Application Note

COMBINING DELAYED FLUORESCENCE AND ULTRAWEAK PHOTON EMISSION TO MONITOR STRESS RESPONSES IN PLANTS USING THE NIGHTSHADE

Abstract

Understanding the effects of stress factors on plants can help developing strategies to mitigate their effects on plant health and crop yield. Delayed fluorescence and ultra-weak photon emission are natural properties of plants. They can be studied non-invasively and in physiologically relevant conditions and respond to a broad range of stress factors. In this application note, imaging of delayed fluorescence and of ultra-weak photon emission are used to assess the stress status of leaves turning brown using the NightSHADE In Vivo Plant Imaging System. Results show a good correlation between these imaging methods and indicate that the combination of both imaging methods using the NightSHADE can be a useful tool for the study of stress factors in plants.

Introduction

Plants are exposed to many stress factors, such as drought, salt, heat, cold, pathogens, contaminants, and others, - all of which can have a negative effect on plant health and crop yield. Studying the effect of stress factors and the mechanisms of defence of the plant against them can help developing strategies to mitigate their effects on plant health and crop yield.

Many methods are available for the study of effects stress factors in plants, but some of them are invasive or require transformation or excessive manipulation of the plant. Methods based on the measurement of natural properties of the plant, requiring minimal manipulation, are more physiologically relevant and easier to perform. Delayed fluorescence (DF) is a natural property of plants which informs about the status of the photosynthetic system, and 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 [1]. However, it's not the only available method.

Ultraweak photon emission (UPE, also known as biophoton emission or low-level chemi-luminescence) is the emission of photons at a very low intensity (10^{1}-10^{3}photons·sec^{-1}·cm^{-2}, or 10^{-16} to 10^{-18} W·cm^{-2} [2]), that seems to happen in all living systems [3]. UPE originates from the oxidative metabolic reaction in microbial, plant and animal cells, and it is generally considered that

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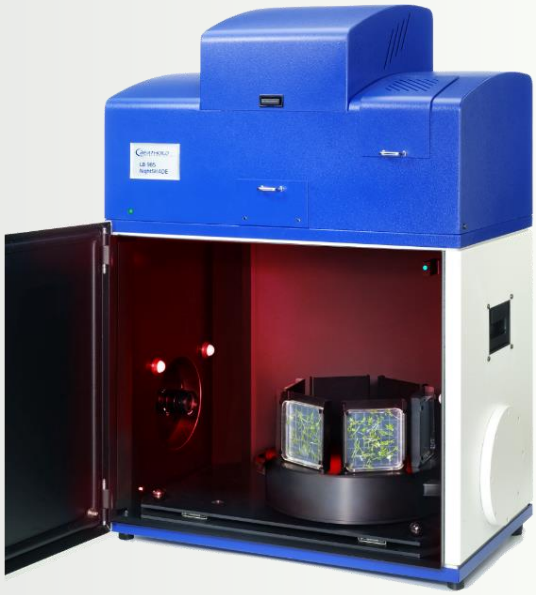
electronically excited species formed during the oxidative metabolic processes are solely responsible for it [4]. UPE is also a non-invasive method, based on natural properties of the cells, and has been demonstrated to respond to many different stress factors in plants, animals, and humans (see [4] and [5] for reviews). Hence, it can be a good alternative or complement to DF for the study of the effects of stress factors.

In this application note, DF and UFE are used to look for the stress signs in leaves turning brown, and the performance of both methods is compared.

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.



Materials and Methods

- NightSHADE evo In Vivo Plant Imaging System with LED panels (Berthold Technologies).
- Powershot G11 digital camera (Canon).
- Leaves from *Carpinus betulus*.
- IndiGO™ image analysis software (Berthold Technologies).

Leaves from *Carpinus betulus* (one healthy, one brownish) were illuminated with the LED panels for 10 minutes with the 470 nm, 660 nm, and 730 nm channels (35 uE·mm^{-2}·s^{-1} in each channel). White LEDs must be avoided because they exhibit afterglow that could mask DF and UPE. LEDs were then turned off and, after a short delay (3 and 10 s were tested), DF images were acquired in Luminescence with 60 s exposure time and 2x2 pixel binning.

After acquiring the DF images, leaves were kept in absolute darkness for 30 minutes to let DF fade out (DF has been described to mask UFE for 5-10 minutes [x]). Then, UFE images were acquired in Luminescence mode with an exposure time of 10 minutes and 8x8 pixel binning.

As last step, a black-and-white picture was taken in Photo mode with 0.1 s exposure time and 10% illumination intensity. DF and UPE images were overlaid over this picture for spatial reference. Colour pictures of the leaves were also taken using the Canon camera.

Images were analysed using indiGO™ image analysis software. Emission intensity was determined using the line tool and expressed as counts per second (cps). In order to make results of DF and UPE comparable, cps values were corrected for the pixel binning: cps were divided by 4 for 2x2 pixel binning and by 64 for 8x8 pixel binning.

Results

Healthy leaves show high levels of DF in the (fig. 1A, right), accompanied with undetectable levels of UPE (fig. 1B, right). Brownish leaves show reduced levels of DF in some areas, and undetectable levels of DF in others (fig. 1A, left). Areas with detectable levels of DF correspond with areas of de leaf that stay green (fig. 1C). UPE is undetectable in most of the surface of the leaf: neither green areas nor brown areas show detectable levels of UPE. However, UPE is high in a damaged area that seems to be transitioning from green to brown (fig. 1C, red box), and it can also be detected in other green-to-brown transition areas, but at lower levels.

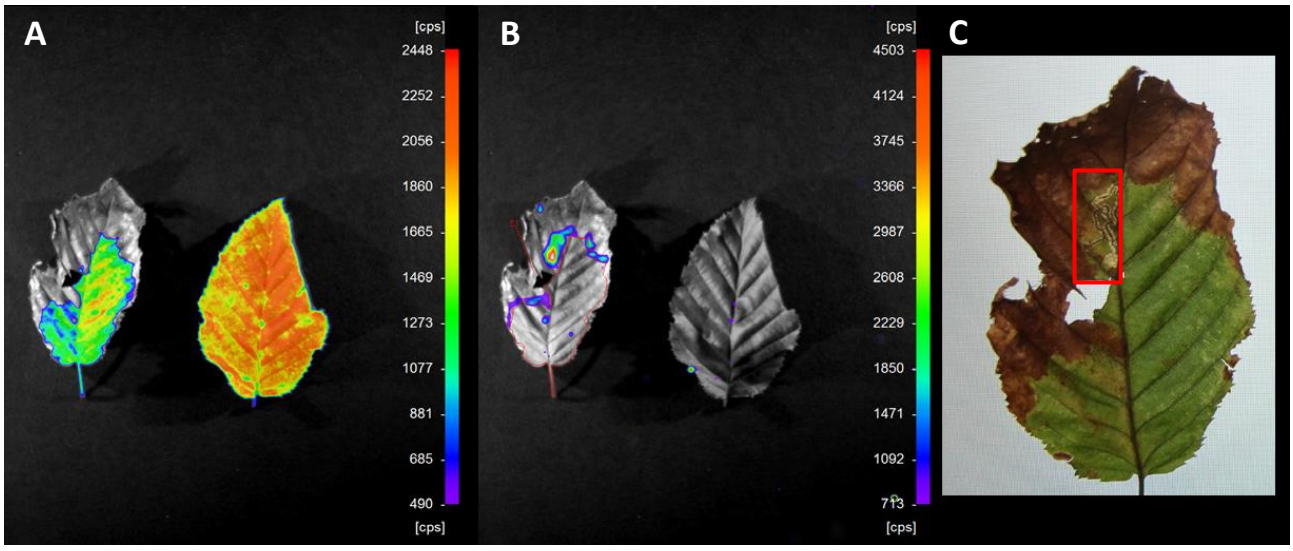


***Figure 1**. A) DF emission of a healthy leaf (right) and a brownish leaf (left). Image was acquired with 60 s exposure time and 2x2 pixel binning. B) UPE of a healthy leaf (right) and a brownish leaf (left). Image was acquired with 10 min exposure time and 8x8 pixel binning. C) Picture of the brownish leaf taken with a standard digital camera (Canon Powershot G11); the box marks an area showing high UPE.*

In order to have quantitative data, light intensity was quantified using the indiGO™ image analysis software. Emission intensity was determined with the line tool, drawing a line that crossed the most representative areas of both leaves (fig. 2, right), including the damaged area of the brownish leaf (fig. 1C, red box). The resulting intensity graph of the DF is shown in figure 2 (left). Results were expressed in counts per second (cps) and corrected for the binning factor (table 1). The quantification shows that, while the effects of leaf damage are visible in the values of DF, with a reduction of a 25%, the effect is much more evident in UPE, which increases by almost 10 times. The combination of low DF and high UPE in those areas is indicative of high levels of stress.

	DF	UPE
Healthy area	8.300	0.013
Damaged area	6.250	0.125

***Table 1**. Quantification of delayed fluorescence (DF) and ultraweak photon emission (UPE) using the line tool of the indiGO™ software. Results expressed as counts per second and corrected for the binning factor.*

The quantification also shows how weak UPE is compared to DF: 5 times lower in damaged areas, and more than 600 times lower in healthy areas.

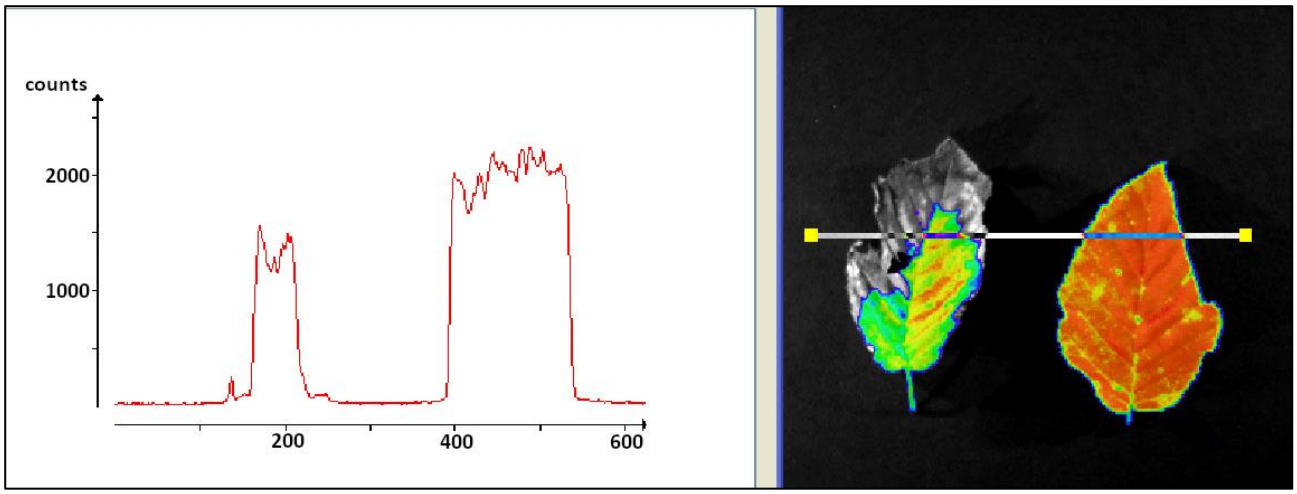


***Figure 2**. Quantification of delayed fluorescence (DF) using the line tool of the indiGO™ software. The graph (left) represents the quantification of the intensity in the areas crossed by the line (left).*

Conclusions

At the settings tested in NightSHADE, the UPE values were almost undetectable in the healthy leaf, but very clearly detectable in the damaged areas of the brownish leaf. On the other hand, the decrease in DF was clearly visible. The low levels of DF and high levels of UPE in areas of the brownish leaf transitioning from green to brown indicate that cells in those areas are highly stressed.

The results shown in this application note demonstrate that the NightSHADE evo In Vivo Imaging System is suitable for the study of the effects of stress factors by imaging DF and UPE in plants.

References

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