Leaf Movement Rhythm in *Arabidopsis thaliana*

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Circadian Rhythms, Cotyledons, Picture Analysis, Video Digitizing

A circadian rhythm of leaf movements of *Arabidopsis thaliana* and its recording in continuous weak light with a video-computer system is described.

The advantages of using *Arabidopsis thaliana* for physiological, genetical and molecular biological questions are well known [1–6]. Recently molecular biological methods have been used to study circadian regulations in this plant [7, 8]. An easily recordable hand of the circadian rhythm would be of advantage in using this plant for further studies of its rhythmic system and for selection of rhythm mutants. We report here the recording of leaf movement rhythms in this plant.

Methods

Plants of *Arabidopsis thaliana* (L.) were grown from seeds obtained from the Botanical Garden in Tübingen. They were raised on a standard soil (Typ T, Patzer KG, D-W-6492 Sinntal-Jossa) under 18:6 LD cycles (natural light supplemented with white light of fluorescence tubes, Osram L 65 W 25 S, Universal white, 120 cm above plants, from 4 to 22:00 o'clock) in a greenhouse. Seedlings with expanded cotyledons were transferred to 4 × 4 cm pots with the same standard soil as above. A few days later the cotyledons were recorded with a video-camera/computer system [9] from above or from the side under LL conditions. The light was provided by one white and one green fluorescence tube (Osram 20 W/32 White de Luxe, Sylvania F20TI2-G, green). Light intensity was 120 $lux = 11.4\ \mu Em^{-2}s^{-1}$). Temperature was 24 ± 1 °C. In some experiments the primary leaves of older plants were recorded.

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The recording system consisted of a video camera coupled to an Atari 1040 ST computer *via* a digitizer. Data were collected with the program OXALIS and period lengths determined with the program OXALDIFI for a nonrecursive digital filter procedure (bandpass for trend removal) [9].

We used in the OXALIS program the facility to determine the size of an object by the number of pixels in a predetermined field. The leaves, as viewed from above, occurred as white contours on a dark background; therefore the number of white pixels was recorded. Every 20 min a picture was digitized and the data stored. The time dependent changes in the number of pixels could be viewed as a graph during recording, and afterwards by the OXALDIFI evaluation program.

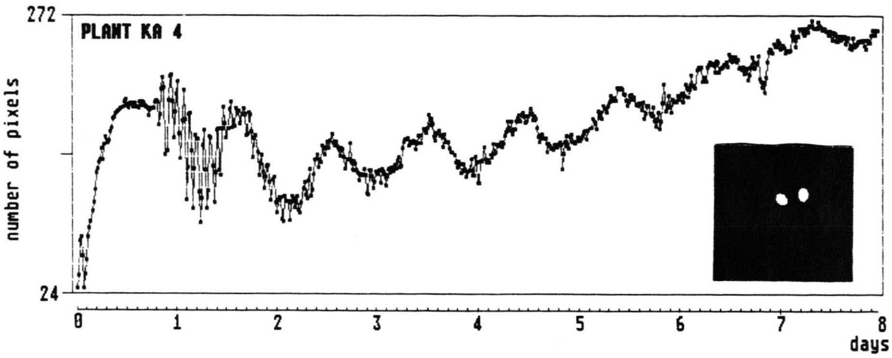
In other experiments we recorded leaf movement as viewed from the side of the plant. Two methods were used. A view field was arranged vertical to the leaf (Fig. 2, inset). The position of the center of the leaf as seen against a dark background was determined as a function of time (scanning every 20 min). Or a small piece of polystyrene was attached to the tip of the leaf with a tiny amount of vaseline. It served as a white marker against a black background and its vertical movements were recorded every 20 min.

Results

Fig. 1a shows a record of the changes in the pixel number of an *Arabidopsis thaliana* plant having expanded cotyledons and no secondary leaves throughout the recording period (see inset of Fig. 1 A). A circadian rhythm with a mean period of 22 h and 29 min (SE = 14 min, n = 6) was found.

Fig. 1b shows a recording from an older plant with the first pair of secondary leaves in addition to the cotyledons (see inset of Fig. 1b). The mean period length was 24 h and 12 min (SE = 11.7 min, n = 7).

Because changes in apparent size could have been brought about by either a bending and expansion of the leaf lamina or by a vertical movement of the lamina, plants were also recorded from the side (Fig. 2). The plant shown in the inset of Fig. 2 is an example. It can be seen from the record of the vertical position of the left leaf that it moves up and down. Mean period length was 26 h 17 min as calculated from 3 days of recording.



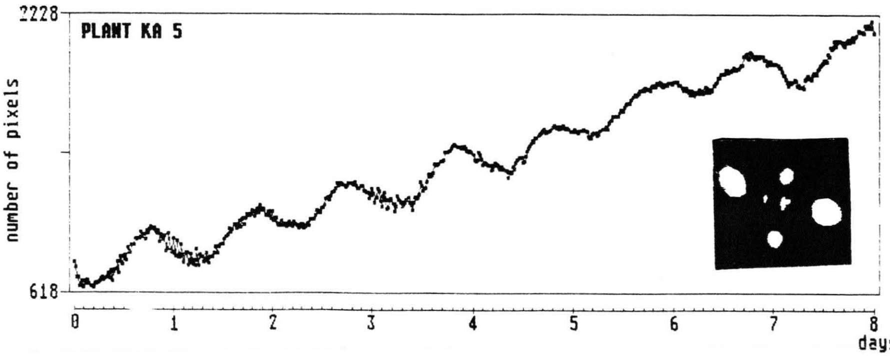


Fig. 1. a: Size (y axis, measured in number of white pixels) of horizontal projection of cotyledons of young *Arabidopsis thaliana* seedling recorded for 8 days (plant KA 4) under constant temperature (24 °C) and continuous light (green and white, 120 lux). Inset shows the two cotyledons as white contours from above, as recorded. Zero is time of transfer from greenhouse conditions to the weak continuous recording light. b: As a, except that an older plant with secondary leaves in addition to the cotyledons (see inset) was recorded.

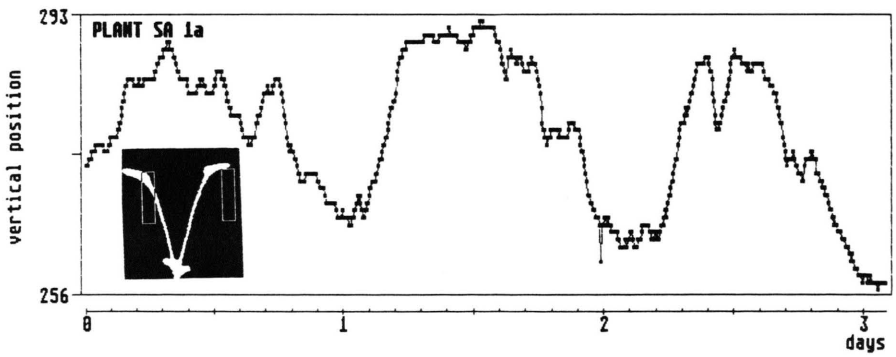


Fig. 2. Leaf movement rhythm of a plant recorded from the side (inset) a day after recording from above (same plant KA 5 as in Fig. 1 b). The y coordinate of the center of white leaf pixels in the left view field (white outlined rectangular) is shown as a function of time (days).

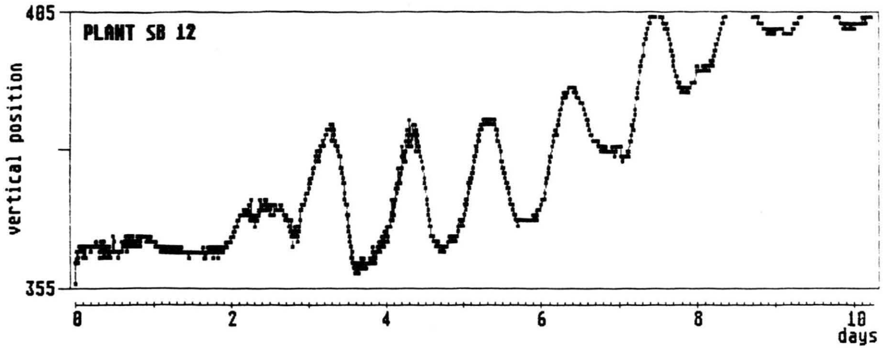


Fig. 3. Rhythmic movement of a leaf, the tip of which was marked with a small piece of polystyrene. Recording from the side. Vertical position of this marker shown as a function of time (days).

Fig. 3 shows an example in which the tip of a leaf was marked with a small polystyrene ball. Recording was from the side again. The light intensity was 120 lux.

Discussion

Among circadian rhythms leaf movements are well studied and documented [10]. Usually leaves with pulvini are used such as from the leguminose family. But leaf movements are known also from plants without special pulvini [11]. *Arabidopsis thaliana* belongs to the latter. With the video system used it was shown that the leaves move up and down in a circadian fashion.

We used a recording system which allows to monitor a larger number of plants and to record the leaves in an uncomplicated way. The method of choice was a video-camera/computer system. Especially the method of viewing the plants from above and to record the horizontal projection of the leaves against a dark background (Fig. 1) is useful. It allows to measure many plants simultaneously without special preparations.

There are, however, several difficulties which should be addressed. When viewed from above, all leaves on a plant were recorded simultaneously. If new leaves are formed during the recording period, their horizontal projection is added to the one of the cotyledons and the allover changes are measured. By using view fields around each individual leaf the changes of single leaves could be monitored.

When recording from above we could not determine whether the leaves were moving up and down or whether the laminae were curling and expanding only. Therefore the plants were recorded from the side also. It was demonstrated that a vertical movement indeed occurred. However, this method needs more preparation time, since plants have to be lined up in several strata if many plants are recorded, and a view field has to be set for each leaf at the onset of recording. The field has to be quite long to cope with the growth of the leaves (see inset of Fig. 2). In the example given the right view field was too short and the leaf was soon outside its area. In this case the recording of the leaf movement ceased. This difficulty can be prevented by marking the tip of a leaf with a small white polystyrene ball and use a rather large view field (the view field of the example given in Fig. 3 was still too small, as shown by the last part of the curve in this figure). The other leaves of the plant were not marked and do therefore not disturb recording.

The method of recording from the side also circumvents the difficulty that the horizontal projections (using the method of viewing the leaves from above) is maximal in a horizontal position of the leaves, but becomes smaller if the leaves either move upward or downward. Thus, during the course of a cycle a leaf moving above *and* below the horizontal position would show reductions in pixel number twice and instead of an about 24 h rhythm an approximately 12 h rhythm would be recorded. If the leaf oscillated in the range *above* the horizontal position, the rhythm would appear to be phase shifted against the rhythm of a leaf which oscillates in the range *below* the horizontal position. In reality, however, the changes in leaf angle would follow a similar time course, but the interpretation of the curves would be confusing. Recording from the side does not show this difficulty.

We have not yet optimized the intensity of the continuous light during the recording nor the quality of the light. We have deliberately used low intensities for two reasons. First, we wanted to avoid fast growth of the leaves, in order to have more pronounced oscillations. Leaf growth would, in both ways of recording, from above and from the side, lead to an upward trend. Secondly, bright light often dampens circadian rhythms. Damping might, however, depend also on the quality of the light used, and more experiments are needed to optimize conditions.

The increase of period length of the leaf movement rhythm found in older leaves could be an effect of age. In Phaseolus coccineus period length decreases with age (Mayer, unpublished).

We realize that our findings are preliminary, but it might be of interest and help for research work on the nature and mechanism of the circadian system and on the possible involvement of circadian rhythms in photoperiodic time measurement in *Arabidopsis thaliana* to know that leaf movements occur in a circadian fashion. For a precise determination of period length the molecular biological approaches are too time consuming and labor intensive. Only automated recording such as the one used here would generate sufficient data of a quality allowing detailed analysis of the rhythm.

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[1] K. Napp-Zinn, in: The Induction of Flowering (Evans, ed.), Macmillan of Australia, S. Melbourne 1969.
[2] E. M. Meyerowitz, Cell **56,** 263–269 (1989).
[3] E. M. Meyerowitz, Trends Gen. **6,** 1–2 (1990).
[4] M. A. Estelle and C. R. Somerville, Trends Gen. **2,** 89–93 (1986).
[5] C. R. Somerville, Pl. Cell **1,** 1131–1135 (1989).
[6] N. Goto, T. Kumagai, and M. Koornneef, Physiol. Plant. **83,** 209–215 (1991).
[7] A. J. Millar and S. A. Kay, Cell **3,** 541–550 (1991).
[8] C. R. McClung, in: The Molecular Genetics of Biological Rhythms (Young, ed.), Marcel Dekker, New York 1992.
[9] J. Schuster and W. Engelmann, in: Chronobiology: Its Role in Clinical Medicine, General Biology, and Agriculture, Part B (Hayes, Pauly, and Reiter, eds.), pp. 389–396, Wiley-Liss, New York, Chichester, Brisbane, Toronto, Singapore 1989.
[10] R. L. Satter, H. L. Gorton, and T. C. Vogelmann (eds.), The Pulvinus: Motor Organ for Leaf Movement, American Society of Plant Physiologists, Rockville, Maryland 1990.
[11] D. F. Wetherell, in: The Pulvinus: Motor Organ for leaf movement (Satter, Gorton, and Vogelmann, eds.), pp. 72–78, American Society of Plant Physiologists, Rockville, Maryland 1990.