

## CHLOROPHYLL A FLUORESCENCE AS A BIOINDICATOR OF THE PLANT ENVIRONMENTAL STRESS

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### Summary

In the work with barley plants acclimated to different light regimes we demonstrate that although light is an essential source of energy for photosynthesis but can also be harmful to plants. The presented data show potential of chlorophyll fluorescence measurements for analysis of photosynthetic performance and plant environmental stress under natural conditions.

**Key words:** barley, photosynthesis, chlorophyll a fluorescence, quenching analysis

### Introduction

Plants are in natural environment exposed to various kinds of natural and anthropogenic stress factors (Lichtenthaler 1996). The quantification of plant stress status is a long-term study problem that affects many physiological processes. Exposure of green plants to strong light and frequently to other environmental stresses potentially results in photoinhibition of photosynthesis (Osmond 1994; Critchley 1998).

Measurement of chlorophyll a fluorescence has become a very useful technique for obtaining a rapid quantitative and qualitative information about photosynthesis, mainly in case when gas-exchange measurements reach their limits (compensation CO<sub>2</sub> point). Progress in understanding and practical use of chlorophyll fluorescence in area of plant ecophysiology was made by development of proper instruments (Schreiber *et al.* 1986) establishment of methodology and terminology. Chlorophyll fluorescence has been widely used for quantify a photochemical efficiency of PSII and electron transfer of many crops, woody shrubs and trees and to assess physiological impairment of vegetables and fruits during storage (Earl and Tollenaar 1999; DeEll *et al.* 1999).

The objective of presented work is to give a look into the modification of light photosynthetic responses in natural conditions. We show a protocol of evaluation of photochemical and non-photochemical fluorescence quenching induced by stress factors, mainly strong irradiation.

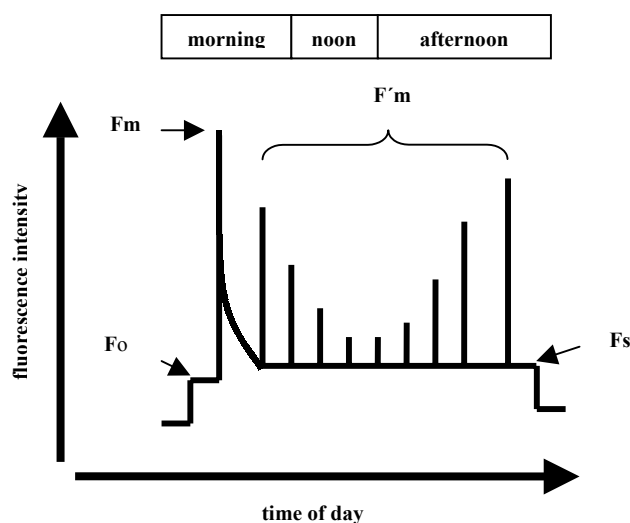
### Material and methods

#### Plant material

Barley plants (*Hordeum vulgare* L., cv. Kompakt) were cultivated naturally in artificially regulated micro-ecosystems in 20 kg pots with soil substrate and watered regularly. As soon as the first leaf had emerged the plants were placed under the shields and cultivated for the rest of vegetation period to be acclimated for two different light regimes, as follows: (i) sun adaptation regime - diurnal incident photon flux density (maximal PPFD level of 1500  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and (ii) shade adaptation regime - 25% PPFD of the sun adaptation (maximal PPFD level of 300  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ).

#### Chlorophyll a fluorescence measurement

Daily modulation of photochemical PSII efficiency were measured by MiniPam fluorometer (Walz, Effeltrich, Germany). An intact leaf was inserted into the leaf-clip holder (2030-HB; Walz, Effeltrich, Germany) and connected to fluorometer. Minimal fluorescence yield ( $F_0$ ) was measured by modulated red light beam with low light intensity ( $0,15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). Maximal fluorescence yield ( $F_m$ ) was determined by application of 800 ms saturation white light pulse of  $7000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  intensity. Both determined parameters were measured pre-dawnly (4.00am). Plants were then illuminated by natural daily light. The steady state value of chlorophyll a fluorescence ( $F_s$ ) was thereafter recorded and the next saturating pulses were applied each hour to determine the maximal fluorescence yield ( $F'_m$ ) in the light adapted leaves (for detail see figure 1). The following calculations were made, such as: (i) maximal photochemical PSII



**Figure 1:** Sequence of the determination of various chlorophyll a fluorescence parameters in barley leaves in the course of the natural day using the MiniPam.

efficiency,  $F_v/F_m$ , after a 30-minute dark adaptation, where  $F_v = F_m - F_o$ ; (ii) actual photochemical PSII efficiency,  $\Delta F/F_m$  according to Genty *et al.* (1989); (iii) electron transport rate,  $ETR = \Delta F/F_m \cdot I_{ABS} \cdot 0.5$ , where  $I_{ABS} = PPFD \cdot 0.84$  is the intensity of absorbed light energy by a leaf, 0.5 reflected that two photons must be absorbed by PSII and PSI per one transported electron and 0.84 represents an absorption coefficient of a leaves; (iv) photochemical,  $qP = (F_m' - F_s)/(F_m' - F_o)$  and (v) non-photochemical fluorescence quenching,  $NPQ = (F_m - F_m')/F_m'$ .

The response of photosynthetic apparatus to increasing PPFD was measured using a light curve with 3-min interval of individual light intensities of PPFD (50-, 100-, 200-, 400-, 550-, 750-, 1000-, 1500- and 2000  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) which were generated by external halogen lamp (Walz, Effeltrich, Germany) connected to leaf-clip holder. All light curve measurements were made in laboratory conditions after previous dark leaf adaptation (30 min.).

## Results and discussion

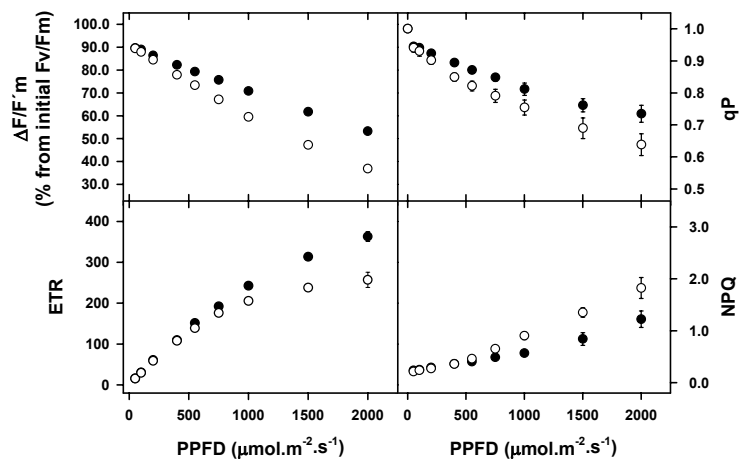
Plants are generally acclimated to different growing environments on morphological, physiological and molecular levels (Percy 1998). Photosynthetically active light quanta are absorbed by the light-harvesting complexes of both PSII and PSI photosystems. The interpretation of chlorophyll fluorescence changes used in account of plant environmental stress is greatly facilitate by the fact, that at room temperature fluorescence originates almost exclusively from PSII.

Light responses of barley leaves to increasing PPFD (known as a light curve) are used to quantify the limits of photosynthetic machinery, as well as quenching analysis which determines the efficiency of individual protective mechanisms realized in stress conditions (figure 2). Photochemical quenching ( $qP$ ) refers to photosynthetic activity of electron transport from PSII and non-photochemical quenching ( $NPQ$ ) refers to thermal dissipation of excitation energy from PSII (Krause and Weis 1991). Generally,  $NPQ$  is considered as a mechanism protecting photosynthetic machinery against the excessive light (Critchley 1999).

In conclusions, the presented results of chlorophyll a fluorescence yield detected by a fluorescence technique is very facile and grateful tool for measurement of plant stress state affected by harmful environmental circumstances. We recommend this technique for determination of plant environmental stress in the area of eco-physiology, ecology and breeding.

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**Figure 2:** Response of actual photochemical PSII efficiency ( $\Delta F/F_m$ ), electron transport rate (ETR), photochemical ( $qP$ ) and non-photochemical ( $NPQ$ ) quenchings on increasing PPFD. Full symbols – sun plants, empty symbols – shade plants.