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Moderate drought stress stabilizes the primary quinone acceptor Q_A and the secondary quinone acceptor Q_B in photosystem II

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This work is dedicated to Jean-Marc Ducruet, who introduced me to the afterglow band and constructed two thermoluminescence machines for us.

Abstract

Drought induces stomata closure and lowers the CO_2 concentration in the mesophyll, limiting CO_2 assimilation and favouring photorespiration. The photosynthetic apparatus is protected under drought conditions by a number of downregulation mechanisms like photosynthetic control and activation of cyclic electron transport leading to the generation of a high proton gradient across the thylakoid membrane. Here we studied photosynthetic electron transport by chlorophyll fluorescence, thermoluminescence and P700 absorption measurements in spinach exposed to moderate drought stress. Chlorophyll fluorescence induction and decay kinetics were slowed down. Under drought conditions an increase of the thermoluminescence AG-band and a downshift of the maximum temperatures of both, the B-band and the AG-band was observed when leaves were illuminated under conditions that maintained the proton gradient. When leaves were frozen prior to the thermoluminescence measurements the maximum temperature of the B-band was upshifted in drought-stressed leaves. This shows a stabilization of the $Q_B/Q_B^{\bullet-}$ redox couple in accordance with the slower fluorescence decay kinetics. We propose that during drought stress photorespiration exerts a feedback control on photosystem II via the binding of a photorespiratory metabolite at the non-heme iron at the acceptor side of photosystem II. According to our hypothesis an exchange of bicarbonate at the non-heme iron by a photorespiratory metabolite such as glycolate would not only affect the midpoint potential of the $Q_A/Q_A^{\bullet-}$ couple as has been shown previously but also that of the $Q_B/Q_B^{\bullet-}$ couple.

Introduction

Drought is one of the most important abiotic stress conditions leading to a loss of crop production. Upon drought, stomata keep closed, CO₂ concentration in the mesophyll decreases and CO₂ assimilation declines. When the CO₂ concentration at the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is low, the oxygenase reaction becomes more important compared to the carboxylase reaction and photorespiration is enhanced.

Photosynthetic electron transport is not considered as primary damage site under water deficit (Kaiser 1987; Cornic and Fresneau 2002). The photosynthetic apparatus has been demonstrated to be highly resistant to drought as shown by an unaltered quantum yield of photosystem II (ΦPSII) under moderate drought stress and a reversible decrease in ΦPSII upon increasing drought stress (Cornic and Briantais 1992; Genty et al. 1987). It has been described that photosynthetic electron flow is down-regulated under drought stress protecting the photosynthetic apparatus against oxidative stress. A number of regulation mechanisms such as nonphotochemical quenching (NPQ), photosynthetic control and cyclic electron transport are operating under drought. Ott and co-workers (1999) reported under drought conditions a slowing down of the reduction of oxidised P700, the primary electron donor of photosystem I (PSI), and interpreted this effect as a pH-dependent regulation of electron transport, most likely at the level of the cytochrome b6/f complex (photosynthetic control). Cyclic electron flow was proposed to be responsible for this higher proton gradient leading to higher non-photochemical quenching and protecting thereby the photosynthetic apparatus (Golding and Johnson 2003). Chlororespiration employs the same pathways like cyclic electron flow leading to a reduction of the plastoquinone pool and a generation of a proton gradient in the dark (Peltier et al. 2016). Induction of cyclic/chlororespiratory pathways can be seen by thermoluminescence upon slow dehydration of leaves (Bürling et al. 2014).

Thermoluminescence can be used as a probe of the behaviour of PSII reaction centres, both in isolated systems and in whole leaves (for review, see Ducruet and Vass 2009). Using this method, samples are first cooled down and then illuminated with a given number of single turnover flashes at the lower temperature. Subsequent warming reveals several peaks of luminescence emission. These emission bands originate from recombination of different types of trapped charge pairs, which can be identified by their maximum emission temperature, T_m. For example, recombination of the semiquinone, Q_B^{•-} with the S₂- or S₃-state of the water-splitting complex yields a TL band centered around 30 °C, the so-called B-band (Rutherford

et al. 1982). In whole leaves, at higher temperatures (40-46 °C at a 0.5°C s⁻¹ heating rate) an additional TL band, the afterglow band, has been reported and ascribed to the afterglow luminescence emission (AG-band) induced by reverse electron flow (Peeva et al. 2012; Bürling et al. 2014). This AG-band is suppressed by an uncoupler or by freezing the sample. Physiological conditions like drought that activate cyclic/chlororespiratory pathways cause a downshift in the maximum temperature of the AG-band (Ducruet et al. 2005; Bürling et al., 2014). The intensity of the AG band depends on the illumination protocol and on the concentration of NADPH and ATP in the stroma, dependent on the dihydroxyacetone phosphate/3-phosphoglycerate ratio (Krieger et al. 1998).

Aim of this study was to show the different regulation mechanisms set in place upon drought stress using chlorophyll fluorescence, thermoluminescence and P700 absorption changes. We investigated whether, in addition to the regulation mechanisms described in the literature, another regulation mechanism at PSII is activated under drought. According to our hypothesis photorespiration protects the photosynthetic apparatus not only by evacuating excess electrons, thereby keeping the electron transport chain oxidized and protecting against PSI acceptor side limitation (Eisenhut et al. 2017) but also by acting directly on PSII. It has been recently shown in a photorespiratory mutant accumulating the photorespiratory metabolite glycolate that PSII electron transport is altered thereby protecting PSII against ¹O₂ production and photo-oxidative damage (Messant et al. 2018). Glycolate was supposed to exchange the bicarbonate ligated to the non-heme iron at the acceptor side of PSII and to shift the midpoint potential of the redox couple Q_A/Q_A⁻ (Messant et al. 2018). Release of the bicarbonate ligated to the non-heme iron shift the midpoint potential of the redox couple Q_A/Q_A⁻ to a more positive potential (Brinkert et al. 2016). Until now it remains an open question whether drought stress inducing photorespiratory conditions evokes a similar modification of the acceptor side of PSII in a wild-type plant as has been observed in the photorespiratory mutant. To show alterations of photosynthetic electron transport and especially of PSII under drought stress, we measured chlorophyll fluorescence induction, decay, thermoluminescence and performed P700 absorption measurements.

Materials and Methods

Plant material. Spinach (*Spinacia oleracea* L.) was used for this study. Individual plants were grown in pots with a diameter of 13 cm in a greenhouse for six weeks and then moved for 1 week to controlled high light condition (8 h light, $I=450 \mu\text{mol quanta m}^{-2}\text{s}^{-1}$, 22°C, 16 h dark, 18°C; light source, fluorescent light tubes; Osram Dulux L 55W/840). Plants in control condition were watered every day while stressed plants were unwatered to attend 35% of total weight pot loss (3-4 days) in stress condition and kept afterwards at the same stress level during the following days by adding the lost water once a day.

Chlorophyll fluorescence. Chlorophyll fluorescence was measured on leaves attached to the plants at room temperature with a Dual-PAM-100 (Walz, Effeltrich, Germany). Fluorescence induction was measured during excitation with a multiple turnover flash (duration 300 ms). Fluorescence decay was measured after excitation with a saturating single turnover flash with a sampling rate of 2.5 μs .

Thermoluminescence (TL) was measured using a homebuilt apparatus. Leaf discs were dark-adapted for 5 min at 20°C. TL was charged by two single turnover flashes with a xenon flash lamp at 1°C or at -7°C when leaf disks frozen at 77K were used. The flashes were spaced with a 1 s dark interval. The TL signal was recorded during warming of the sample to 70°C at a heating rate of 0.4°C s⁻¹.

P₇₀₀ absorption. The redox state of primary donor of photosystem I (PSI) P₇₀₀ was monitored by following the changes in absorbance of 2 min dark-adapted leaves at 830 versus 875 nm using the Dual-PAM-100. Attached leaves were used. To probe the maximum extent of P₇₀₀ oxidation, leaves were illuminated with far red light superimposed on the saturating pulse of red light. Oxidation of P₇₀₀ was measured in leaves of WT and *hpr1-1* as a function of the intensity of the actinic light. $Y(\text{NA}) = (P_m - P_{m'})/P_m$ is a measure of the limitation of electron transport at the PSI acceptor side. P_m represents the maximum oxidation of P₇₀₀ and $P_{m'}$ the maximum oxidation at a given light intensity after application of a saturating light pulse. $Y(\text{ND})$ is a measure of the donor side limitation and it is calculated as $Y(\text{ND})=1-P_{700\text{red}}$, where $P_{700\text{red}}$ represents the fraction of reduced P₇₀₀ at a given state (Klughammer and Schreiber, 2008).

Immunoblots Thylakoid membranes were prepared as in Shimakawa et al. (2020) and used for analysis by SDS-PAGE (12% acrylamide) and immunoblotting. Proteins were blotted onto nitrocellulose membrane. Labelling of the membranes with antibodies was carried out at room temperature in 1x TBS (50 mM Tris-HCl pH 7.6, 150 mM NaCl), 0.1% Tween 20 and 1% non-fat powder milk). Primary antibodies were purchased from Agrisera (Vännäs, Sweden) with the exception of the antibodies raised against PTOX, kindly provided by M. Kuntz (CEA Grenoble). After washing, bound antibodies were revealed with a peroxidase-linked secondary anti-rabbit antibody (Agrisera, Vännäs, Sweden) and visualized by enhanced chemiluminescence.

Results

Drought stress in spinach plants was achieved by withholding water for 3-4 days until 35% of the initial weight of the pots was lost. This moderate drought stress did not induce a decrease in the absolute quantum yield of PSII (Fv/Fm). Well-watered plants had an Fv/Fm value of 0.81 ± 0.02 and stressed plants of 0.80 ± 0.01 . Also the water content of the stressed leaves remained unchanged. Prolonged drought stress induced a decrease in Fv/Fm. Since we are interested in studying a regulation mechanism and not an irreversible damage of the photosynthetic apparatus upon drought stress, mature leaves of moderately stressed plants (about 35% of loss of initial pot weight) were used for the following measurements.

Alterations of the PSII acceptor side in the drought-stressed plants compared to the control were followed by chlorophyll fluorescence measurements. As shown in Fig. 1, in a leaf from a stressed plant the different phases of the induction curve were flattened, and the area above the induction curve reflecting the number of available electron acceptors was smaller. Similar effects have been observed earlier under drought stress for other species and have been interpreted as reduced electron demand by PSI under these conditions (Oukarroum et al. 2009).

The kinetics of $Q_A^{\bullet-}$ reoxidation can be followed by analysing the fluorescence decay (Fig. 2). According to de Wijn and van Gorkom (2001), different phases of $Q_A^{\bullet-}$ reoxidation are assigned to electron transfer to Q_B (or $Q_B^{\bullet-}$) which is bound to the Q_B site at the time of the flash (fast and slower phases) and to electron transfer to Q_B but rate-limited by the exchange of a reduced quinone (Q_BH_2) in the Q_B -site for a PQ from the pool (slow phase). We fitted

here the decay curves by a sum of two exponential functions (time zero to 20 ms). The slow phase was not analysed since there was no longer a difference observed between the two conditions. As shown in Fig. 2 and Table 1, moderate drought stress led to an increase in the life-times of the fast phase (t_1) and the slower phase (t_2). The increase in the life-times indicates a slowdown of the electron transport between Q_A and $Q_B/Q_B^{\cdot-}$ supporting the hypothesis that photorespiratory activity affects the PSII acceptor side shifting the midpoint potential of both quinone acceptors, Q_A and Q_B , to a more positive value.

Drought stress has been described to affect thermoluminescence emission in barley and wheat leaves favouring the appearance of the AG-band compared to well-watered leaves (Peeva et al, 2012; Bürling et al. 2014). This is indeed the case also for spinach. Fig. 3 shows representative TL curves after excitation of the samples by two flashes at 1°C. The well-watered plant showed only a very small AG-band while a strong increase of the AG-band was observed in the stressed plant. A decrease in both, the maximum temperature of the B-band and the AG-band was found in stressed samples similar to previous reports (Bürling et al., 2014; Peeva et al., 2012). The downshift of the maximum temperature of the TL-bands under drought conditions is attributed to a larger proton gradient across the thylakoid membrane and a stimulation of cyclic electron transport according to Ducruet (Miranda and Ducruet 2005; Bürling et al., 2014; Peeva et al., 2012). An increase in cyclic electron transport activity may depend on a biochemical activation of this pathway by, for example, redox regulation (Courteille et al. 2013) or by an increase in the amount of the proteins implicated in cyclic electron transport. Western Blot analysis (Fig. 4) showed in thylakoids from stressed leaves a higher amount of proteins involved in the two pathways of cyclic electron flow via the NDH complex and the Ferredoxin Quinone Reductase (FQR) pathway mediated by the proteins PGR5/PGRL1. In addition, higher levels of the Plastid Terminal OXidase, PTOX, involved in chlororespiration, and of Early Light-Induced Proteins, ELIP, a stress-related protein (Heddad et al. 2006), were found.

As detailed in the introduction, under drought accumulation of the photorespiratory metabolite glycolate may affect the acceptor side of PSII. A large ΔpH may overlay the effect of glycolate accumulating under drought on the midpoint potentials of the quinone acceptors Q_A and Q_B . To destroy the ΔpH leaves were frozen in liquid nitrogen prior to the TL measurement. Frozen leaves showed a B-band at a higher temperature in stressed leaves than in control leaves (Fig. 5). A temperature difference of 3°C was found between the samples (Table 2), indicating that the $S_{2,3}Q_B^{\cdot-}$ couple was stabilized in stressed leaves. It would have been interesting to study a possible shift of T_m of the Q-band ($S_2Q_A^{\cdot-}$ recombination) upon

drought stress. This would give access to modifications of the midpoint potential of the redox couple $Q_A/Q_A^{\cdot-}$. However, to obtain a Q-band addition of DCMU is required that binds to the Q_B -binding site and inhibits electron transfer between Q_A and Q_B . We did not infiltrate leaves with DCMU because such an infiltration procedure could remove photorespiratory metabolites like glycolate from the non-heme iron at the acceptor side of PSII.

It has been reported previously that drought stress affects the redox state of P700 (Ott et al. 1999). As seen in Fig. 6, we observed a stronger donor-side limitation of PSI in stressed leaves compared to the control. A clear trend is shown in Fig. 6, although the differences are statistically not significant. No sign of acceptor side limitation was observed in the stressed plants showing that photorespiration activity was sufficient to keep the PSI acceptor side open.

Discussion

Photosynthetic electron transport is regulated at a number of different sites during moderate drought stress according to the data shown here and previously published work (Ott et al. 1999; Golding and Johnson 2003; Peeva et al. 2012; Böhrling et al. 2014). In Figure 7, we present a model showing four sites of regulation of photosynthetic electron transport: PSII, PTOX, cyclic flow and electron donation to PSI. As shown in Fig. 6, P700 is in a slightly higher oxidation state under steady state conditions in stressed leaves although this difference is statistically not significant. A higher oxidation state of P700 has been reported previously in different species (Ott et al. 1999; Wada et al. 2019). Accumulation of $P700^+$ protects PSI against photo-oxidative damage (Shimakawa and Miyake, 2018; Wada et al. 2019). Ott and coworkers (1999) reported a slowing down of the reduction of $P700^+$ that can be explained either by a slower electron donation by plastocyanin or by a limitation of electron transport at the cytochrome *b₆f* complex, the so-called photosynthetic control. Plastocyanin diffusion may become limited under severe drought conditions since the thylakoid lumen may shrink. This has been shown to be the case in desiccated cyanobacteria from a desert crust (Bar-Eyal et al. 2015). In these cyanobacteria $P700^+$ accumulates and acts as a quencher of excess energy, protecting the photosynthetic apparatus against oxidative stress. Under moderate drought conditions, however, limitation of plastocyanin seems to be very unlikely and instead photosynthetic control, i. e. electron transport limitation at the cyt *b₆f* complex when the pH in the lumen drops below a certain threshold value, is the likely reason for slowing down electron transport towards PSI.

An increase in the proton gradient can be achieved by cyclic electron transport that has been shown to be enhanced under drought stress (Golding and Johnson 2003). Cyclic electron flow takes place via two different pathways: the ferredoxin quinone reductase (FQR) pathway which likely involves the proteins PGRL1 and PGR5 and via the NDH complex (for a recent review see Nawrocki et al., 2019). During slow dehydration, Lehtimäki et al. (2010) reported an increased amount of PGR5 and PGRL1 proteins but not of NDH-H. Ibanez et al. (2010) observed that PTOX and both, the FQR and NDH-dependent pathway of cyclic flow, were activated under a combination of drought, heat and high illumination stress. Under the moderate drought stress applied here, both PGR5 and NDH-B protein levels were increased (Fig. 4) supporting the upregulation of cyclic electron transport pathways upon drought stress. The amount of PTOX is also clearly increased indicating activation not only of cyclic but also of chlororespiration. In stressed plants PTOX may also play a role as a safety valve during illumination. Thermoluminescence measurements revealed under drought stress an increase in the AG-band (Fig. 3) that has been attributed to an activation of cyclic/chlororespiratory flow (Peeva et al. 2012; Bürling et al. 2014). The lumen acidification leads to a downshift of both, the B-band and the AG-band (Fig. 3; see also Bürling et al. 2014). The peak temperature of a TL-band is indicative of the energy stored in the charge-separated state and is determined by redox potentials of both the recombination partners, in this case, S_2/S_3 and $Q_B/Q_B^{\cdot-}$. The $Q_B/Q_B^{\cdot-}$ couple involves proton release when $Q_B^{\cdot-}$ is reoxidized and is expected to follow Nernst behaviour, with the redox potential changing by -59 mV per pH unit (Rutherford et al. 1984; Vass and Inoue 1986; De Causmaecker et al. 2019).

In addition to these previously described regulation mechanisms activated upon drought stress and described above, we show here that the electron transport between Q_A and Q_B is slowed down (Figs 1, 2). We propose that this modification is caused by a photorespiratory metabolite accumulating under drought stress, most likely glycolate that replaces the bicarbonate ligated to the non-heme iron situated between Q_A and Q_B at the acceptor side of PSII. As shown by Messant et al. (2018), accumulation of glycolate affects the electron transport between Q_A and Q_B and protects PSII against 1O_2 generation. 1O_2 is highly toxic and able to damage PSII. Brinkert et al. (2016) have shown that the midpoint potential of the redox couple $Q_A/Q_A^{\cdot-}$ is shifted towards a more positive value in the absence of bicarbonate, allowing for a direct charge recombination pathway in PSII that does not lead to chlorophyll triplet formation and subsequent 1O_2 production. A protection mechanism at PSII avoiding triplet chlorophyll and 1O_2 generation is important to protect PSII against photoinhibition under conditions when the electron transport chain is highly reduced as it is

the case under drought stress. In addition to Q_A the midpoint potential of the $Q_B/Q_B^{\cdot-}$ couple seems to be stabilized under drought stress. As seen in the fluorescence decay, all phases are slowed down, including the slow phase that reflects according to de Wijn and van Gorkom (2001) the exchange of Q_BH_2 in the Q_B -site for a PQ molecule from the PQ pool. The binding efficiency of PQ to the Q_B site might be affected by the ligand change at the non-heme iron, but is also influenced by the concentration of oxidized PQ. If enhanced cyclic electron flow reduces the PQ pool, the concentration of oxidized PQ is lowered, which limits its availability for binding to the Q_B site and contributes to the decreased rate of fluorescence decay in the ms phase. The rate of forward electron transfer between $Q_A^{\cdot-}$ and Q_B is determined by free energy difference between the $Q_A/Q_A^{\cdot-}$ and $Q_B/Q_B^{\cdot-}$ states. Upon drought stress, also the fast phase of the fluorescence decay is slower showing that the free energy difference between the quinone acceptors is decreased. This can arise either from stabilization of $Q_A/Q_A^{\cdot-}$ or destabilization of $Q_B/Q_B^{\cdot-}$. Since $Q_B/Q_B^{\cdot-}$ is stabilized according to the T1 data (Fig. 5), we assume that $Q_A/Q_A^{\cdot-}$ is even more stabilized compared to $Q_B/Q_B^{\cdot-}$. Stabilization of the $Q_B/Q_B^{\cdot-}$ couple has been described previously in *Synechocystis* PCC 6803 mutants, mutated in the D-de loop of the D2 protein, which showed an upshift of the B-band (Kless et al. 1993; Mäenpää et al. 1995) and in the *Synechocystis* PCC 6803 ΔP_{sbT} strain. Stabilization of $S_2Q_B^{\cdot-}$ has been correlated with increased light sensitivity (Fagerlund et al. 2020). Further studies are required to show whether mild drought stress protects higher plants against photoinhibition or increases the susceptibility to photoinhibition.

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Table 1

Fitted parameters for fluorescence decay of control and drought-stressed plants (Fig. 2). Life-times (t) were obtained after fitting the fluorescence decay curves by a sum of two exponential functions ($n=9$). The difference between the life-times of control and drought-stressed plants are statistically different (*) according to Student's t -test ($p < 0.05$).

	Fluorescence decay ($t_{1/2}$ in ms)	
	t1	t2
control	0.303 ± 0.019	4.17 ± 0.50
drought stress	$0.565 \pm 0.051^*$	$6.57 \pm 0.94^*$

Table 2

Thermoluminescence measurements performed on leaf discs of control and drought-stressed plants. Leaf discs were frozen at 77K, excited at -7°C with 2 single turnover flashes. The maximum temperature of the B-band was determined ($n=6$). The difference between the T_m of control and drought-stressed plants are statistically different (*) according to Student's t -test ($p < 0.05$).

	TL
	T_{\max} B-band ($^\circ\text{C}$)
control	31.2 ± 1.9
drought stress	$34.8 \pm 2.0^*$

Figure legends

Figure 1. Chlorophyll fluorescence induction curves in control and drought-stressed leaves. A: representative curves (black: control, red: stressed); B: Area above the induction curves. Average values with standard deviation are shown. Statistically significant differences between the area above the induction curves of leaves of control and stressed plants were determined by Student's *t*-test ($*p < 0.01$; $n=11$).

Figure 2. Chlorophyll fluorescence decay in control and drought-stressed leaves. Variable fluorescence was induced by saturating a single turnover flash. A: representative traces (black: control, red: stressed). B: half-times of the fast, middle and slow phase. Decays were fitted by the sum of two exponential decay functions (fast and middle phase) and a linear function (slow phase). Statistically significant differences between leaves of control and stressed plants were determined by Student's *t*-test ($*p < 0.01$; $n \geq 9$).

Figure 3. Thermoluminescence bands in control and drought-stressed leaves. Samples were excited at 1°C by two single turnover flashes. Black: control, red: stressed leaf. Red line: Fit of the experimental curves by two Gaussian functions. The B-band and AG-band intensities are indicated by the green curves, respectively.

Figure 4. Immunoblots of proteins involved in alternative electron transport in membrane fractions isolated from control and drought-stressed leaves. Immunoblots were conducted with antibodies directed against the plastid terminal oxidase (PTOX), PGR5, the subunit B of the NDH complex (NDH-B), Early Light-Induced Proteins (ELIP). RuBisCo large subunit (RbcL) was used as loading control. In addition, the Coomassie stained gel is shown. $2.5 \mu\text{g}$ chlorophyll was loaded for both samples.

Figure 5. Thermoluminescence bands in control and drought-stressed leaves frozen at 77K. Leave disks were frozen at 77K prior to the insertion into the thermoluminescence cuvette. Samples were excited at -7°C by two single turnover flashes. Black: control, red: stressed leaf.

Figure 6. The redox state of the PSI primary donor P_{700} was monitored through the changes in absorbance at 830 versus 875 nm. Following the initial determination of maximal oxidation of

P₇₀₀, actinic light of the indicated intensities was given for 60 s. PSI donor-side limitation Y(ND) and acceptor-side limitation Y(NA) are based on saturating pulse analyses. Black symbols: control, red symbols stressed leaves, squares: Y(ND), circles Y(NA); (mean ± SD, n=6; 3 biological replicates).

Figure 7. Model of the regulation of electron transport under stress conditions. Four different sites slow down electron transport and protect the photosynthetic electron transport. 1: the acceptor side of PSII is modified, most likely by binding of a photorespiratory metabolite at the non-heme iron between Q_A and Q_B. Dashed line shows the ligation of bicarbonate or glycolate to the non-heme iron. According to our model, this induces a stabilization of the midpoint potentials of the redox couples Q_A/Q_A^{•-} and Q_B/Q_B^{•-} and slows down forward electron transport and protects PSII against photooxidative damage by ¹O₂. 2: PTOX activity acting as a safety valve to keep the redox poise of the plastoquinone pool. 3: cyclic electron transport via the NDH complex or via the FQR pathway involving PGR5/PGRL1 is stimulated under drought leading to an increase in the ΔpH. 4: a high ΔpH slows down the turnover of the cytochrome *b₆f* complex and the electron donation to PSI. Accumulation of P700⁺ protects PSI against photooxidative damage.

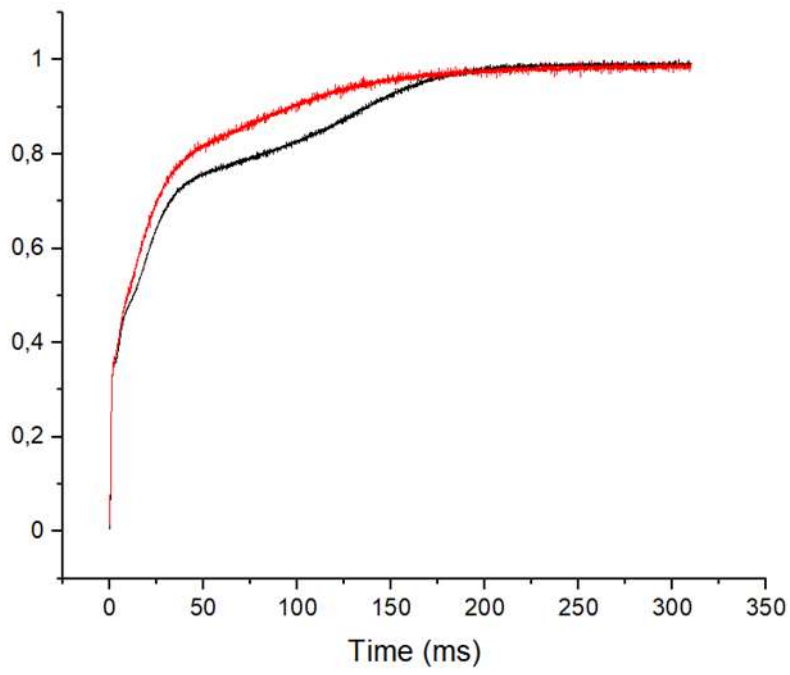


Figure 1

Figure 2

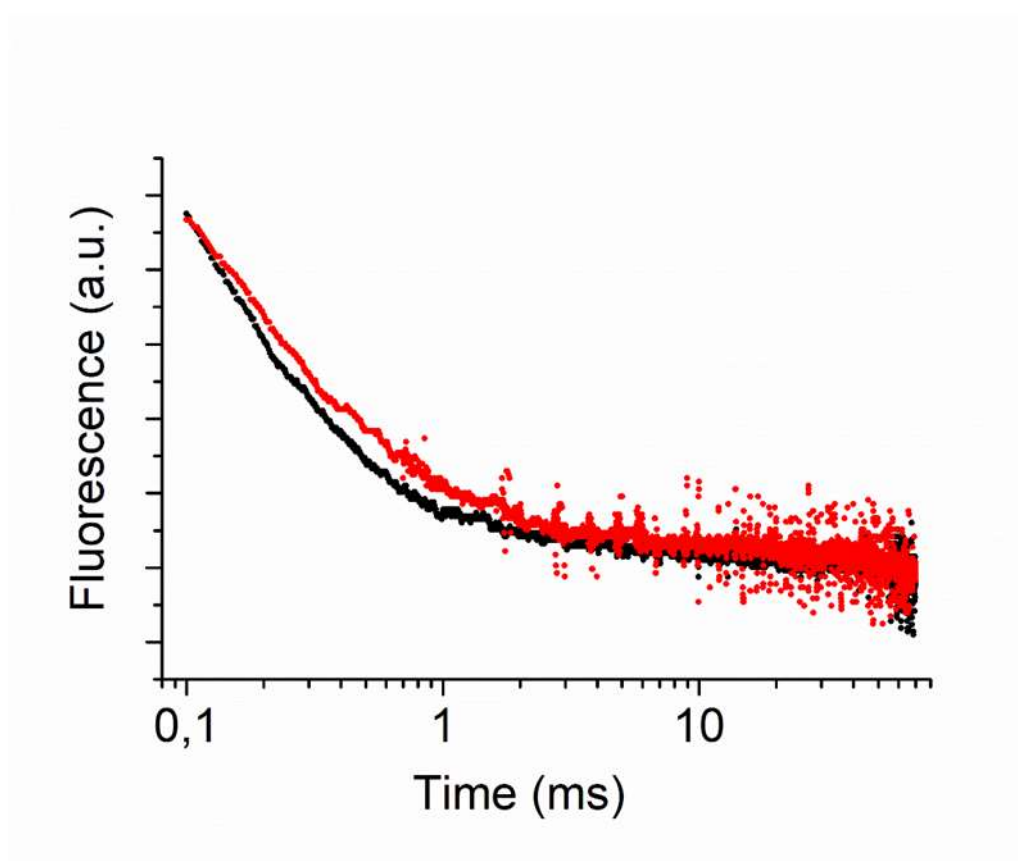
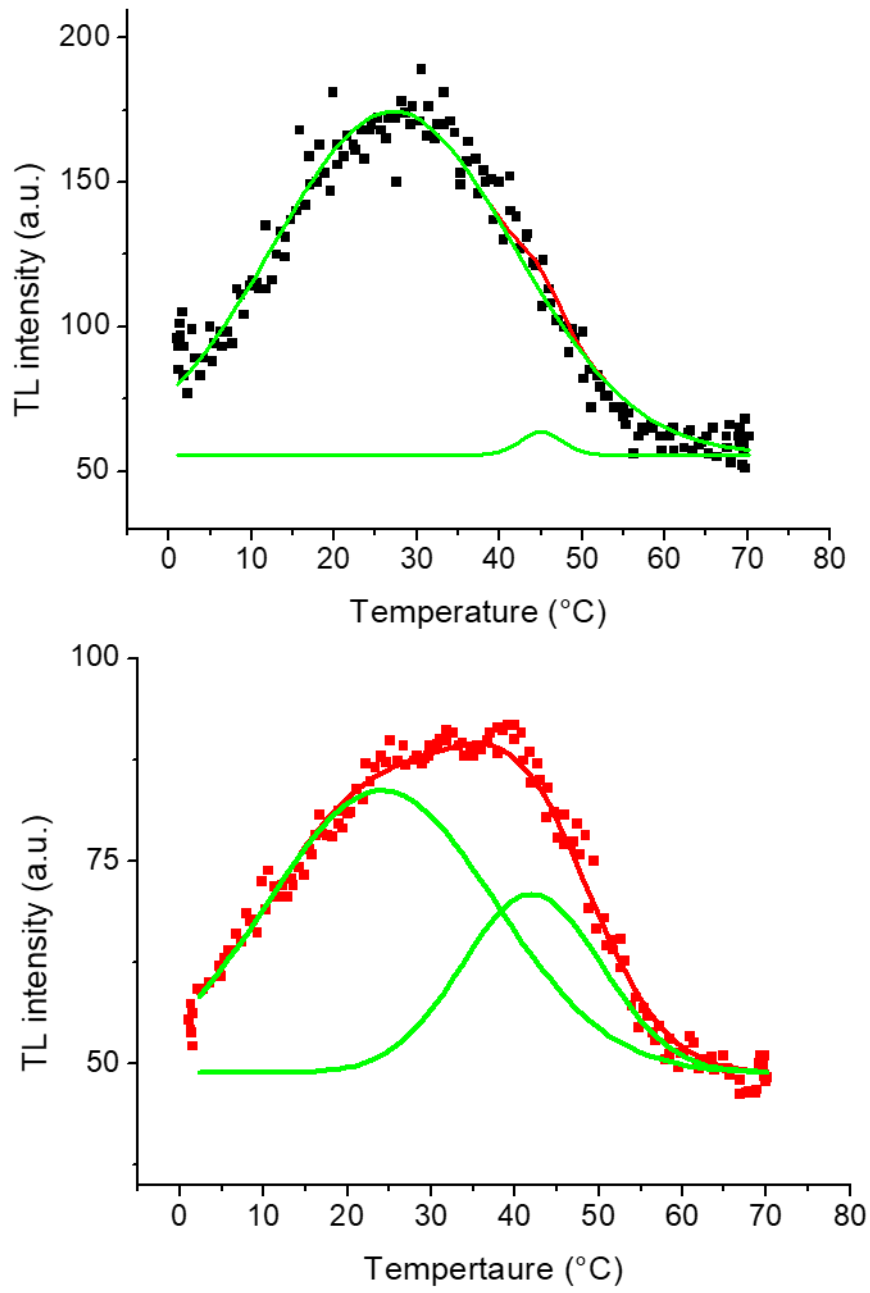


Figure 3



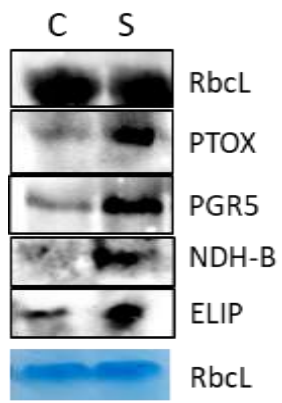
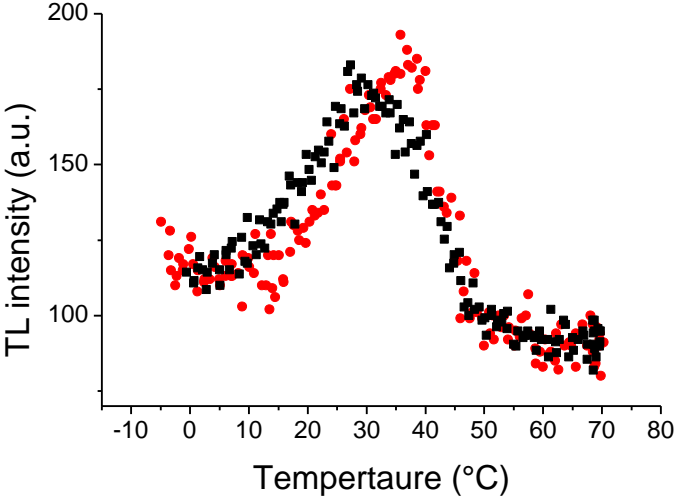


Figure 4

Figure 5



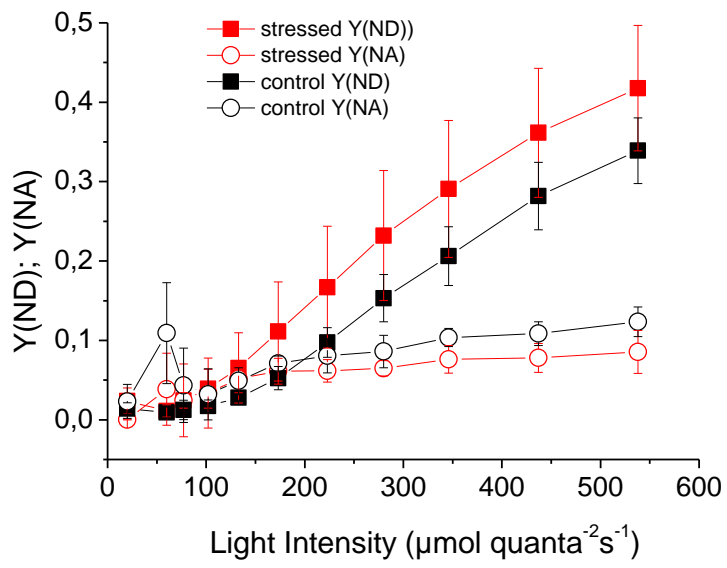


Figure 6

Figure 7

