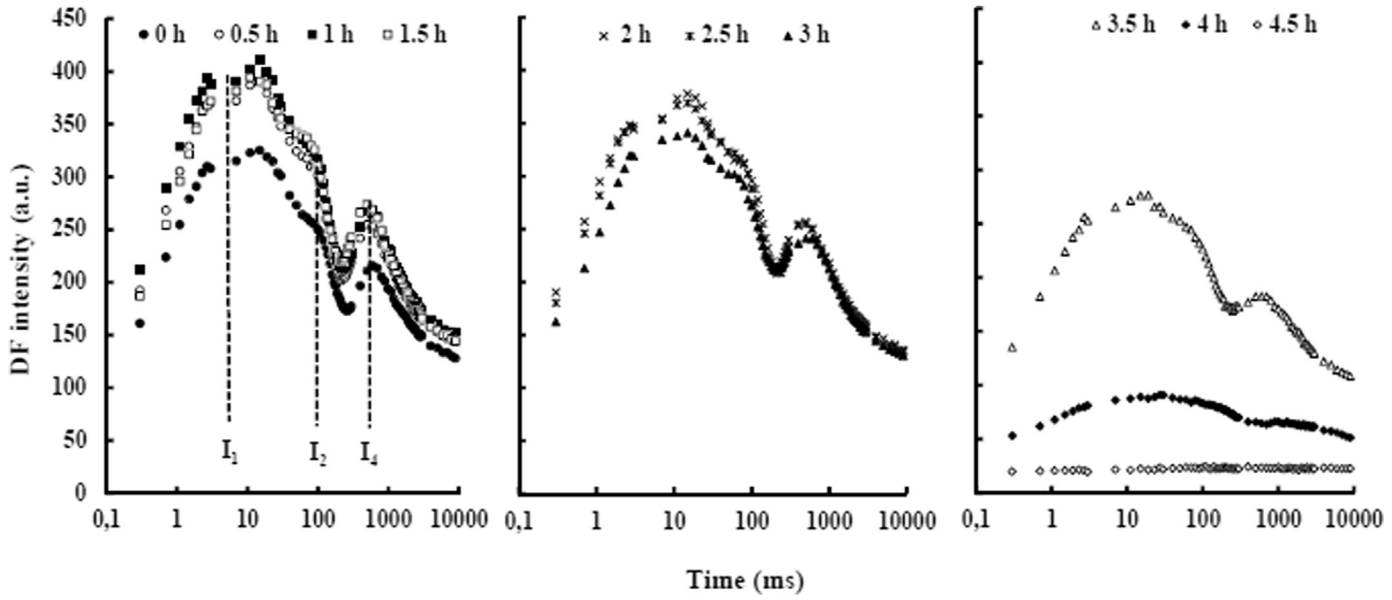

Fig. 3. Delayed fluorescence induction of desiccated *P. tiliacea* thalli kept in dark at room temperature. The curves were recorded during the dark period after interruptions of the red actinic light of 5000 µmol photons m^{-2} s^{-1} This delayed fluorescence curve measured at 10–30 µs delay-time and plotted on a logarithmic time scale.

desiccation treatment and subsequently totally lost. As observed in PF and DF, at 4.5 h desiccation treatment, any 820 nm change was not detected.

4. Discussion

In this study, simultaneous measurements of PF (OJIP transition), DF and MR were used to carry out *P. tiliacea* thalli response to short desiccation period at room temperature and in dark. It is noteworthy to highlight that PF is emitted during dark to light transition while DF is detected during light-to-dark transition (Glotsev et al. [16] and references therein). PF is mainly emitted from PSII and PSI contributes very little to the PF transient in I-P phase [25,28]. PF depends on the redox state of the PSII reaction centers (RC) [21,28,30], however, from microseconds to milliseconds, DF has been thought to depend on the recombination between the reduced electron acceptor Q_A^- and the oxidized secondary electron donor, Z^+ of PSII [16,20]. MR at 820 nm indicates the redox states of P700 and PC [32]. In this study, desiccation treatment caused a down-regulation at the PSII activity in *P. tiliacea* thalli; revealed by loss of variable fluorescence and strong decrease in DF intensity. Furthermore and using JIP-test approach, the PI_{ABS} and PI_{total} fluorescence parameters as a measure of an overall photosynthetic thalli performance showed a negative effect of desiccation treatment on photosynthetic activity (Fig. 2B). The concentration of reaction centers per chlorophyll (estimated as RC/ABS by JIP-test), the yield of primary photochemistry $\varphi_{Po}/(1-\varphi_{Po})$ and the yield of the electron transport $\psi_o/(1-\psi_o)$ are the expressions that form the PI_{ABS}. These three expressions decreased during desiccation time mainly below 2 h desiccation treatment. This means that changes in antenna properties, trapping electron efficiency or electron transport beyond Q_A were negatively affected in desiccated thalli. In the other words, a decline in PI_{ABS} was related to a down-regulation of electron transport

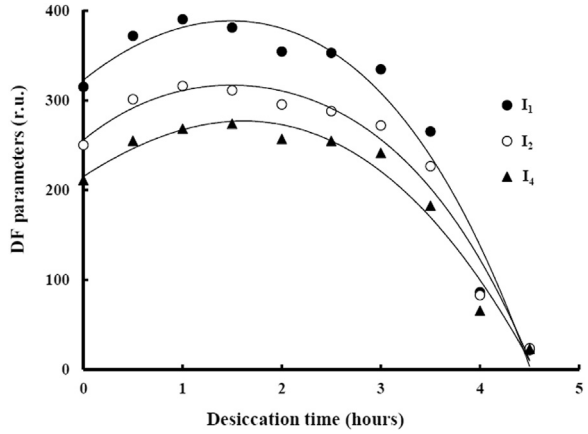

Fig. 4. Change of I_1, I_2 and I_3 peaks measured from $DF_{10\text{-}30\mu s}$. (A) I_1, I_2 and I_3 peaks were plotted vs. desiccation time treatment.

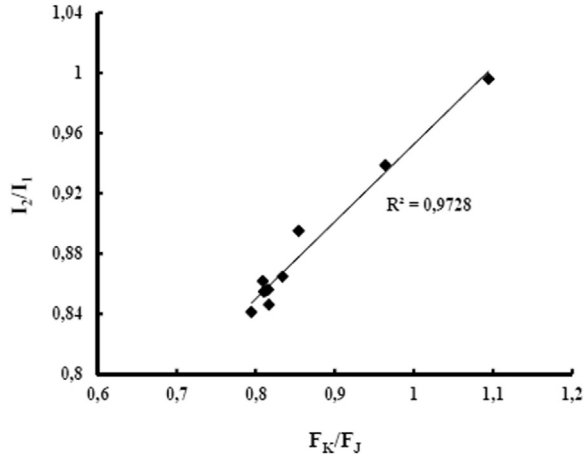

Fig. 5. Relation between F_K/F_J and I_2/I_1 parameters. F_K and F_J are intermediates step in PF transient recorded at 0.3 and 2–3 ms respectively. I_1 and I_2 are peaks values in the delayed fluorescence induction measured at 7 and 100 ms respectively.

chain. These results are consistent with the findings observed by Strasser et al. [18] and Georgieva et al. [2–4] in drying resurrection plant *Haberlea rhodopensis* and Goltsev et al. [17] in detached bean leaves (*Phaseolus vulgaris* cv. Cheren Starozagorski) during drying (decrease of the relative water content). PI_{total}, another sensitive fluorescence parameter, is created out of two independent expressions: the performance index PI_{ABS} and the yield of the efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors. The strong decrease of PI_{total} in desiccated thalli was mainly due to the decline of PI_{ABS}. In fact, the efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors (δ_{Ro}) was less affected in desiccated *P. tiliacea* thalli (Fig. 2A) and as observed in drying resurrection plant *Haberlea rhodopensis* [18]. However, this δ_{Ro} parameter seems to be sensitive to drying treatment in detached bean leaves [17]. This result, point out the variability sensitivity in plant species to desiccation-induced change of electron transport chain between tolerant and non tolerant organisms. In previous studies, *P. tiliacea* thalli have the capacity to recover all photosynthetic capacity after re-watering [14,21]. Here, the question arise: how *P. tiliacea* thalli (and in poikilohydric organisms in general) maintain their physiological processes in a reversible state?. It is known that in plant cells, photosynthesis is regarded as one of the most important metabolic processes [38] and counteract a stressed state such as a limitation of water, cells maintain an equilibrium balance between energy production and consumption. To tolerate desiccation state, *P. tiliacea* thalli reduced light energy excitation expressed by a loss of variable fluorescence PF and DF amplitude (Figs. 1 and 3). This loss of variable fluorescence might be due to an increase in inactive reaction centers (silent RCs) which dissipate excitation energy to heat energy but still capable of reversible photochemical activity [14]. This mechanism of energy dissipation in desiccated plants has been reported to prevent light damage (photo-oxidative damage) [50]. In the other words, a photoprotection strategy has been established. Furthermore, in Fig. 1, desiccation treatment was accompanied by a decrease in F_o, this decline in F_o could be modified by the excitation quenching processes within chlorophyll antennae. It means that during desiccation situation, any processes are developed that lead highly effective fluorescence quenching. A plausible explanation is that in desiccated state, the chlorophyll molecules became in close contact with external quenchers that are capable efficiently to

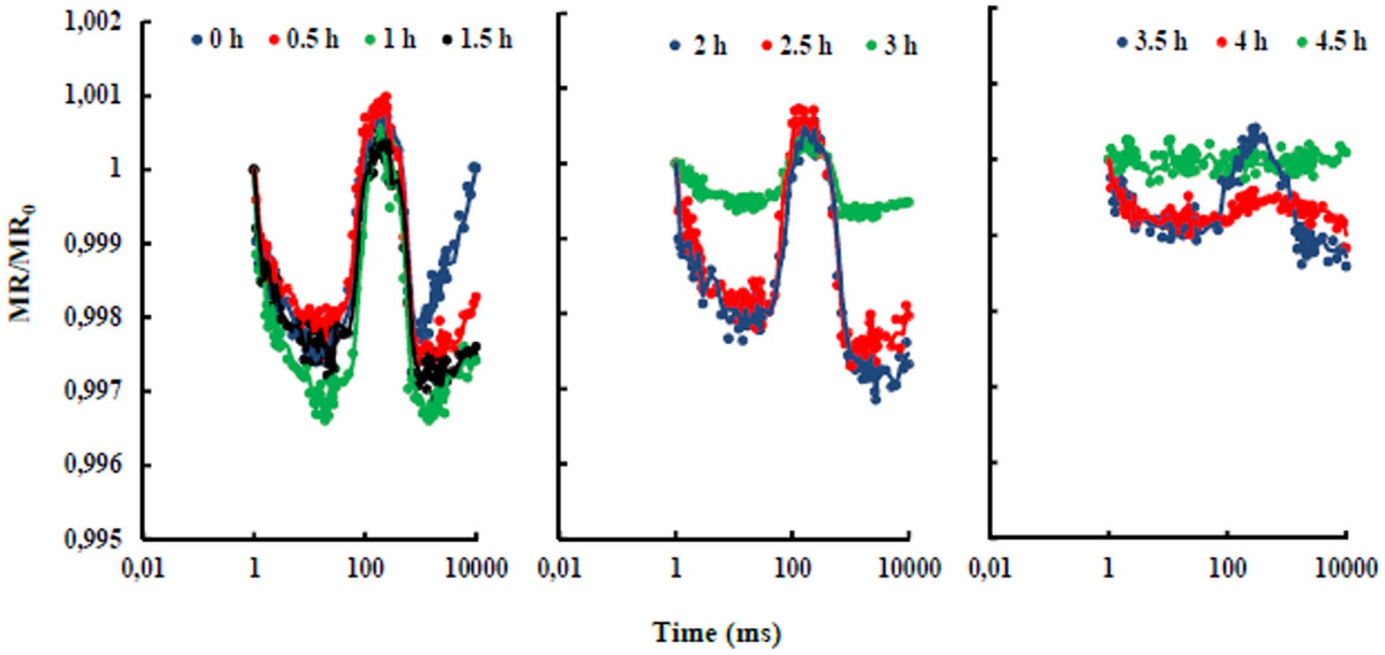

Fig. 6. Kinetics of modulated reflection at 820 nm (MR) induced by red actinic light of 5000 µmol photons m^{-2} s^{-1} in desiccated thalli of *P. tiliacea* at room temperature.

quench the excitation energy.

As mentioned before, the efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors was less affect by desiccation treatment in *P. tiliacea* thalli (Fig. 2A). This finding can be interpreted as a limitation of linear electron transport to PSI. Moreover and in respect to redox change of P700 and PC, we observe in Fig. 6 the amplitude of re-reduction phase from MR/MR_o decrease (connected to I-P phase from PF) which reveals a disconnection between the two photosystem (PSII and PSI) or in other words a decline rate of electron transport from reduced plastoquinone pool to $P700^+$. This result was also mentioned in desiccated resurrection plant *Haberlea rhodopensis* [18] and in a chlorolichen, *Ramalina yasudae* [51]. Further, the second re-reduction phase (corresponding to S-M phase from PF) decreased also during short desiccation in dark. At 0 h desiccation time, this second re-reduction was suggested to be connected to an activation of cyclic electron transport (CET) [14.23]. Therefore, we can suppose that desiccation induces a limitation in CET.

The DF-signal recorded between 10 and 30 μs of darkness (this delay time was used in this study) probes the recombination between Q_A^- and $P680^+$ [52,53] and depends on their concentration [16]. Also, Schansker et al. [54] demonstrated that the DF-signal in the tens of μs time is determined by reduction of the photosynthetic electron transport. Here, we can contribute the decrease in DF intensity at least to an increase in inactive RCs (formation the so called heat sinks or silent reaction centers). Fig. 5 showed an increase in the parameter F_K/F_J which indicates a limitation of electron donation on the donor side of PSII. This decrease in efficiency of fluorescence emission might be due to an inhibition of stable charge separation in RCs. The linear correlation observed between the independents parameters F_K/F_J (calculated from PF) and I_2/I_1 ratio (calculated from DF) indicated that the limitation of electron donation on the donor side of PSII was responsible in the increase of inactive RCs. We noted here that I_1/I_2 ratio was considered as a sensitive DF parameter to predict relative water content in detached leaves of bean plants [17]. It is important to note also that in detached bean plants the I_1/I_2 ratio in desiccated state decrease while in this study the I_1/I_2 ratio increase. However the shape and DF amplitude measured in detached leaves of bean plants (Fig. 1B in [17]) were different compared to *P. tiliacea* thalli response.

In conclusion, desiccated state led to loss of variable fluorescence and DF amplitude, limitation in CET and electron donation on the donor side of PSII and a disconnection between PSII and PSI. These strategies allow to *P. tiliacea* a photoprotection mechanism from photooxidative damage. However, in order to clarify in details the whole response of *P. tiliacea* to desiccation and maintain a reversible photochemical activity, it is interesting to study the response of isolated *Trebouxia jamesii* algae (the photosynthetic partner of *P. tiliacea*) to the same short desiccation treatment.

Acknowledgements

This work was supported by the Swiss National Science Foundation, Project no. 200021-116765.

References

[1] P. Alpert, Constraints of tolerance: Why are desiccation tolerant organisms so small or rare? J. Exp. Bot. 209 (2006) 1575–1584.
[2] K. Georgieva, Z. Szigeti, E. Sarvari, L. Gaspar, L. Maslenkova, V. Peeva, E. Peli, Z. Tuba, Photosynthetic activity of homoiochlorophyllous desiccation tolerant plant *Haberlea rhodopensis* during dehydration and rehydration, Planta 225 (2007) 955–964.
[3] K. Georgieva, S. Lenk, C. Buschmann, Responses of the resurrection plant *Haberlea rhodopensis* to high irradiance, Photosynthetica 46 (2008) 208–215.
[4] K. Georgieva1, E. Sárvári, A. Keresztes, Protection of thylakoids against combined light and drought by a lumenal substance in the resurrection plant Haberlea rhodopensis, Ann. Bot. 105 (2010) 117–126.
[5] L. Maslenkova, Thermoluminescence from photosynthesizing systems as a method for detection of early plant stress symptoms. Effect of desiccation on thermoluminescence emission parameters in mesophytic and poikilohydric plants, Gen. Appl. Plant Physiol. 36 (2010) 87–99.
[6] D.F. Gaff, Desiccation-tolerant flowering plants in Southern Africa, Science 3 (1974) 1033–1034.
[7] H. NagyDeri, E.R. Peli, K. Georgieva, Z. Tuba, Changes in chloroplast morphology of different parenchyma cells in leaves of *Haberlea rhodopensis* Friv. during desiccation and following rehydration, Photosynthetica 49 (2011) 119–126.
[8] B. Fernandez-Marin, A. Holzinger, J.I. Garcia-Plazaola, Photosynthetic strategies of desiccation-tolerant organisms, Chap. 38 ed, in: M. Pessarakli (Ed.), Handbook of Photosynthesis, 3rd Ed., CRC Press, Boca Raton, FL, 2016, pp. 719–737.
[9] J. Wolfe, G. Bryant, Freezing, drying, and/or vitrification of membrane–solute–-water systems, Cryobiology 39 (1999) 103–129.
[10] Z. Tuba, M.C.F. Proctor, Z. Csintalan, Ecophysiological responses of homoiochlorophyllous and poikilochlorophyllous desiccation-tolerant plants: a comparison and an ecological perspective, Plant Growth Regul. 24 (1998) 211–217.
[11] U. Heber, Photoprotection of green plants: a mechanism of ultra-fast thermal energy dissipation in desiccated lichens, Planta 228 (2008) 641–650.
[12] F.I. Woodward, Do plants really need stomata? J. Exp. Bot. 49 (1998) 471–480.
[13] L. Nybakken, K.A. Solhaug, W. Bilger, Y. Gauslaa, The lichens Xanthoria elegans and Cetraria islandica maintain a high protection against UV-B radiation in Arctic habitats, Oecologia 140 (2004) 211–216.
[14] A. Oukarroum, R.J. Strasser, G. Schansker, Heat stress and the photosynthetic electron transport chain of the lichen *Parmelina tiliacea* (Hoffm.) Ach. in the dry and the wet state: differences and similarities with the heat stress response of higher plants, Photosynth. Res. 111 (2012) 303–314.
[15] V. Goltsev, I. Zaharieva, P. Lambrev, I. Yordanov, R.J. Strasser, Simultaneous analysis of prompt and delayed chlorophyll a fluorescence in leaves during the induction period of dark to light adaptation, J. Theor. Biol. 225 (2003) 171–183.
[16] V. Goltsev, I. Zaharieva, P. Chernev, R.J. Strasser, Delayed fluorescence in photosynthesis, Photosynth. Res. 101 (2009) 217–232.
[17] V. Goltsev, I. Zaharieva, P. Chernev, M. Kouzmanova, H.M. Kalaji, I. Yordanov, V. Krasteva, V. Alexandrov, D. Stefanov, S.I. Allakhverdiev, R.J. Strasser, 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, Biochim. Biophys. Acta 1817 (2012) 1490–1498.
[18] R.J. Strasser, M. Tsimilli-Michael, S. Qiang, V. Goltsev, 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*, Biochim. Biophys. Acta 1797 (2010) 1313–1326.
[19] M. Brestic, M. Zivcak, H.M. Kalaji, R. Carpentier, S.I. Allakhverdiev, Photosystem II thermostability in situ: Environmentally induced acclimation and genotype-specific reactions in Triticum aestivum L, Plant, Physiol. Biochem. 57 (2012) 93–105.
[20] H.M. Kalaji, V. Goltsev, K. Bosa, S.I. Allakhverdiev, R.J. Strasser, Govindjee, Experimental in vivo measurements of light emission in plants: a perspective dedicated to David Walker, Photosynth. Res. 114 (2012) 69–96.
[21] H.M. Kalaji, G. Schansker, R.J. Ladle, V. Goltsev, K. Bosa, S.I. Allakhverdiev, M. Brestic, F. Bussotti, A. Calatayud, P. Dąbrowski, N.I. Elsheery, L. Ferroni, L. Guidi, S.W. Hogewoning, A. Jajoo, A.N. Misra, S.G. Nebauer, S. Pancaldi, C. Penella, D.B. Poli, M. Pollastrini, Z.B. Romanowska-Duda, B. Rutkowska, J. Serôdio, K. Suresh, W. Szulc, E. Tambussi, M. Yanniccari, M. Zivcak, Frequently asked questions about in vivo chlorophyll fluorescence: practical issues, Photosynth. Res. 122 (2014) 121–158.
[22] A. Oukarroum, V. Goltsev, R.J. Strasser, Temperature effects on pea plants probing by simultaneous measurements of the kinetics of prompt fluorescence, delayed fluorescence and modulated 820 nm reflection, PLoS One 8 (3) (2013) e59433.
[23] A. Oukarroum, M. El Gharous, V. Goltsev, R.J. Strasser, Delayed fluorescence emission as a probe for the response of photosynthetic organisms to high temperature exposure: a comparative study, J. Lumin. 180 (2016) 321–327.
[24] M. Pollastrini, R. Desotgiu, F. Camin, L. Ziller, G. Gerosa, R. Marzuoli, F. Bussotti, 2Severe drought events increase the sensitivity to ozone on poplar clones, Environ. Exp. Bot. 100 (2014) 94–104.
[25] E. Salvatori, L. Fusaro, E. Gottardini, M. Pollastrini, V. Goltsev, R.J. Strasser, F. Bussotti, Plant stress analysis: Application of prompt, delayed chlorophyll fluorescence and 820 nm modulated reflectance. Insights from independent experiments, Plant Physiol. Biochem. 85 (2014) 105–113.
[26] H. Kautsky, A. Hirsch, NeueVersuchezur Kohlensaureassimilation, Naturwissenschaften 19 (1931) 964.
[27] R.J. Strasser, A. Srivastava, Govindjee, Polyphasic chlorophyll *a* fluorescence transient in plants and cyanobacteria, Photochem. Photobiol. 61 (1995) 32–42.
[28] R.J. Strasser, A. Srivastava, M. Tsimilli-Michael, Analysis of the chlorophyll *a* fluorescence transient. Chlorophyll fluorescence: a signature of photosynthesis, in: G.C. Papageorgiou, Govindjee (Eds.), Advances in Photosynthesis and Respiration Series, Vol 19 Springer, Dordrecht, 2004, pp. 321–362.
[29] G. Schansker, S.Z. Tóth, R.J. Strasser, Methylviologen and dibromothymoquinone treatments of pea leaves reveal the role of photosystem I in the Chl *a* fluorescence rise OJIP, Biochim. Biophys. Acta 2005 (1706) 250–261.
[30] A. Stirbet, Govindjee, Chlorophyll a fluorescence induction: A personal perspective of the thermal phase, the J-I-P rise, Photosynth. Res. 113 (2012) 15–61.
[31] B.L. Strehler, W. Arnold, Light production by green plants, J. Gen. Physiol. 34 (1951) 809–820.
[32] G. Schansker, A. Srivastava, Govindjee, R.J. Strasser, Characterization of the 820-nm transmission signal paralleling the chlorophyll a fluorescence rise (OJIP) in pea leaves, Funct. Plant. Biol. 30 (2003) 785–796.
[33] A. Oukarroum, M. El Gharous, R.J. Strasser, Does *Parmelina tiliacea* lichen photosystem II survive at liquid nitrogen temperatures? Cryobiology 24 (2017) 160–162.
[34] A. Oukarroum, R.J. Strasser, Phenotyping of dark and light adapted barley plants by the fast chlorophyll *a* fluorescence rise OJIP, S. Afr. J. Bot 70 (2004) 277–283.

[35] M. Tsimilli-Michael, R.J. Strasser, In vivo assessment of stress impact on plant's vitality: applications in detecting and evaluating the beneficial role of mycorrhization on host plant, in: A. Varma (Ed.), Mycorrhiza:Genetics and Molecular Biology, Eco-function, Biotechnology, Eco-physiology, and Structure and Systematics, Springer, Berlin, Germany, 2008, pp. 679–703.
[36] K. Yan, P. Chen, H. Shao, C. Shao, S. Zhao, Dissection of photosynthetic electron transport process in sweet sorghum under heat stress, PLoS ONE 8 (5) (2013) e62100, http://dx.doi.org/10.1371/journal.pone.0062100.
[37] S. Silvestre, S.S.0 Araújo, M.C.V. Patto, J.M. Silva, Performance index: an expeditious tool to screen for improved drought resistance in the Lathyrus genus, J. Integr. Plant Biol. 56 (2014) 610–621.
[38] N.R. Baker, A possible role for photosystem II in environmental perturbation of photosynthesis, Physiol. Plant. 81 (1991) 563–570.
[39] G.H. Krause, E. Weis, Chlorophyll fluorescence and photosynthesis: the basics, Annu. Rev. Plant Physiol. Plant Mol. Biol. 42 (1991) 313–349.
[40] C. Neubauer, U. Schreiber, The polyphasic rise of chlorophyll fluorescence upon onset of strong continuous illumination: 1. Saturation characteristics and partial control by the photosystem II acceptor side, Z. Naturforsch C 42c (1987) 1246–1254.
[41] G. Schansker, S.Z. Tóth, R.J. Strasser, Dark-recovery of the Chl *a* fluorescence transient (OJIP) after light adaptation: the qT-component of non-photochemical quenching is related to an activated photosystem I acceptor side, Biochim. Biophys. Acta 1757 (2006) 787–797.
[42] P. Ilík, G. Schansker, E. Kotabová, P. Váczi, R.J. Strasser, M. Barták, A dip in the chlorophyll fluorescence induction at 0.2–2 s in *Trebouxia*-possessing lichens reflects a fast reoxidation of photosystem I. A comparison with higher plants, Biochim. Biophys. Acta 1757 (2006) 12–20.
[43] G. Schansker, Y. Yuan, R.J. Strasser, Chl a fluorescence and 820 nm transmission changes occurring during a dark-to-light transition in pine needles and pea leaves: a comparison, in: J.F. Allen, E. Gantt, J.H. Golbeck, B. Osmond (Eds.), Photosynthesis: Energy from the Sun, Springer, Dordrecht, The Netherlands, 2008, pp. 945–949.
[44] C.G. Papageorgiou, M. Tsimilli-Michael, K. Stamatakis, The fast and slow kinetics of chlorophyll *a* fluorescence induction in plants, algae and cyanobacteria: a viewpoint, Photosynth. Res. 94 (2007) 275–290.
[45] R. Kaňa, E. Kotabová, O. Komárek, B. Šedivá, G.C. Papageorgiou, Govindjee, O. Prášil, The slow S to M fluorescence rise in cyanobacteria is due to a state 2 to state 1 transition, Biochim. Biophys. Acta 1817 (2012) 1237–1247.
[46] S. Kodru, T. Malavath, E. Devadasu, S. Nellaepalli, A. Stirbet, R. Subramanyam, Govindjee, The slow S to M rise of chlorophyll a fluorescence reflects transition from state 2 to state 1 in the green alga Chlamydomonas reinhardtii, Photosynth. Res. 125 (2015) 219–231.
[47] V. Goltsev, I. Yordanov, Mathematical model of prompt and delayed chlorophyll fluorescence induction kinetics, Photosynthetica 33 (1997) 571–586.
[48] V. Goltsev, P. Chernev, I. Zaharieva, P. Lambrev, R.J. Strasser, Kinetics of delayed chlorophyll a fluorescence registered in milliseconds time range, Photosynth. Res. 84 (2005) 209–215.
[49] A. Srivastava, R.J. Strasser, How do land plants respond to stress temperature and stress light? Stress and stress management of land plants during a regular day, Archs. Sci. Genève 48 (1995) 135–146.
[50] U. Heber, Conservation and dissipation of light energy in desiccation tolerant photoautotrophs, two sides of the same coin, Photosynth. Res. 113 (2012) 5–13.
[51] M. Kosugi, M. Arita, R. Shizuma, Y. Moriyama, Y. Kashino, H. Koike, K. Satoh, Responses to desiccation stress in lichens are different from those in their photobionts, Plant Cell Physiol. 50 (2009) 879–888.
[52] G. Christen, F. Reifarth, G. Renger, On the origin of the '35 μs kinetics' of $P680^+$ reduction in photosystem II with an intact water oxidizing complex, FEBS Lett. 249 (1998) 49–52.
[53] M. Grabolle, H. Dau, Efficiency and role of loss processes in light-driven water oxidation by PSII, Physiol. Plant. 131 (2007) 50–63.
[54] G. Schansker, S.Z. Tóth, L. Kovács, A.R. Holzwarth, G. Garab, Evidence for a fluorescence yield change driven by a light-induced conformational change within photosystem II during the fast chlorophyll *a* fluorescence rise, Biochim. Biophys. Acta 1807 (2011) 1032–1043.