Getting to Know The Circadian Clock and Plant Growth With NightSHADE

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INTRODUCTION

In plants, complex integration of light signaling mechanisms and the circadian clock influence many aspects of growth and development. Sensitive imaging systems have allowed researchers to use promoter::firefly Luciferase (LUC) reporter constructs to study gene expression patterns over long periods of time in living plants. Growth-rate of seedlings and a structure called hypocotyl has been used extensively to characterize various mechanisms involved in environmental control of plant growth and morphogenesis[1,2,3]. Performing such studies poses challenges to combine controlled growth environments with highly sensitive imaging systems. Here we show the NightSHADE imaging chamber can be used for multiple applications, including bioluminescence and growth-rate analysis under dark or light-controlled environment.

METHODS

Plant materials (*prCCA1::LUC*, CS9382; *prCAB::LUC*, CS9381; Col-0, CS28168) were obtained from TAIR[4] Arabidopsis stock center. After stratification seeds were germinated[3,5] and grown on plates containing MS[6] medium. All images were obtained with a CCD camera installed in the NightSHADE[7] chamber. The CCD camera was used from its housing on the top of the chamber for bioluminescence images and was moved to its housing on the side for real-time images of young seedlings growing on vertical plates (as shown in Figure 1).

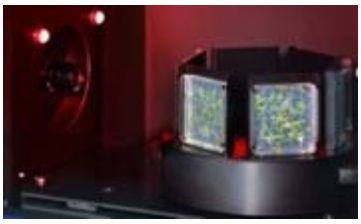

Figure 1. Rotating platform inside the NightSHADE holds up to six square plates. The indiGO™ software allows complete hardware control of the rotor to image each plate consecutively multiple times over a period of days. The inside chamber has white (or infra-red) LEDs for imaging, and red, blue, or far-red LEDs for light treatment. Picture courtesy of BERTHOLD TECHNOLOGIES.

RESULTS

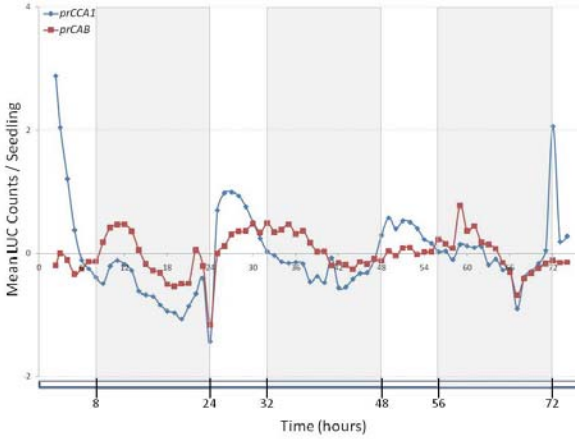

Figure 2 (Left). *CCA1* gene expression preceded activation of *CAB* promoter. Seedlings (*prCCA1::LUC* & *prCAB::LUC*) were entrained under 8-h-light (cool white fluorescents, 50 µmol m^{-2} s^{-1}) / 16-h-dark photoperiods for 7 days, sprayed with luciferin and imaged for bioluminescence every hour for 3 days under dim constant light in the NightSHADE.

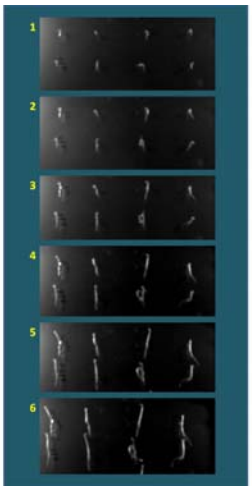

Figure 3 (Right). Arabidopsis (Col-0) seedlings were grown as described[3] prior to imaging in the NightSHADE every 4 hours (images 1-6). The indiGO™ software was used to control the CCD camera, the rotor and light/dark conditions. Only the top-half of the plate is shown here in each image. These images were used to measure hypocotyl lengths using ImageJ (NIH) (data not shown).

CIRCADIAN CLOCK ASSOCIATED 1 (*CCA1*) is involved in the central feedback loop in Arabidopsis and is thought to act around dawn to activate *CAB* gene expression[8,9,10]. Seedlings entrained under short-day conditions were imaged for *LUC* expression in the NightSHADE. *CCA1* gene expression preceded activation of *CAB* promoter (Figure 2), consistent with CCA1 protein playing a positive role in *CAB* gene expression. In a separate experiment, Arabidopsis (Col-0) seedlings were entrained under short-day conditions for 3 days and imaged (Figure 3) with the CCD camera housed on one side of the chamber. These results display two of the multiple imaging applications of the NightSHADE chamber, which provides a unique combination of controlled growth environment with a sensitive CCD camera.

REFERENCES

[1]Vandenbussche, F. et al. D. *Bioessays* 27, 275–284 (2005).
[2]Nozue, K. & Maloof, J. N. *Plant Cell Environ.* 29, 396–408 (2006).
[3]Nozue, K. *et al. Nature* 448, 358–361 (2007).
[4]The Arabidopsis Information Resource (TAIR) (http://www.arabidopsis.org/index.jsp).
[5]Millar, A.J. *et al. Plant Cell* 4, 1075-1087 (1992).
[6]Murashige, T. & Skoog, F. *Physiol. Plant*. 15, 493-497 (1962).
[7]For more information on NightSHADE LB 985, visit: http://www.berthold.com/ww/en/pub/bioanalytik/produkte/nightshade.cfm
[8]Millar, A.J. & Kay, S.A. *Plant Cell* 3, 541-550 (1991).
[9]Wang, Z.-Y. & Tobin, E.M. *Cell* 93, 1207-1217 (1998).
[10]Alabadi, D. *et al. Science* 293, 880-883 (2001).