Application Note

IMPROVED EXPERIMENTAL SETUP FOR ANALYSIS OF CIRCADIAN RHYTHMS USING THE NIGHTSHADE

Abstract

Endogenous biological clocks drive daily rhythms enabling organisms to anticipate environmental changes as well as to coordinate and adapt their physiology in a synchronized manner. Research on circadian rhythms benefits from real-time monitoring of reporter lines in which the promoter of a gene of interest drives the expression of luciferase *pGENE::LUC+* in combination with sensitive imaging systems [1]. However, in multicellular organisms, circadian clocks are naturally variable at individual, tissue as well as cellular level [2, 3], culminating in noisy or inaccurate data. Therefore, robustness is required to accurately address key questions in circadian biology. For this purpose, we developed a simple protocol for circadian rhythms experiments with *Arabidopsis thaliana* reporter lines using the NightShade LB 985. Our experimental setup improves data quality, reduces luminescence variation between replicates and highly correlates with modelling predictions.

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Introduction

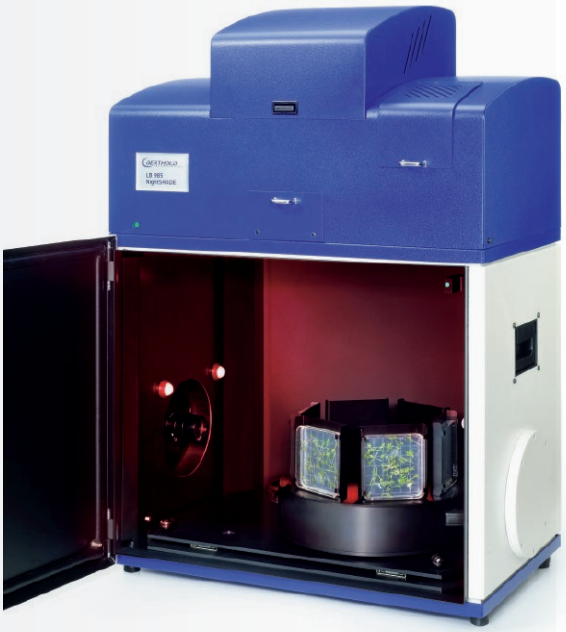
The circadian clock enables plants to anticipate as well as to respond to environmental variations and thus, improves their fitness [4]. Both, environmental and metabolic signals feed into the clock, which is comprised of a network of genes and keep it synchronized with day/night cycle. In return, the clock controls various pathways and ensures they get activated at the appropriate time of the day.

Light is one of the so-called Zeitgebers, which can reset the clock. In this work, we looked at the effect of light on the complex system of clock-genes in *Arabidopsis thaliana* to explore the mechanisms by which the plant clock adapts to day length variation.

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 darkroom – 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

Arabidopsis seedlings bearing *pCCA1::LUC*+ construct were used for circadian rhythm analysis. Eight-day-old seedlings entrained in short days were transferred into the NightSHADE chamber for luminescence recording during eight additional days. Further details about the device, CCD camera, growth condition, light sources and luciferin manipulation can be found elsewhere (http://www.berthold.com/ww/en/pub/bioanalytik/produkte/nightshade.cfm and https://doi.org/10.1016/j.jtbi.2017.03.005) [5]. Luminescence measurements were performed in 10 – 15 pooled seedlings using top-housed CCD camera in darkness during 600s (binning 1x1, high gain) after dark-adaptation for 120 s prior to photon acquisition. Plant culture was performed in horizontally-oriented plates and placed around 20 cm below the camera for enhanced signal acquisition (Fig.1). Manual focus of CCD camera is required.

Results

Dark-adaptation for 120s prior to photons counting prevented the incidence of chlorophyll auto-fluorescence, such as demonstrated by the lack of signal in Col-0 wild-type in contrast to reporter seedlings (Fig 1). The *CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)* gene encodes a master regulator of the central circadian oscillator [6]. This gene is highly expressed at dawn and repressed through dusk over the night [6]. The activity of the *CCA1 promoter::LUC+* was recorded during eight days. The period of the oscillations was 23.910 ± 0.004 h (Fig 2). Robustness of rhythms is shown by the Relative Amplitude Error (Fig 3). In addition to 24 h cycles, this experimental setup allowed to accurately test mathematical predictions of light inputs to the plant circadian clock in a large range of entrainment cycles, such as 8, 13 and 16 h [5].

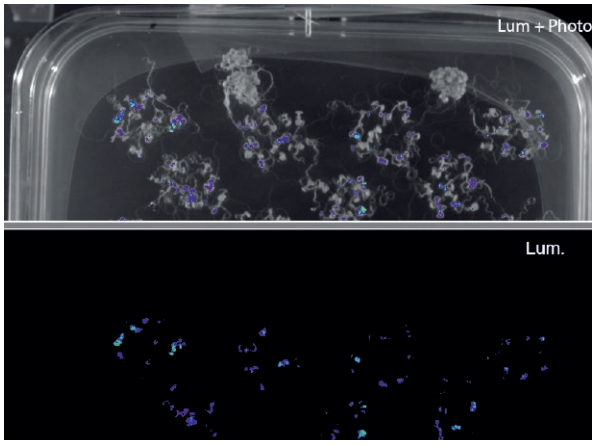


Figure 1. A. thaliana pCCA1::LUC+ (glowing) and Col-0 wild type (negative control) seedlings 7h after light onset (when luminescence measurements started).

Conclusion

We developed a significantly improved robust protocol for circadian experiments using the NightShade LB 985. The results of our experiments with *Arabidopsis thaliana* reporter lines agree with previous reports that the light-sensitive Arabidopsis clock gene network provides the plant with the ability to adapt to seasonal changes in day length. In addition, our experimental setup reduces luminescence variation between replicates and highly correlates with modelling predictions, thus, improving data quality.

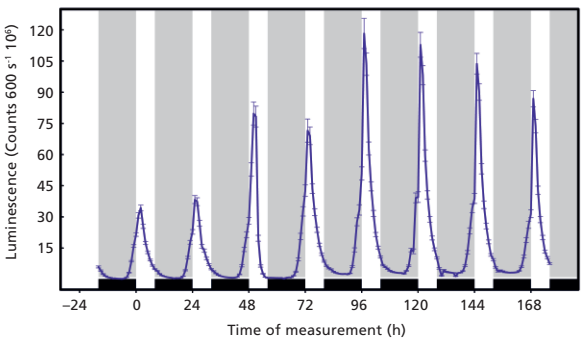


Figure 2. Luc reporter activity of A. thaliana pCCA1::LUC+ seedlings under short-day cycles (8h light/16h dark).

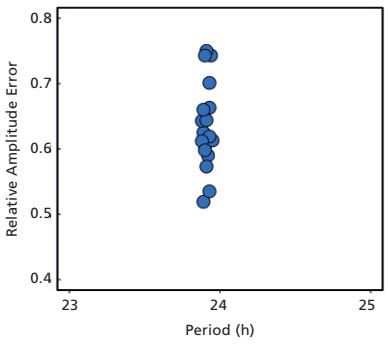


Figure 3. Relative Amplitude Error demonstrating the robustness of the circadian oscillations.

References

1. Millar, A.J., et al. Plant Cell, 1992. 4(9): p. 1075-1087.
2. Endo, M. Curr Opin Plant Biol, 2016. 29: p. 44-49.
3. Endo, M., et al. Nature, 2014. 515(7527): p. 419-422.
4. Green, R.M., et al. Plant Physiol. 2002. 129: p. 576-584.
5. De Caluwe, J., et al. J Theor Biol, 2017. 420: p. 220-231.
6. Alabadi, D., et al. Curr Biol, 2002. 12(9): p. 757-761.

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