Measurement of Delayed Fluorescence in Plants - a Monitoring System for Stress Factors

Manfred Hennecke€; Bernd Hutter€; Angela Brüx€*

€Communicated by BERTHOLD TECHNOLOGIES GmbH & Co. KG, Calmbacher Str. 22, D-75323 Bad Wildbad, Germany
*Address all correspondence to: angela.bruex@berthold.com

- Delayed Fluorescence as a Way of Monitoring Plant Stress Status
- Mycotic Infections + Drought Tolerance

Abstract

Delayed fluorescence acts as an indicator not only for chlorophyll content, but also for the physiological state of the plant which can vary based on environmental influences such as drought, high saline levels or mycotic infections. In this study we used delayed fluorescence for *in vivo* imaging of plants as a monitoring system for stress factors.

Introduction

In photosynthesis light is absorbed within the photosystem II. The excited reaction center P680, which consist of a "special pair" chlorophyll *a* (P680), reduces pheophytin (Pheo) which then transfers the electron to plastoquinone (PQ) and further down the cascade towards photosystem I. After light excitation of photosystem I, the electron is used to NADPH. In parallel an H^+-gradient is formed that is used for ATP synthesis (Figure 1).

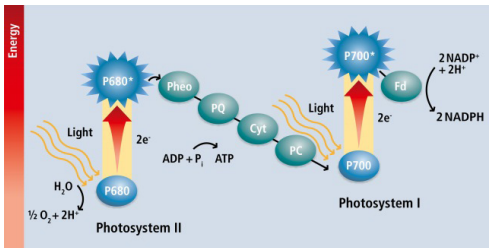

Figure 1: Scheme of Electron Flow from Photosystem II to Photosystem I

Chlorophyll fluorescence is the remaining way for the plant to dissipate excessive light energy collected by the photosynthetic apparatus which cannot be utilized for photosynthesis.

Delayed fluorescence, also called afterglow, is the extremely weak light emitted by pre-illuminated intact plants. It is an ubiquitous and well-studied process in photosynthetic organisms[2] (for review[3]), closely related to the photosynthetic reactions. It is emitted by the chlorophyll *a* molecules with the same emission wavelength as its counterpart prompt fluorescence. The signal of prompt fluorescence lasts for nanoseconds whereas delayed fluorescence can be detected seconds and minutes later. Upon termination of the illumination photons are emitted, presumably as the result of charge recombination between excited PQ and the P680 of photosystem II[3]. Although delayed fluorescence represents only a small portion of the fluorescence emitted, it provides a powerful tool to study stress reactions in plants. Herbicides, pathogens and other stress factors can act on the chloroplasts and thereby alter the delayed fluorescence reaction. Therefore measurement of delayed fluorescence kinetics is a fast and simple way to study the effects of stress factors and to obtain dose-response curves. Here we study the influence of fungal infection and drought on delayed fluorescence and its value as a monitoring system for stress factors on plants.

Experimental Procedures and Instrument Settings

Mycotic Infection

Delayed fluorescence was measured in tomato leaves 8 days post infection with a fungus. Leaves were cut into discs, inserted into a 24 well plate and illuminated for 30 s with a LED panel. Immediately

after switching off the light delayed fluorescence was measured using a Berthold Technologies NightSHADE. Exposure time was 20 s using a pixel binning of 4 by 4. Intensities of light were converted into counts per second (cps) with indiGO™ software (Figure 2).

Drought Tolerance

Soy bean plants were illuminated for 30 s with a LED panel. Immediately after switching off the light delayed fluorescence was measured in NightSHADE. Exposure time was 30 s using a pixel binning of 4 by 4. Subsequently 50 % of the plants were kept dry whereas the other 50 % of the plant were watered. The plants were measured again after 2 days. Intensities of light were converted in counts per second (cps) with indiGO™ software (Figure 2).

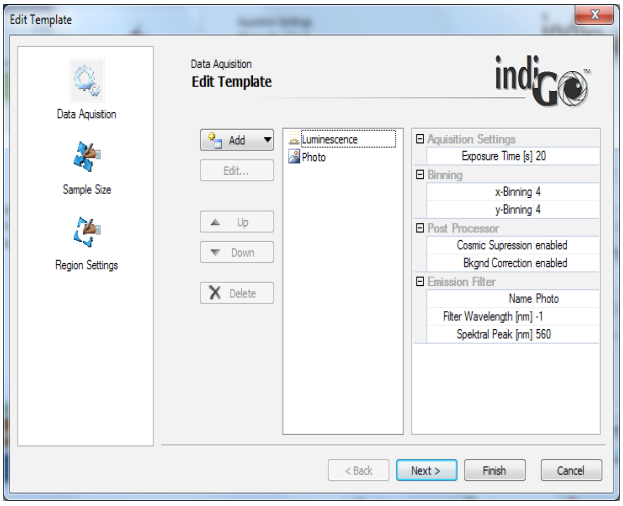

Figure 2: Instrument Settings in indiGO™ software: Template for delayed fluorescence measurement with defined settings.

Material

- NightSHADE LB 985 (figure 3)
- LED panel
- 24 well micro plate

Results

Mycotic infections

Untreated tomato leaves exhibited strong signals of delayed fluorescence as a direct indicator for chlorophyll content. On the contrary infected leaves did not display any delayed fluorescence signals (Figure 4).

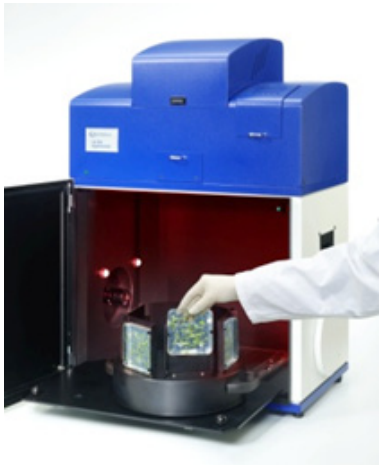

Figure 3: NightSHADE LB985 Plant Imaging System

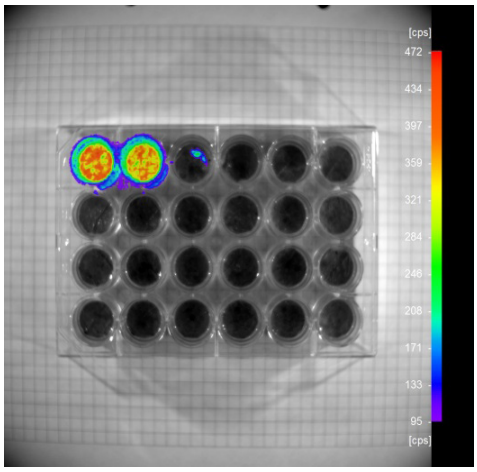

Figure 4: Delayed Fluorescence of Tomato Leaves after Fungal Infection. Well A1+A2: untreated leaves, well A3-D6: leaves infected with fungus, 8 days after infection. No delayed fluorescence is visible due to destroyed chlorophyll.

Drought Tolerance

In response to drought, delayed fluorescence was decreased in soybean plants whereas watered soybean plants exhibited the same intensity of signals in delayed fluorescence imaging as two days earlier (Figure 5).

Conclusion

Delayed fluorescence measurement is a straight-forward and rapid method to follow plant viability *in vivo*. In our experiments the physiological state of the photosynthetic apparatus was either affected by mycotic infection or by drought. In

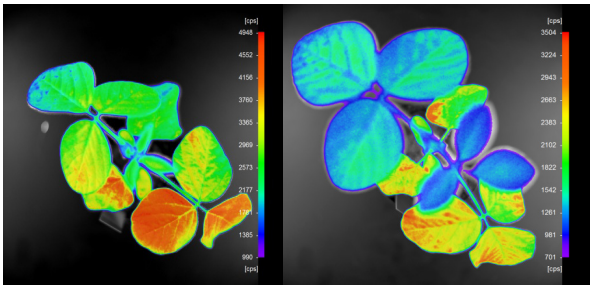

Figure 5: Delayed Fluorescence of Soybean Plants after Drought Stress. Left: watered plant, Right: Fluorescence in the same plant after 2d of drought. Red colour shows high intensities representing high chlorophyll content, blue colour shows low intensities of fluorescence, indicating low amounts of chlorophyll.

both cases the impaired viability let to the absence respectively reduction of chlorophyll delayed fluorescence due to the degradation of chlorophyll. Delayed fluorescence can also be used to study the effects of herbicides[1], hormones, circadian rhythm[2], growth inhibitors and other stress factors on plants. Furthermore it is an easy and fast tool to determine dose-response curves of plants towards inhibitors such as herbicides[1].

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3. **Jursinic (ed.)** (1986): Delayed Fluorescence: Current Concepts and Status. New York: Academic Press