Non-photochemical fluorescence quenching and quantum yields in PS I and PS II: Analysis of heat-induced limitations using Maxi-Imaging-PAM and Dual-PAM-100

Ulrich Schreiber and Christof Klughammer

1 Introduction

Different forms of non-photochemical quenching (NPQ) can be induced by illumination depending on light intensity and the physiological state of the investigated sample. At moderate light intensities in leaves mostly energy-dependent quenching is observed that increases with internal acidification of the thylakoids. At a given light intensity, internal acidification is favored by a high rate of electron flow and counteracted by efficient transduction of the ΔpH by ATP synthesis. NPQ is known to reflect dissipation of excess radiation energy by heat emission. Whether a given photon flux density (PAR) is excessive for a particular plant or not, depends on its physiological state. Environmental stress lowers the threshold above which PAR becomes excessive.

The most stress-sensitive components of the photosynthetic apparatus are the reversible ATP-ase and the Calvin-Benson cycle. When these key functions are affected, CO_2-reduction is suppressed and alternative electron transport pathways are stimulated, particularly the Mehler-Ascorbate-Peroxidase cycle (MAP cycle, water-water cycle; Schreiber *et al*. 1995, Asada 1999). As the MAP cycle does not require ATP (in contrast to Calvin cycle), a large ΔpH can be formed, which is reflected by high NPQ. The other way around: when at constant light intensity stimulation of NPQ is observed, this often indicates suppression of CO_2-reduction and stimulation of the MAP cycle. Alternatively, increased NPQ can also be caused by decreased efficiency of ATP synthesis. In any case, stimulation of NPQ is a convenient "indicator" for excess excitation energy that cannot be used in CO_2 fixation.

The same transthylakoidal ΔpH which causes NPQ, also controls the rate of electron transfer from PS II to PS I and, hence, acts as a limiting factor at the donor side of PS I. Here we present comparative measurements on the same heat treated samples using the Maxi-Imaging-PAM Chlorophyll Fluorometer for visualization of heat-induced heterogeneities in PS II parameters and the Dual-PAM-100 for assessment of PS I parameters.

2 Experiments

2.1 Material and Methods

Plant material. Detached, field-grown rose leaf featuring 5 pinna.

Heat treatment. Four of the 5 pinna were heated for 5 min at 44, 46, 48 and 50°C by submersion of the tip parts in a constant temperature water bath. After treatment the leaf was kept at room temperature between moistured tissue. The experiments were carried out between 1 and 3 h after the treatment.

Instruments. Measurements were carried out with the Maxi-version of the Imaging-PAM and the Dual-PAM-100 (Heinz Walz GmbH).

2.2 Measurements

2.2.1 Fo, Fm and Fv/Fm images measured with Maxi-Imaging-PAM

Five different areas of interest (AOI) were defined in the 5 tip regions of the pinnate leaf. An Fo-Fm measurement was carried out (Saturation Pulse applied following dark-adaptation), resulting in images of Fo, Fm and Fv/Fm. The observed heat effects on these parameters were relatively weak (not shown in the figures), suggesting that PS II reaction centers are not severely damaged by the applied heat doses. With increasing treatment temperature there is a tendency for an increase of Fo, decrease of Fm and decrease of Fv/Fm.

2.2.2 Dark-light Induction Curve measured with Maxi-Imaging-PAM

Information on reaction steps beyond PS II can be obtained *via* dark-light induction curves. In particular, the kinetics and relative extent of non-photochemical quenching allows conclusions on transduction of the transthylakoidal proton gradient in ATP-synthesis and utilization of ATP in the Calvin-Benson cycle. Fig. 1 shows a standard dark-light Induction Curve recorded with the heat-treated rose leaf. A moderate actinic light intensity (70 µmol quanta/(m^2·s) was applied in order to assure light limitation at steady state illumination. For the sake of clarity only 3 out of 5 AOIs and only the parameters Fm', and qN (coefficient of non-photochemical quenching) were selected for display.

In the control qN first increases and then declines towards the steady-state. This may be considered the "fingerprint" of a physiologically healthy sample with normal CO_2 fixation activity. While the increase is driven by O_2-

PAM Application Notes (2008) 1: 15 - 18

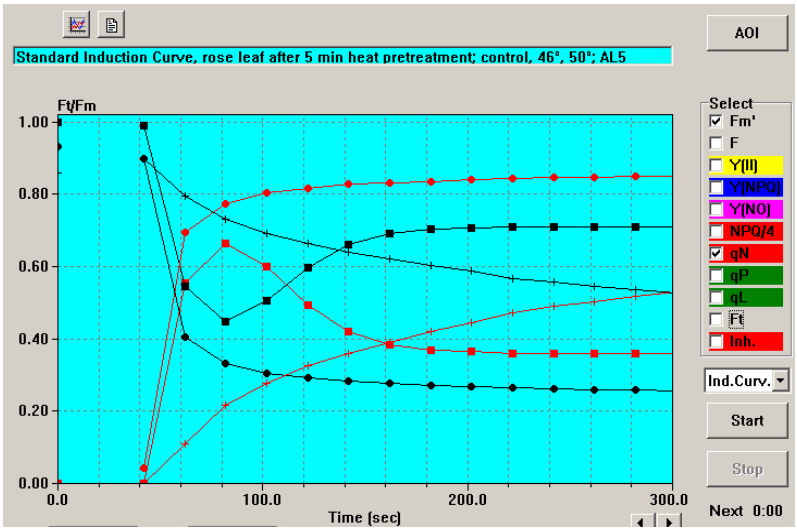

Fig. 1. Effect of 5 min heat pretreatment on dark-light Induction Curve of rose leaf. Displayed parameters are Fm' (in black) and qN (in red). Three AOI were selected on control (squares), 46°C treated (diamonds) and 50°C treated (crosses) parts of the leaf.

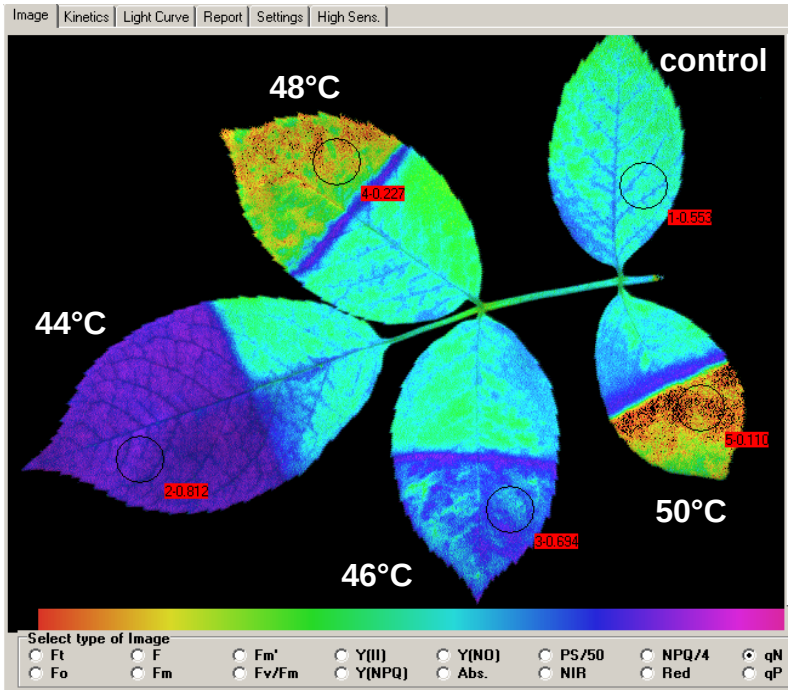

Fig. 2. Effect of 5 min heat pretreatment on light-induced non-photochemical quenching (qN parameter). Tip parts of the various leaflets submersed for 5 min into water at the indicated temperatures. Image taken 20 sec after onset of AL at 70 µmol quanta/(m²·s). The false color scale ranges from 0.000 (black) to 0.999 (purple).

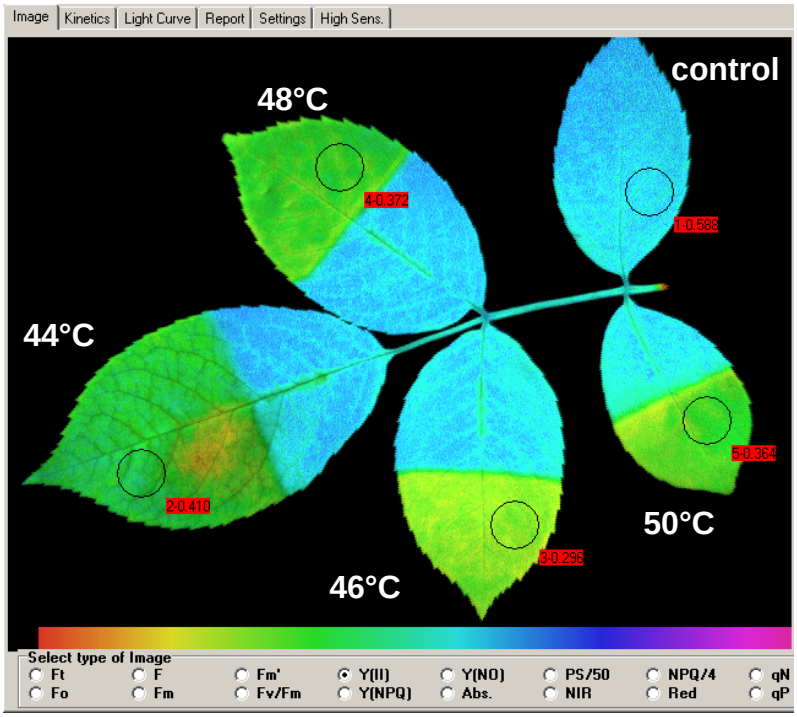

Fig. 3. Effect of 5 min heat pretreatment on effective PS II quantum yield, Y(II). Sample identical to that of Figs. 1 and 2. Image taken after 4 min illumination at 70 µmol quanta/(m²·s) (steady-state).

PAM Application Notes (2008) 1: 15 - 18

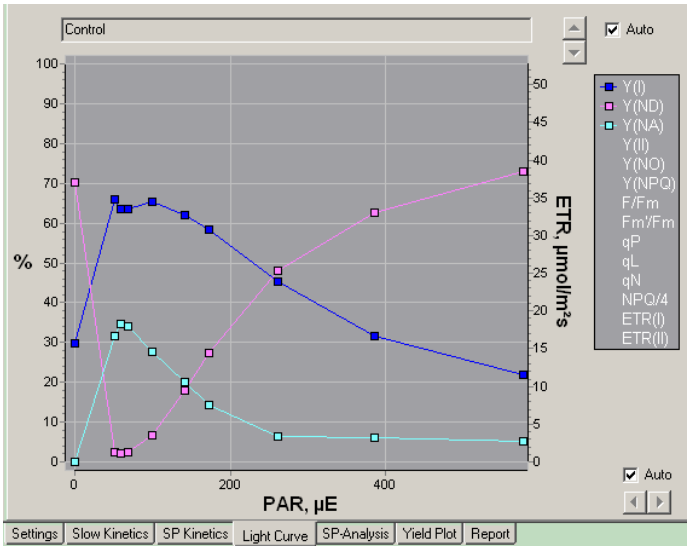

Fig. 4. Light response curve of complementary PSI quantum yields Y(I), Y(ND) and Y(NA) in the control.

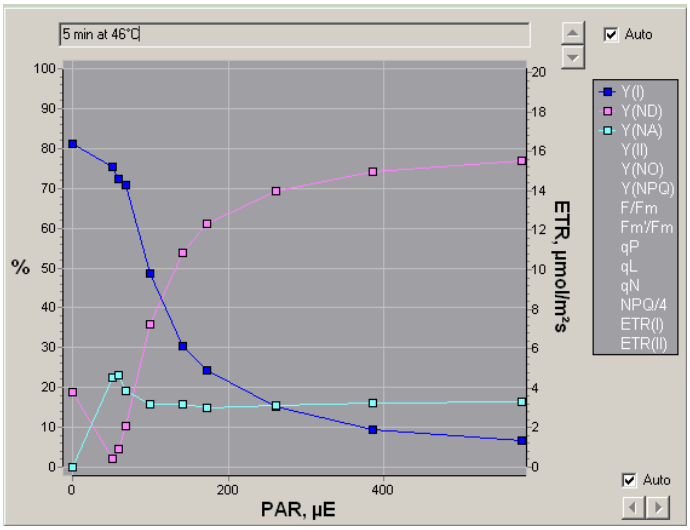

Fig. 5. Light response curve of complementary PSI quantum yields Y(I), Y(ND) and Y(NA) in sample area heated for 5 min at 46°C.

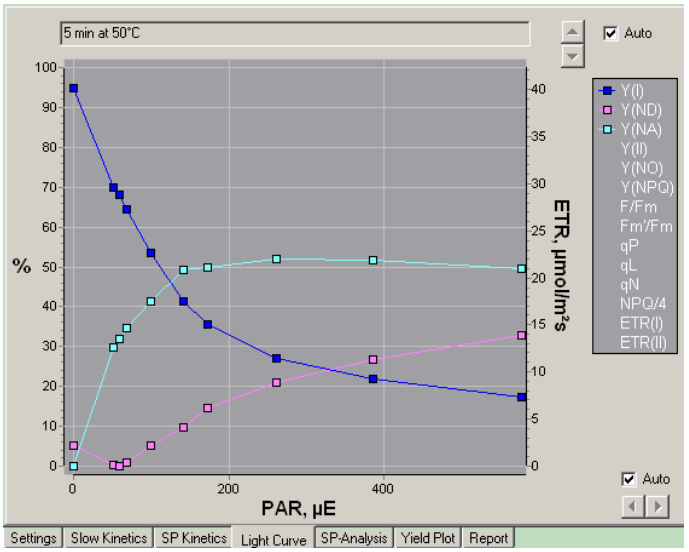

Fig. 6. Light response curve of complementary PSI quantum yields Y(I), Y(ND) and Y(NA) in sample area heated for 5 min at 50°C.

dependent electron flow (MAP cycle) before Calvin-Benson cycle is light-activated, the decline reflects onset of CO_2 dependent electron flow, which consumes ATP and thus stimulates utilization of the ΔpH in ATP synthesis. Heat pretreatment affects both the first rise and the secondary decline. After 5 min at 46°C the increase is stimulated and the secondary decline suppressed, resulting in high qN. In contrast, after 5 min at 50°C the increase is substantially slowed down.

It may be concluded that:

a) Mild heat stress inhibits CO_2 dependent electron flow and at the same time enhances O_2 dependent electron flow, which is responsible for internal acidification, which again stimulates non-photochemical quenching. The latter reflects down-regulation of PS II and protection against damage by excess PAR.

b) Strong heat stress suppresses O_2 dependent electron flow and thus inhibits the protection mechanism reflected by NPQ.

Fig. 2 shows the screenshot of a qN image taken 20 s after onset of illumination in the course of the Induction Curve presented in Fig. 1. The strong stimulation of non-photochemical quenching in the 44°C and 46°C treated tip areas are obvious, as well as its suppression in the 48°C and 50°C samples. It may be noted that the latter two display a band of highly stimulated qN between the heated and non-heated parts, where the effective temperature presumably was 44-46°C.

During a standard Induction Curve images are measured repetitively every 20 s and a large amount of information is collected on heterogeneities in fluorescence parameters. Comparison of the images taken after different illumination times reveals that qN is a dynamic parameter that is controlled by complex regulation mechanisms. Additional information may be obtained by analyzing the images of various other fluorescence parameters, like Y(II).

Fig. 3 show a Y(II) image taken close to the steady state after 4 min illumination. Comparison with the qN image reveals that maximal stimulation of qN in the 46°C sample goes along with maximal suppression of Y(II). While the heat induced damage should further increase in the 48 and 50°C samples, the steady-state Y(II) values are higher. This finding is in agreement with the current concept of non-photochemical quenching reflecting an effective mechanism of down-regulation of PS II.

2.2.3 Measurements with the Dual-PAM-100

The Dual-PAM-100 allows simultaneous measurements of Chl fluorescence and P700 absorbance changes. P700 provides analogous information on PS I as Chl fluorescence provides on PS II. A special Saturation Pulse method was developed for assessment of PS I quantum yield (Klughammer and Schreiber 1994). With alternative emitter-detector modules besides Chl fluorescence and P700 various other parameters can be measured that pro-

vide additional information on photosynthesis (e.g. Acridine fluorescence, NADPH fluorescence and P515 electrochromic shift).

In the following, some measurements with the Dual-PAM-100 are presented, using the same sample as for the experiment of Figs. 1-3. The effect of heat treatment on the properties of PS I quantum yield is analyzed, which can be limited either by the donor side (P700 oxidized) or by the acceptor side. Based on the original concept of excitation energy partitioning in PS II of Kramer *et al*. (2004) three complementary quantum yields are defined for PS I:

Y(I) + Y(ND) + Y(NA) = 1

Y(I) Photochemical quantum yield.

Y(ND) Quantum yield of non-photochemical energy dissipation in PS I due to donor side limitation.

Y(NA) Quantum yield of non-photochemical energy dissipation in PS I due to acceptor side limitation.

The light intensity dependence of these complementary quantum yields was measured with the help of automated light response curves (Light Curves). Screenshots of the Light Curves measured with control, 46°C and 50°C treated samples are presented.

In the control, at low PAR energy conversion at PS I reaction centers is limited by the acceptor side, as Y(NA) > Y(ND). At higher PAR values (in the given example above 100 µmol quanta/ m^2s), an increase of Y(ND) indicates increasing donor-side limitation, while at the same time Y(NA) declines to a low level. The decline of photochemical quantum yield, Y(I), at high PAR is correlated with a complementary rise of Y(ND), which is characteristic for a physiologically healthy, well-regulated sample.

The light response characteristics are substantially changed by heat treatment and distinctly different in the case of 46°C and 50°C treated samples, as shown in Figs. 5-6.

Acceptor side limitation of PS I, as reflected by the Y(NA) parameter, is increased by mild heat stress (almost by a factor of 3 in the steady-state) and substantially further enhanced by strong heat stress. Conversely, donor side limitation, as reflected by the Y(ND) parameter, is strongly stimulated by mild heat stress and suppressed by strong heat stress.

The P700 measurements confirm the conclusion derived from fluorescence measurements that mild heating inhibits CO_2 dependent electron flow. This leads to a limitation at the PS I acceptor side, as reflected by an increase in Y(NA). While the alternative O_2-dependent electron flow displays a lower capacity, it induces a large ΔpH that not only causes down-regulation of PS II (reflected by non-photochemical quenching), but also down-regulation of PS I, as reflected by high Y(ND). A large ΔpH acts like a brake on intersystem electron flow, as electron transfer from reduced plastoquinone *via* the cytochrome bf complex is obligatorily coupled to proton translocation into the acidic intra-thylakoid space.

2.2.4 Outlook

During the past 20 years countless studies have been carried out using PAM fluorometers on the protective role of down-regulation of PS II. It has been shown that without such protection excess energy leads to formation of triplet excited states and reactive oxygen species. The question arises whether any protective role can be attributed to the observed down-regulation of PS I as well. Measurements with the Dual-PAM-100 should help to clarify this question.

In addition to the presented type of measurements, a wide range of other measurements can be carried out on the same sample with the Dual-PAM-100 that provide additional information:

- P700 reduction kinetics following single turnover saturating flash in the presence of far-red background light giving information on intersystem electron transport.
- Polyphasic fluorescence rise upon onset of saturating continuous illumination.
- Fluorescence relaxation kinetics following a saturating single turnover flash, which reflect the reoxidation kinetics of the primary acceptor Q_A.
- Using the P515 emitter-detector module: Measurement of the flash induced electrochromic absorbance shift and its relaxation kinetics.

3 References

[1] Schreiber U, Hormann H, Asada K and Neubauer C (1995) O_2-dependent electron flow in spinach chloroplasts: Properties and possible regulation of the Mehler-Ascorbate Peroxidase Cycle. In: Photosynthesis: from Light to Biosphere. (Mathis P ed), Vol II, pp 813-818, Kluwer Academic Publishers, Dordrecht

[2] Asada K (1999) The water-water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. Ann Rev Plant Physiol Plant Mol Biol 50: 601-639

[3] Klughammer C and Schreiber U (1994) An improved method, using saturating light pulses, for the determination of photosystem I quantum yield *via* $P700^+$-absorbance changes at 830 nm. Planta 192: 261-268

[4] Kramer DM, Johnson G, Kiirats O and Edwards GE (2004) New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes. Photosynth Res 79: 209-218