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Effects of Light on Ultradian Rhythms in the Lateral Leaflets of *Desmodium gyrans*

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Rhythmic up–down movements were studied in the lateral leaflets of *Desmodium gyrans* (L.F.) DC. These were recorded with a video–computer system, whereby the digital video signals from a CCD camera were processed with special software. Under control conditions (24°C and 0.1 nM cm^{-2} s^{-1} of stable, dim light), the average period of lateral leaflet movement was 3.5 min. In the presence of light stimuli (for 2 min), those leaflets always moved toward the light, regardless of where it was applied to any axial part of the pulvinus. The strongest effect was manifested by a reduced amplitude of movement and, thus, a shortened period, which could be up to ~43% less under moderate light intensity (5 nM cm^{-2} s^{-1}). Oscillations regained their original regularity over ~10 cycles after the light stimulus was removed. In addition, these oscillations temporarily disappeared after long exposure (~10 min) under moderate light, or when the leaflets were quickly exposed to a higher intensity (~12 nM cm^{-2} s^{-1}). Therefore, we have now demonstrated that light can affect physiological parameters that are involved in the control of oscillations.

Keywords: Desmodium gyrans, lateral leaflet, light stimulus, oscillation, pulvinus

Plants of *Desmodium gyrans* have a terminal leaflet (3 to 7 cm long) and one or two lateral leaflets (approx. 1 cm long). In the daytime, the terminal leaflets orient themselves horizontally and pointed upward (Fig. 1A), but they hang down during the night (Fig. 1B). These up-and-down movements occur with a periodicity approximating 1 d. This rhythm is circadian, and the period (*T*) is temperature-compensated (i.e., *T* values do not change much with fluctuations in temperature). In contrast, such movements by the lateral leaflets exhibit an ultradian rhythm, with a temperature-dependent periodicity of just a few minutes (Lewis and Silyn-Roberts, 1987; Engelmann and Antkowiak, 1998; Miah et al., 2002; Miah, 2004). For those tissues, an increase in temperature reduces the period. These oscillations can also be synchronized by temperature cycles (Lewis and Silyn-Roberts, 1987). In the lateral leaflets, the oscillations are generally believed to be generated by the rhythmic swelling and shrinking of motor cells located in the pulvinus at the leaflet base. Electrical potentials across those motor cells oscillate, maintaining a constant phase relationship with the leaflet positions (Antkowiak and Engelmann, 1995; Engelmann and Antkowiak, 1998). Fluctuations in the pulvini electrical potentials result from the uptake and release of ions, especially K^+ and Cl^- (Kumon and Tsurumi, 1984; Lowen and Satter, 1989; Starrach and Mayer, 1989; Lee, 2006). A considerable amount of potassium is also shuttled from one part of the pulvinus to another, acting as a cation reservoir (Freudling et al., 1988). The motor cells oscillate between electrically polarized and depolarized states. The state of depolarisation causes an efflux of K^+ and H^+ while hyperpolarization promotes an influx of K^+ and H^+ to the cells. These K^+ fluxes are believed to be responsible for the osmotic movement of water across the pulvinus, which in turn results in volume changes within the pulvinus and noticeable leaflet movement. The periodicity of these oscillations can be changed by a number of stimuli, both chemical and magnetic (Kumon and Tsurumi, 1984; Starrach and Mayer, 1989; Weber et al., 1992; Johnsson et al., 1993; Antkowiak and Engelmann, 1995; Fostad et al., 1997; Engelmann and Antkowiak, 1998; Miah et al., 2002; Miah, 2004). Here, we used white light stimuli to perturb the rhythmic lateral leaf movements of *D. gyrans*. Our objective was to determine whether visible light could influence rhythm parameters, especially the period of oscillations.

Figure 1. *D. gyrans* plant (**A**) in day position and (**B**) in night position, and (**C**) petiole with terminal and two lateral leaflets.

MATERIALS AND METHODS

Plants of *D. gyrans* [alt. *Desmodium motirium* (Houtt.)

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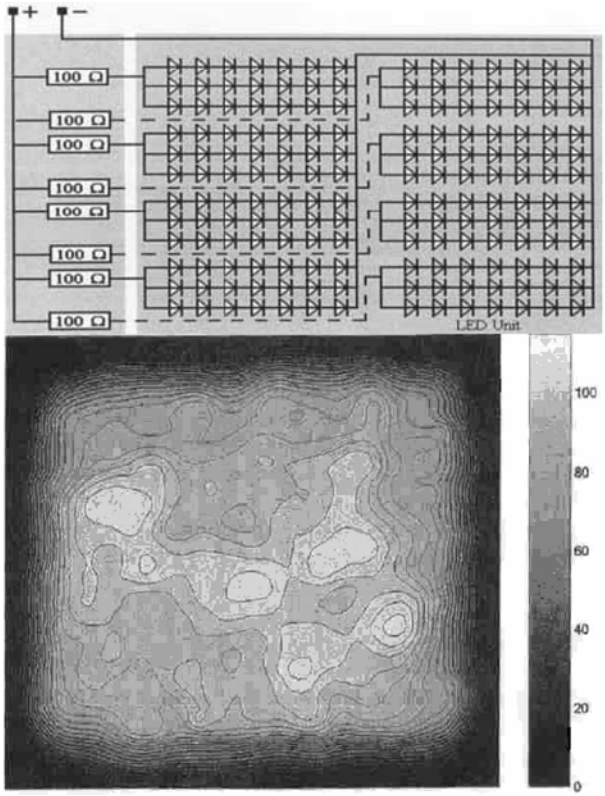

Figure 2. Light source (LED array) with contour plot of typical light intensity for circuit current, at ~2 cm from array. Right bar shows intensity distribution, scaling in mM m^{-2} s^{-1}. 1 mM m^{-2} s^{-1} = 0.1 nM cm^{-2} s^{-1}. Light intensity, i.e., emitted photons in mM m^{-2} s^{-1}, was measured with a quantum sensor at different locations in plane from LED array. A central uniform light intensity was used to expose pulvinus.

Merril] were cultivated under a 12-h photoperiod in a greenhouse at ~28°C and ~65% humidity (Engelmann and Antkowiak, 1998). Their terminal and lateral leaflets, which showed regular oscillation, were removed when the mother plants were about 4 months old and approximately 70 cm tall. To minimize temperature fluctuations, these tissues were kept in distilled water in an acrylic glass holder inside of an acrylic glass box. As an optical marker, a small white Styrofoam ball was attached to the tip of each lateral leaflet, which was then illuminated from above by an array of white LEDs (EL 383UBC/H2). This light source was constructed as described by Miah (2001). The LED array, along with a contour representation of the typical light intensity, is shown in Figure 2. The lights were clamped above the pulvinus, ~5 cm from the lateral leaflet. Intensity was measured with a quantum sensor (LI-190SA; LI-COR, USA). Different light levels were adjusted and obtained by changing the current through the circuit. The stimulus was applied to the pulvinus for 2 min. Leaflet movement was then recorded by a video CCD camera (TCZ-250E; Fujitsu, Japan) positioned in front of the box containing the lateral leaflets. The video signal was converted by a digitizer (VIDEO ST 1000, Germany) and processed by computer with special software. The horizontal and vertical positions of the Styrofoam marker were plotted by the program at 5-sec intervals over 10 h, and the data were stored on disk. The period of movement was determined by using a digital filter on these data and calculating the time between successive leaf position maxima.

RESULTS AND DISCUSSION

Figure 3 presents a typical example of lateral leaflet movements (for 1 out of 10 plants), as recorded by our video-computer system. The position of the lateral leaflet was recorded as a function of time, starting at Time 0. In the absence of a light stimulus, i.e., under control conditions (0.1 nM cm^{-2} s^{-1}), the period of movement was 3.5 min at ambient temperature (24°C). A light intensity of 5 nM cm^{-2} s^{-1} for 2 min was then directed onto the abaxial and adaxial parts of the pulvinus. A strong effect was found in both instances -- namely, a reduced amplitude of movement and a shortened period. If only the abaxial part was illuminated, the leaflet oscillated to a lower position, vice versa for adaxial exposure. When the stimulus was applied for 2 min to the lateral leaflet, the period of the oscillations changed. Figure 4 depicts this period, plotted as a function of cycle, with Cycle 1 being the start when the pulvinus was first illuminated. For the lateral leaflets, the period, at a given intensity, declined rapidly from the time of application, and continued to decrease even when the light was turned off. The period of oscillations was reduced as much as ~43% under a moderate light intensity of 5 nM cm^{-2} s^{-1}, but regained its normal regularity after about 10 cycles from the time the stimulus was removed. This effect of light on lateral leaflet oscillations was completely reversible (Fig. 4). However, when the leaflets were exposed to longer durations (approx. 10 min), the oscillations either halted or temporarily disappeared.

We also observed the effects of lateral leaflet oscillations on period with increasing light intensity (*I*; Fig. 5). The period remained almost constant up to an *I* value of ~ 1 nM cm^{-2} s^{-1}, then rapidly decreased with increasing intensity. These oscillations stopped when the intensity was greater than 12 nM cm^{-2} s^{-1}. Although we kept the temperature constant throughout these experiments, it should be noted that photon reactions within the lateral leaflets can cause a rise in temperature. Nevertheless, we do not suspect that these period changes were caused by temperature fluctuations. We also measured the temperature at the lateral leaflet position and found no alterations in temperature for the light source-lateral leaflet separation, exposure time, or light intensities tested here.

Results from previous electrophysiological studies have suggested that proton pumps, changes in membrane potential, and ion transport are responsible for water uptake and loss in motor cells, i.e., creating a volume change in those cells. Movement of the lateral leaflets of *Desmodium gyrans* is believed to originate from these volume changes in the pulvinus, which occur due to ionic movement between different parts of the motor cells. The depolarization of motor cell membranes causes K^+ and H^+ efflux, while their hyperpolarization is associated with K^+ and H^+ influx. Movement of water across the pulvinus occurs due to potassium fluxes, which in turn result in volume changes in the pulvinus and

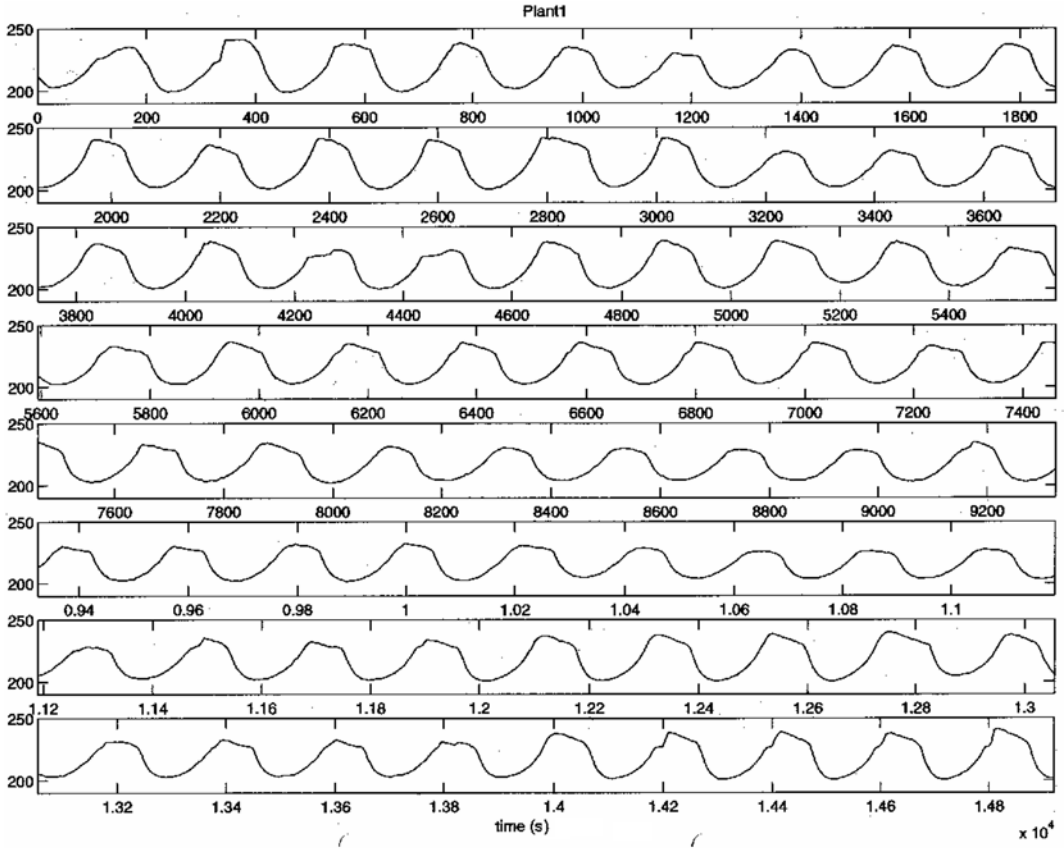

Figure 3. Typical oscillatory movements over time by lateral leaflets of *D. gyrans* under controlled conditions (24°C; 0.1 nM cm^{-2} s^{-1} dim light). Average period of oscillations is 3.5 min.

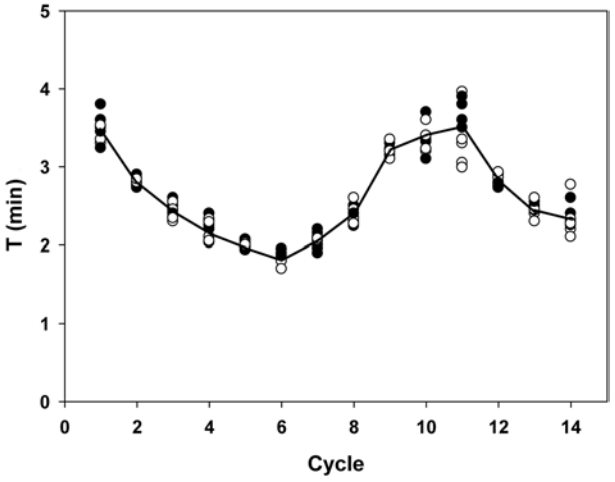

Figure 4. Period of oscillation (*T*) as a function of cycle. Cycle 1 is starting point, when LED light stimulus (intensity $I = 5$ nM $cm^{-2}s^{-1}$ for 2 min) was first applied to pulvinus. Light was then reapplied when oscillation regained the same regularity after ~10 cycles. A line is drawn through means of data from several experiments.

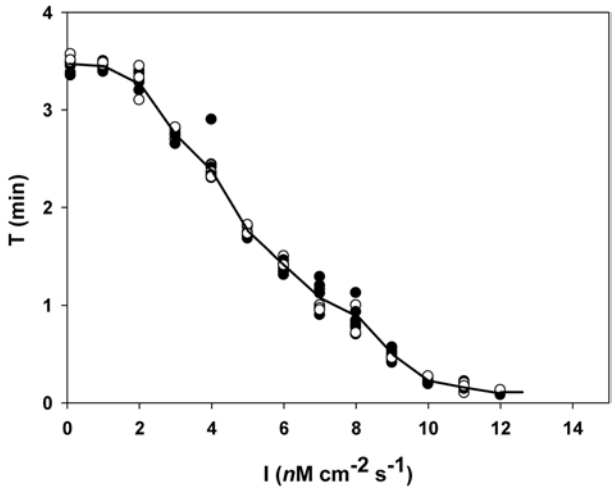

Figure 5. Period of oscillation as a function of light intensity, based on several experiments at each level. Line drawn indicates average value of period for intensities ranging from 0.1 (dim light) to 12 nM $cm^{-2}s^{-1}$ applied for 2 min. Because of large deviation, data for intensity level of 4 nM $cm^{-2}s^{-1}$ are not considered here.

observable leaflet movements. Exposure to various stimuli can alter the period of lateral leaflet movements in *Desmodium* (Engelmann and Antkowiak, 1998). The slowing of that rhythm in the presence of static magnetic fields or electromagnetic fields can possibly be mediated by manipulating the ionic movements within the motor cells of the pulvini (Reina and Pascual, 2001). The effects of electric currents on leaflet movement also have been studied. Both Bose (1913) and Johnsson et al. (1993) have reported that these stimuli can directly affect ion movement across the pulvinus motor cells. There, a direct current (10 to 100 μA) that is applied for a few seconds to the tips of the lateral

leaflets can delay the phase of the rhythm. Pulses of DC current can also prohibit those leaf movement rhythms if applied at the appropriate phase.

Ellingsrud and Johnsson (1993) have used pulses of 27 MHz to perturb the leaf movement rhythm of *D. gyrans*. Such pulses change the amplitude, phase, and period length. Radio frequencies also can stop this movement. The results from experiments where electrical stimuli affect the rhythm of ultradian lateral leaf movement further support a model that can explain this rhythm in terms of proton pumps, membrane-potential changes, and ion transport. Therefore, the effect of light on the rhythm of ultradian leaflet movement in *D. gyrans,* as demonstrated by our experiments, suggests that the shuttling of ions across pulvini motor cells can also be manipulated by light exposure. Although the effect of illumination seen here is not yet completely understood, we propose that light may influence physiological parameters involved in the control of oscillations. This also opens up the possibility of investigating light-induced phase re-setting and the entrainment of ultradian rhythms. The fact that our leaflets oscillated with different frequencies under various levels of illumination, and that these oscillations were halted by a high light intensity, suggests that the biological clock that regulates the ultradian rhythm of lateral leaflet movement oscillates with different frequencies in the natural environment, where light intensity first rises to a mid-day maximum before declining toward evening. This means that the oscillation period for certain plant species varies with local time (if not grown in a greenhouse). Furthermore, because solar light intensity at the same time is different in separate locations, it also varies with the geographic positioning of those plants.

In conclusion, when the lateral leaflet pulvini of *D. gyrans* were illuminated, their period of movement was reduced. Those leaflets always moved toward the direction of the light, whether it was applied to the abaxial or adaxial part of the pulvinus. Oscillations could be halted either after long-time exposure to a moderate light intensity or when plants were quickly treated with high-intensity light. Our findings suggest a possible role for the light/dark cycle in modulating lateral leaflet oscillations for species (known as telegraph plants) that are found primarily in tropical regions, such as in India.

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