DETECTION OF PLANT STRESS BY CHLOROPHYLL FLUORESCENCE

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Abstract: Plant growth and development are dynamic processes which are continuously changed by environmental conditions. Agricultural crops are subjected to abiotic stresses, such as increasing temperature, water, salinity, heavy metals and ozone. Nowadays, modern non-invasive methods allow rapidly monitor and evaluate plant stress responses. Chlorophyll fluorescence imaging has become one of the most powerful and popular tools to track changes in the photosynthetic capacities of plants in response to abiotic and biotic factors. In contrast to traditional methods, chlorophyll fluorescence is less laborious, less time consuming and thereby highly useful for large scale screening experiments. Here, we show its application in the evaluation of *Arabidopsis thaliana* plants in response to various abiotic stressors.

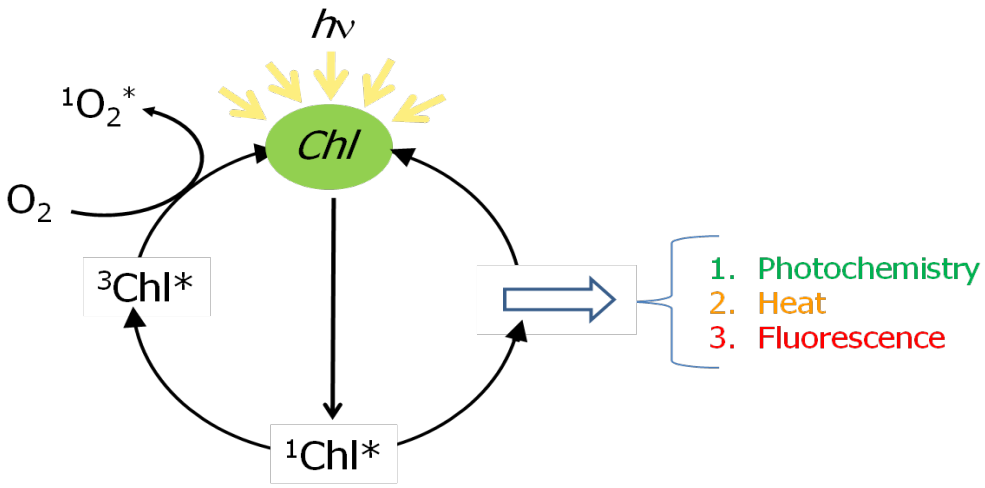
Keywords: chlorophyll fluorescence, stress, salinity, cytokinin, cadmium, heat stress

INTRODUCTION

Chlorophyll is the ubiquitous plant green pigment fundamental for photosynthesis. In a simplified overview, light energy absorbed by chlorophylls associated with photosystem II (PSII) is used to drive photochemistry in which an electron is transferred from the reaction centre (P680) to the primary quinone acceptor (QA) of PSII. Alternatively, absorbed light energy can be lost from PSII as chlorophyll fluorescence or heat. The processes of photochemistry, chlorophyll fluorescence, and heat dissipation are in the direct competition for excitation energy. If the rate of one process increases the rates of the other two will decrease (Figure 1) (Baker 2008). The amount of fluorescence is measured using fluorometer and the initial or absolute value is measured in the absence of light (Baker, Rosenqvist 2004). It is used to monitor the process of photosynthetic energy conversion in plants in order estimate plant´s biosynthetic performance (Bresson et al. 2015).

The parameters employed in many fields of plant physiology could be obtained from pulse amplitude modulation (PAM) of the chlorophyll emission (e.g. Novák et al. 2013, Baldrianová et al. 2015, Novák et al. 2015). In this analysis, the dark adapted leaf is suddenly exposed to light which causes a huge change in value of fluorescence. The PAM-fluorescence analysis provides both qualitative and quantitative information on organization and function of photosynthetic apparatus. One of parameters that can be used to evaluate plants stress response is NPQ (non-photochemical quenching). It correlates with the major process involved in protection against photodamage. In this mechanism, zeaxanthin dissipates the excess energy in chloroplasts via non radiative processes which alleviates excitation pressure (Gray et al. 1996) on PSII centres by diverting light energy into heat and reduces the relative quantum yield of PSII in order to maintain an adequate balance between photosynthetic electron transport and carbon metabolism. Since the chlorophyll fluorescence can give insights into the ability of a plant to tolerate environmental stresses and into the extent to which those stresses have damaged the photosynthetic apparatus, it can be an excellent tool to study stress-induced changes in PSII, which is believed to play a key role in the response of leaf photosynthesis to environmental stresses (Zribi et al. 2008).

Figure 1 Simple model of the possible fate of light energy absorbed by PSII.



MATERIAL AND METHODS

Experiments were performed using *Arabidopsis thaliana* ecotype Col–0 as wild-type. For salinity and hormone experiments, seeds were sown on the surface of 90-mm Petri dishes containing wet filter paper. The Petri dishes containing seeds were kept in the dark at 4°C for 2 days, and then the seeds were transferred into 6×6×6 cm pots containing soil substrate. The plants were cultivated in the greenhouse with day/night temperature 23°C/19°C. After seven days, plants were watered with NaCl (80–120 mM) or 6-benzylaminopurine (BAP) (1–25 µM) up to soil field capacity (~100 ml). The treatment was repeated twice a week for 28 days.

The plantlets for cadmium toxicity were cultivated as follows. Seeds were surface-sterilized and cca 100 seeds per a 12cm square Petri dish were sown on a polyamide mesh (Uhelon 120 T, Silk&Progress, Czech Republic) placed on 1% (w/v) agar solidified half-strength Murashige and Skoog (MS) medium (pH 5.7). Seeds were kept in the dark at 4°C for 2 days and cultivated vertically in a growth chamber (AR36LX, Percival) under a long-day 16 h light/8 h dark cycle at day/night temperature 21°C/19°C, 60% relative humidity, and light intensity 120–150 µmol $m^{-2}s^{-1}$. For treatments, the mesh with 14 days-old plants was shortly immersed in liquid medium (with composition corresponding to new medium) and transferred onto new medium supplemented with 200 µM $Cd(NO_3)_2$ and left to grow for 14 days.

For the heat experiments, the *Arabidopsis* seedlings cultivated as described above were transferred into seed holders containing 0.7% agar and grown hydroponically on liquid half-strength Murashige and Skoog (MS) medium (pH 5.7). Then, cultivated in a growth chamber (AR36LX, Percival) under day 12 h light/12 h dark cycle at day/night temperature 21°C/19°C, 60% relative humidity, and light intensity 120–150 µmol m^{-2} s^{-1} After 28 days, plants were subjected to heat stress (i) SH (shoots and roots 40°C), (ii) S (shoots 40°C and roots 21°C), and (iii) R (shoots 21°C and roots 40°C). Plants were analysed after 20, 90 and 180 minutes of heat stress.

Chlorophyll fluorescence emission from the upper surface of the leaves of intact plants was measured by a modulated fluorometer in which Maximum quantum efficiency of PSII in the dark (QY–max), max efficiency of PSII in light (Fv/Fm–Lss), the quantum efficiency of PSII electron transport at steady state (QY), the photochemical quenching (QP), and None photochemical quenching at the steady state (NPQ) were recorded using a FluorCam 700MF imaging system (Photon Systems Instruments, Czech Republic).

RESULTS AND DISCUSSION

We evaluated fluorescence parameters in response to four contrasting stressors: salinity, heavy metal toxicity, heat, and hormonal toxicity. These factors are relevant to plant breeding and biotechnology, as it is of paramount interest to produce plants resistant to salinity (arid regions),

heavy metal ions (phytoremediation) or heat (global warming). The hormonal stimuli represent model of toxicity arising e.g. from fertilization.

In our experiment, only NPQ value was increased by salinity stress. Parameters QY–max, Fv/Fm–Lss, or QP were not significantly affected (Table 1).

Table 1 The effect of NaCl on chlorophyll fluorescence

	QY–max	Fv/Fm–Lss	QY	NPQ	QP
control	0.86 ± 0.01	0.71 ± 0.02	0.19 ± 0.03	1.53 ± 0.10b	0.27 ± 0.04
80mM	0.87 ± 0.01	0.7 ± 0.01	0.18 ± 0.02	1.82 ± 0.05a	0.25 ± 0.03
100mM	0.87 ± 0.01	0.69 ± 0.01	0.16 ± 0.02	1.86 ± 0.04a	0.24 ± 0.02
120mM	0.88 ± 0.01	0.68 ± 0.01	0.17 ± 0.02	1.75 ± 0.05a	0.24 ± 0.03

Most plant hormones circulate in low nanomolar concentrations. Thus, the exogenously supplied micromolar concentrations will disturb the homeostasis. Indeed, in our experiments, we observed a significant decrease in QY, and significant changes in NPQ and Qp.

Table 2 The effect of exogenous BAP application

	QY–max	Fv/Fm–Lss	QY	NPQ	Qp
control	0.86 ± 0.01	0.71 ± 0.02	0.19 ± 0.03b	1.53 ± 0.34c	0.27 ± 0.04b
1μM	0.85 ± 0.00	0.71 ± 0.03	0.23 ± 0.05a	1.45 ± 0.27d	0.33 ± 0.06a
5μM	0.85 ± 0.01	0.70 ± 0.01	0.22 ± 0.05a	1.50 ± 0.19c	0.31 ± 0.07a
10μM	0.86 ± 0.01	0.69 ± 0.04	0.22 ± 0.05a	1.78 ± 0.21a	0.26 ± 0.06c
15μM	0.86 ± 0.01	0.70 ± 0.01	0.22 ± 0.01a	1.57 ± 0.03b	0.31 ± 0.01a
25μM	0.85 ± 0.01	0.70 ± 0.03	0.22 ± 0.02a	1.52 ± 0.26c	0.31 ± 0.02a

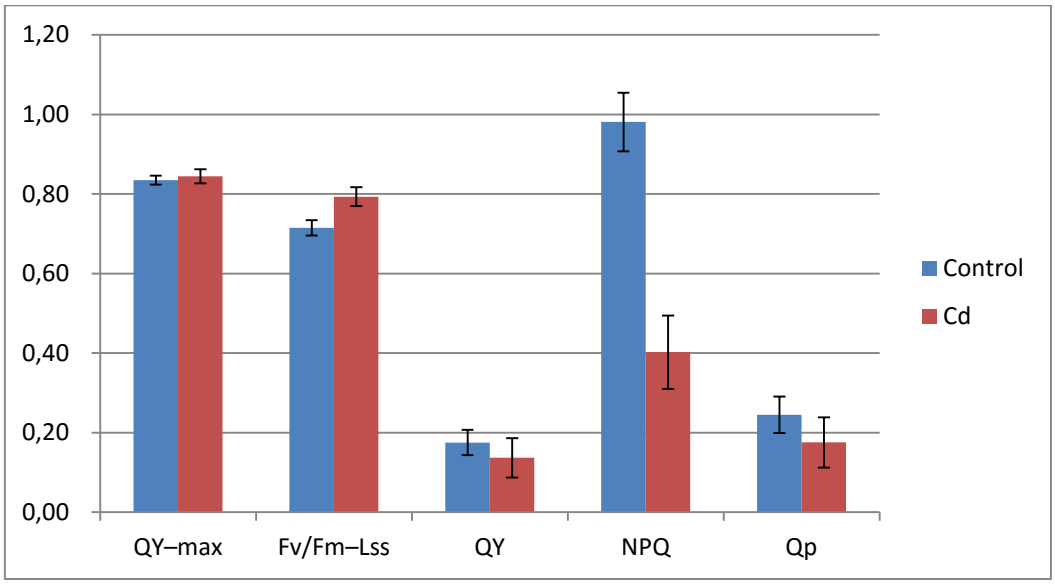
As illustrated in Table 3, heat stress treatments affected values of QY, NPQ and QP.

Table 3 The effect heat stress on chlorophyll fluorescence

	Time (min)	QY–max	Fv/Fm–Lss	QY	NPQ	Qp
control	0	0.9 ± 0.01a	0.81 ± 0.02a	0.46 ± 0.06c	1.32 ± 0.15a	0.57 ± 0.06c
	20	0.84 ± 0.01c	0.75 ± 0.02c	0.60 ± 0.03b	0.69 ± 0.17b	0.79 ± 0.03a
SR	90	0.86 ± 0.01b	0.82 ± 0.01a	0.66 ± 0.04a	0.28 ± 0.06d	0.8 ± 0.04a
	180	0.85 ± 0.01c	0.82 ± 0.01a	0.64 ± 0.04a	0.28 ± 0.06d	0.78 ± 0.05a
	20	0.87 ± 0.01b	0.82 ± 0.02a	0.65 ± 0.04a	0.47 ± 0.13bc	0.78 ± 0.04a
S	90	0.87 ± 0.01b	0.84 ± 0.01a	0.69 ± 0.03a	0.22 ± 0.04d	0.82 ± 0.03a
	180	0.87 ± 0.01b	0.84 ± 0.02a	0.67 ± 0.02a	0.33 ± 0.08d	0.8 ± 0.02a
	20	0.88 ± 0.01b	0.81 ± 0.02a	0.63 ± 0.04b	0.76 ± 0.18b	0.78 ± 0.03a
R	90	0.87 ± 0.01b	0.81 ± 0.02a	0.61 ± 0.04b	0.62 ± 0.17c	0.75 ± 0.03b
	180	0.87 ± 0.01b	0.81 ± 0.02a	0.62 ± 0.03b	0.59 ± 0.12c	0.76 ± 0.02b

In our experiment, cadmium ions had no effect on maximum efficiency of PSII in dark, induced a low increase in Fv/Fm–Lss and a significant decrease in NPQ (Figure 2).

Figure 2 The effect of Cd on chlorophyll fluorescence



PSII efficiency and excitation energy dissipation in leaves were examined by modulated fluorescence techniques. QY estimates directly the efficiency of light used for electron transport by PSII. A major factor determining this efficiency is the ability of the leaf to remove electrons from the quinone acceptors of PSII (Baker, Rosenqvist 2004), while the excitation capture efficiency of PSII has been shown to reflect the efficiency with which excitation energy reaches to PSII reaction centres (Schulze, Caldwell . 1995). The decrease in Fv/Fm–Lss could be attributed to two possible factors. One is the decrease in the maximal efficiency of PSII photochemistry (QY–max) which may be caused by damage in the PSII reaction centres. The other is an increase in the non-photochemical quenching deactivation of PSII (Genty et al. 1990) which serve to reduce the rate of excitation of the PSII reaction centres and prevent the PSII quinone acceptors from becoming highly reduced (Baker, Rosenqvist 2004). In the conditions of our experiment, QY–max was not affected by any stress treatment after 28 days indicating that the decrease in Fv/Fm–Lss was caused mainly by the increase in energy dissipation in the antennae.

CONCLUSION

In this work, we utilize modern non-invasive technique to evaluate four different stressors. Our results confirm that the fluorescence measurement is useful tool to monitor responses to different abiotic stressors.

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