Application Note

MEASUREMENT OF DELAYED FLUORESCENCE IN PLANTS - A MONITORING SYSTEM FOR STRESS FACTORS

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.

Manfed Hennecke, Bernd Hutter, Angela Brüx

Berthold Technologies GmbH & Co. KG – Calmbacher Str. 22, D-75323, Bad Wildbad, Germany
https://www.berthold.com

Introduction

In photosynthesis light is absorbed within the photosystem II. The excited reaction centre 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 generate NADPH. In parallel an H^+-gradient is formed, which is used for ATP synthesis (Figure 1).

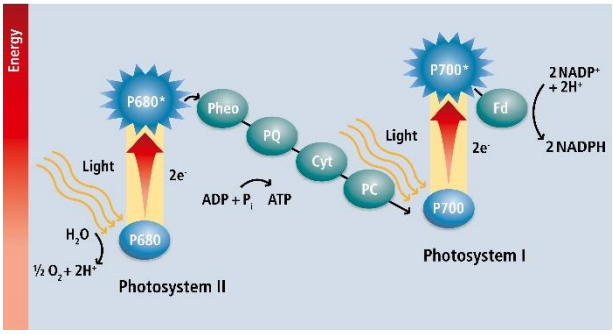


Figure 1: Scheme of electron flow from Photosystem II to Photosystem I

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.

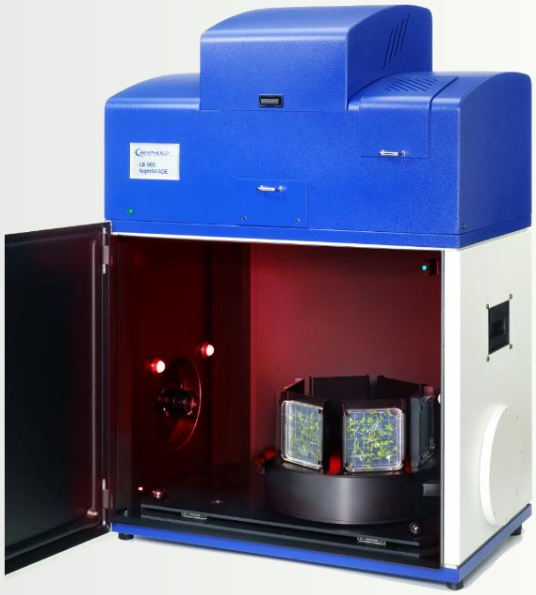
Delayed fluorescence, also called afterglow, is the extremely weak light emitted by pre-illuminated intact plants. It is a ubiquitous and well-studied process in photosynthetic organisms [1] ([2] for review), closely related to the photosynthetic reactions. It is emitted by the chlorophyll a molecules 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 [2]. 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.

The Berthold Technologies NightShade LB 985 In Vivo Plant Imaging System

The NightShade LB 985 In vivo Plant Imaging System is a modular, easy to use optical imaging system dedicated to in vivo analysis of plants. Equipped with an absolutely light-tight cabinet and a cooled CCD camera it enables sensitive luminescence and fluorescence monitoring in tissues, seedlings, and whole plants.

The camera can be attached either to the ceiling or the side walls of the dark room – the sample chamber – to facilitate imaging from above and from the side. The latter position of the camera enables processing of multiple seedlings in parallel while growing plants vertically oriented to enable observation of the complete plant. Furthermore, key environmental conditions like temperature or humidity as well as daylight can be simulated to provide a controlled growth environment.



Materials and Methods

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

Soybean 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).

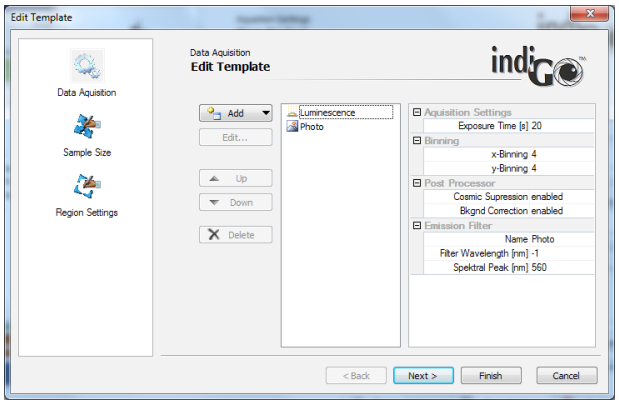


Figure 2: Instrument settings in indiGO™ software: template for delayed fluorescence measurement with defined settings.

Materials

- NightSHADE LB 985 *In vivo* Imaging System for plants
- LED panel
- 24 well microplates

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 3).

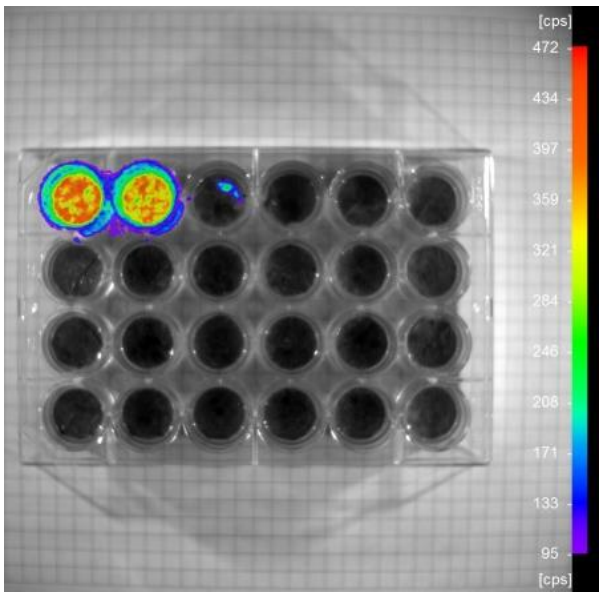


Figure 3: Delayed fluorescence of tomato leaves after fungal infection. Well A1+A2: untreated leaves, well A3-D6: leaves infected with fungus, 8 days after infection. No delayed fluorescence is visible due to destroyed chlorophyll.

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 4).

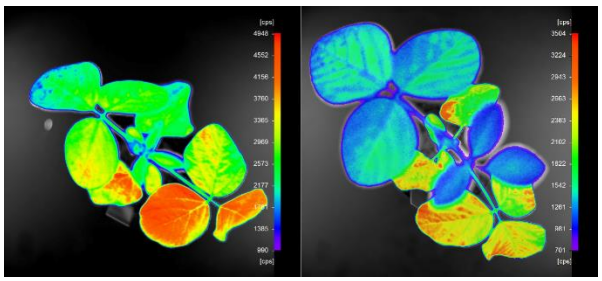


Figure 4: Delayed fluorescence of soybean plants after drought stress. Left: watered plant, right: fluorescence in the same plant after 2 days of drought. Red colour shows high intensities representing high chlorophyll content, blue colour shows low intensities of fluorescence, indicating low amounts of chlorophyll.

Conclusions

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 both cases the impaired viability let to the absence respectively reduction of chlorophyll delayed fluorescence due to the degradation of chlorophyll. As it can be seen in the literature, delayed fluorescence can also be used to study the effects of hormones, circadian rhythms [1], 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 [3] or heavy metals such as cadmium [4].

References

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

3. Lambrev (2004): Delayed fluorescence as a Screening Tool (http://www.bio21.bas.bg/ibf/lambrev/df/).

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Berthold Technologies GmbH & Co. KG
Calmbacher Straße 22
75323 Bad Wildbad
GERMANY
Phone: +49 7081 177 0
Email: bio@berthold.com

www.berthold.com