Application Note

MONITORING OF THE EFFECTS OF STRESS IN PLANTS USING DELAYED FLUORESCENCE AND THE NIGHTSHADE IN VIVO IMAGING SYSTEM

Abstract

Delayed fluorescence is an indicator of the physiological state of plants and can be used to study the effects of stress factors. Because it is a natural process of plants, it can be measured in a non-invasive way without special labelling or treatment of the specimens being studied.. In this application note, DF imaging with the NightSHADE was tested with plants under mycotic infection and under drought conditions. The effects of both stress factors on DF were easily detectable with the CCD camera of the NightSHADE.

Introduction

Preilluminated and undamaged plants release light particles known as delayed fluorescence (DF). DF arises from the radiative deactivation of secondary excited chlorophyll molecules within the antenna complexes of Photosystem II (PS II). These excitations are generated through backward electron-transfer reactions occurring on both the donor and acceptor sides of PS II [1].

DF is coupled with the processes of forward photosynthetic activities, and this means that it is informative about plant physiological states and plant-environment interactions. DF is affected by many factors, such as nutritional status of the plant, salt stress, chilling stress, heat stress, drought stress, acid rain, herbicides, metals, and others [2]. And, as it is a natural process of plants, it can be measured without any special labelling or treatment of the specimens to be studied, and in a non-invasive way.

In this application note we test the imaging of DF with the NightSHADE In Vivo Plant Imaging System under two different conditions: fungal infections and drought.

Materials and Methods

- NightSHADE evo In Vivo Plant Imaging System (Berthold Technologies).
- 24-well plates (Greiner Bio-One).
- indiGO™ image analysis software (Berthold Technologies).

For the fungal infection test, discs were cut from tomato leaves from uninfected plants or from plants 8 days post infection with a fungus. Discs were inserted into the wells of a 24-well plate and illuminated for 30 s with the LED panels. Immediately after switching the light off, images were acquired in the NightSHADE in Luminescence mode with an exposure time of 20 s and 4x4 pixel binning.

For the drought stress test, two groups of soybean plants were used: 50% of the plants were kept dry, and the other 50% were watered. All of them were imaged before starting the experimental period of 2 days to measure their initial (baseline) DF. For DF imaging, plants were illuminated for 30 s with the LED panels. Immediately after switching the light off, images were acquired in the NightSHADE in Luminescence mode with an exposure time of 30 s and 4x4 pixel binning. After 2 days, all plants were imaged using the same settings.

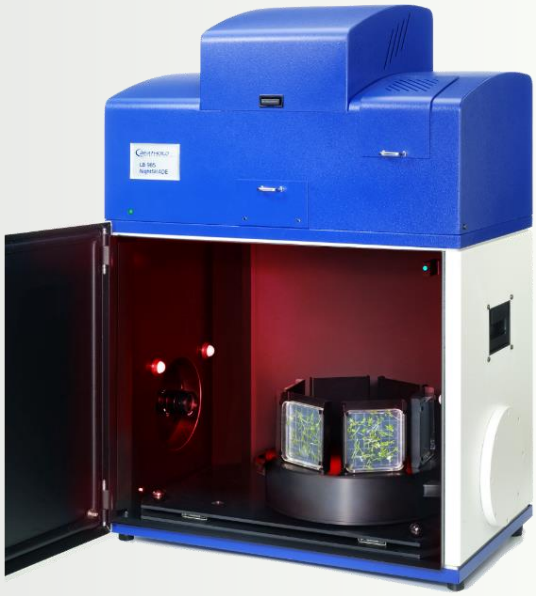
In all cases, pictures in Photo mode were also acquired and are used in the figures to show the position of the fluorescence in the leaves.

Images were analysed using the indiGO™ image analysis software and fluorescence intensity was expressed in counts per second (cps).

The Berthold Technologies NightSHADE evo LB 985N In Vivo Plant Imaging System

The NightSHADE evo LB 985N 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.



Results

The effects of both stress factors tested (mycotic infection or drought stress) had clearly visible effects on DF in both experiments.

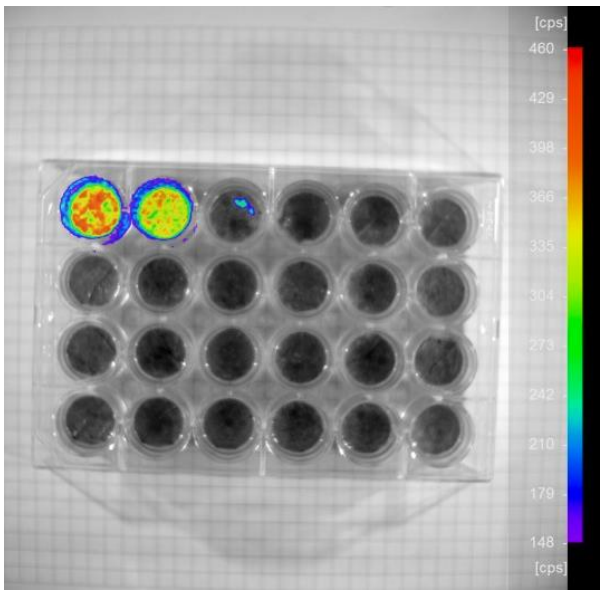


***Figure 1**. Delayed fluorescence of tomato leaves, untreated (wells A1 and A2) or after 8 days of fungal infection (rest of the wells). Image was acquired with 20 s exposure time and 4x4 pixel binning.*

In the image of tomato leaves (Fig. 1), uninfected leaves (wells A1 and A2) showed strong signals of DF, as expected from healthy plants. However, infected leaves showed very low DF (well A3) or no detectable DF (rest of the wells), indicating a heavily damaged photosynthetic system as a consequence of the mycotic infection.

The images of the drought stress test (Fig. 2) also show a clear effect, but not as pronounced. DF content is reduced in most leaves, with leaves with high DF content (approx. 3500-4500 cps) dropping to a medium level (2000-2400 cps) and leaves with medium DF content (approx. 2500 cps) dropping below 1000 cps after two days of drought.

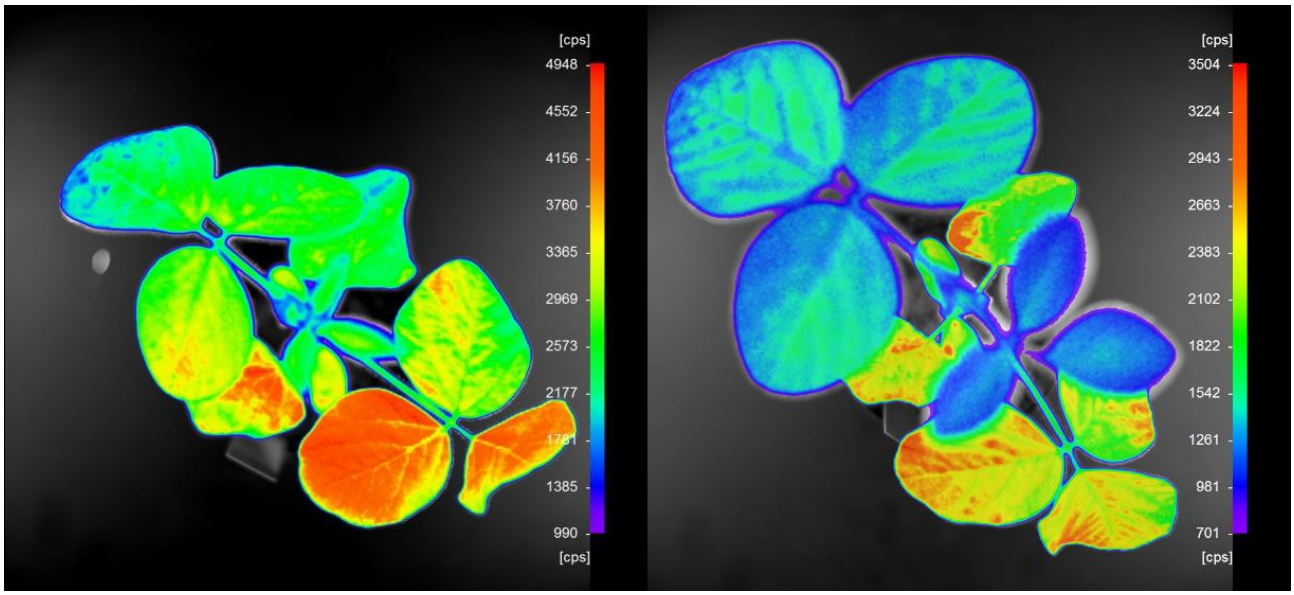


***Figure 2**. Delayed fluorescence in soybean plants after 2 days watered (left) or dry (right). Both pictures are from the same plant. Images were acquired with a 30 s exposure time and 4x4 pixel binning.*

Conclusions

Imaging of DF is a straightforward and non-invasive method to assess the effect of different types of stress factors. Using the NightSHADE evo In Vivo Imaging System, even the effect on DF of mild stress factors, such as 2 days of drought stress, could be easily detected. The ability to image the whole plant in a single image is more informative than methods performing the measurement in a smaller part of the plant, such as a single leaf. Imaging settings can be adjusted if necessary to recognise lower DF values (by increasing the exposure times and pixel binning).

In conclusion, the NightSHADE In Vivo Plant Imaging System is a valuable tool to assess the effect of different types of stress factors using DF imaging.

References

1. Goltsev, V., Zaharieva, I., Chernev, P. and Strasser, R. (2014). Delayed Chlorophyll Fluorescence as a Monitor for Physiological State of Photosynthetic Apparatus. Biotechnology & Biotechnological Equipment, 23:sup1, 452-457. https://doi.org/10.1080/13102818.2009.10818461
2. Guo, Y., and Tan, J. (2013). Applications of Delayed Fluorescence from Photosystem II. Sensors (Basel) 13(12): 17332–17345. https://doi.org/10.3390%2Fs131217332

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