Expression of Circaseptan and Circannual Rhythmcity in the Imbibition of Dry Stored Bean Seeds

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ABSTRACT

Water uptake of seeds of Phaseolus vulgaris L. cv Limburg was monitored in a controlled atmosphere every day at solar noon for a period of 2 years. The imbibition rate, determined as the mean of five replicates, was related to the lunar cycle, maximal water uptake occurring between the new phases of the cycle. The monthly mean value of imbibition rate, calculated from the daily measurements, displayed a circannual rhythm reaching maximal values in August-September and minimal values in February-March. These monthly values were positively correlated with the mean of the maximal temperatures recorded daily outside.

MATERIALS AND METHODS

Seeds. Phaseolus vulgaris L. cv Limburg seeds were obtained at the 'Verenigde tuinbouwers' seed company in Roeselare, Belgium. A large stock was purchased and stored at 4°C in darkness. At regular intervals, part of the stock was transferred into an incubator at 25°C in darkness, so that seeds were kept in these conditions for at least 1 month prior to the experiments. The experiments were also carried out in an incubator at 25°C in the basement of the building. The temperature of the incubator was regularly controlled and never found to oscillate. The ambient temperature in the basement room was fairly constant throughout the year at about 20°C. The utmost care was taken to perform identical manipulations every day for the measurements of water uptake.

Imbibition. Seeds were imbibed in prewarmed distilled water at 20°C. A fixed amount of 30 g (about 100 seeds) was submerged in 250 ml of water in a Petri dish with a diameter of 28 cm. Seeds were weighed to the nearest 0.1 mg with a Sartorius 1601 analytical balance before and after imbibition. Seeds were wetted before imbibition started. Before weighing, the seed coats were dried with laboratory paper towels.

Experimental Arrangement. Water uptake was determined every day over a period of more than 2 years. Five replicates were carried out each time. Imbibition started 2 h before solar noon and ended 2 h after solar noon. Water uptake of the seeds was expressed as the percent (w/w) increase of weight compared to the initial dry weight of the seeds. The mean of the 5 values was calculated and used as the value for that day. The amplitude of the ultradian rhythm (26) is smaller than that of the circaseptan rhythm described above. It can thus be expected that ripples of the ultradian rhythm would not disturb the circaseptan rhythm.

This was checked by starting imbibition experiments every hour over a 6 h period (the wavelength of the ultradian rhythm) each day for a fortnight (14 d). The calculated mean daily water uptake values in these experiments paralleled the curve of the standard measurements at noon (results not presented). This observation allowed us to use the protocol of doing one experiment per day at solar noon.

Presentation of Results. The mean daily value was plotted as a function of the date of the experiment. It was also used to calculate mean values for plotting water uptake against the lunar cycle day and the calendar month. Values obtained using a three point moving mean technique are also presented for the lunar cycle day plot (Fig. 2). The values of the daily maximal temperatures were obtained from the Royal Meteorological Institute (Brussels).

RESULTS

Circaseptan Rhythm. After establishing the existence of an ultradian rhythm in the rate of water uptake of dry stored bean seeds (26), we have subsequently looked for the occurrence of
rhythms with a longer period length.

Figure 1 shows the results for the first weeks of 1985, from January 3 until February 6, along with an indication of new phases of the moon during that period. Minimal values for water uptake coincide with the phases of the moon, maximal values are reached on the days in between. The curve clearly shows a regular pattern of troughs and peaks with a period length of about 7 d.

To confirm the circaseptan character of that rhythm, we calculated a mean value for each day of a theoretical lunar cycle, using the measurements of 25 lunar cycles. All water uptake values were related to the lunar cycle by assigning each value a day number, number 1 being the day of the new moon. The results are presented in Figure 2B. The curve is very similar to that of Figure 1, higher imbibition values being obtained during the days between the phases of the moon which are indicated in Figure 2A.

Circannual Rhythm. Measurements taken over the 2 year period were also used to calculate a mean value for water uptake for each calendar month of the experimental period. The results are presented in Figure 3.

The monthly mean imbibition values produce a smooth curve. High rates of water uptake of the seeds occur in August-September (50%), while minimal values (10–20%) are attained in February. This pattern was observed in 1984 to 1985 and in 1986 to 1987 and a third summer peak was reached in July 1986, thus establishing a circannual rhythm.

Effect of External Temperature. The values of the daily maximal temperature were obtained from the Royal Meteorological Institute (Brussels). A mean temperature was calculated per month and plotted against the monthly mean values for water uptake. The results are shown in Figure 4. High maximal tem-
peratures are correlated with a higher water uptake. The positive correlation is significant at 0.999. No relationship with the atmospheric pressure, nor with the course of the tides in Antwerp could be demonstrated.

**DISCUSSION**

The important feature of our previous results on rhythms in 9 d old etiolated bean seedlings was their occurrence in plants which had been grown in strictly constant environmental conditions from sowing. This fact induced our search for expressions of rhythmicity in younger sporophytes, closer to the seed stage, resulting in the discovery of rhythms in germination vigor and ATP-synthesis rate after imbibition (30) in Phaseolus seeds previously stored in constant conditions.

Rhythmicity in seeds has been a topic of research for several decades. These studies have mostly pointed to circannual or seasonal rhythms in germination (e.g. 10, 23) and have been supported by more recent findings (e.g. 4). Occasionally a parallelism between endogenous water content of the seeds and the circannual rhythm in germination vigor has been noted (12). Several publications have stressed the importance of water uptake in controlling germination of seeds (16), and water uptake has been suggested to control dormancy (19).

In this paper we present results on long-term rhythmicity in the imbibition rate of Phaseolus seeds. Previous work in this laboratory has already shown that an ultradian rhythm with a period length of 6 h (26) governed water uptake by bean seeds after a standardized imbibition period of 4 h. We have now determined the water uptake of bean seeds at noon over a 2 year period.

Two rhythmic components were evident: the individual monthly graphs (Fig. 1) as well as the composed results on 25 lunar cycles (Fig. 2B) clearly showed that the lunar cycle influences the water uptake by bean seeds. Water uptake was faster on the days between the phases of the moon (Figs. 1 and 2, A and B), and slower in the periods of new phases, thus demonstrating a circaseptan rhythm.

A seasonal rhythm in water uptake was even more pronounced, pointing to an underlying circannual rhythm. During the experimental period the highest monthly mean values of water uptake were recorded in August-September, while minimal values were observed in February (Fig. 3). The same shape of curve could be found when plotting mean values of weeks or lunar cycles (not shown).

Notwithstanding the precautions taken to keep the seeds in a constant environment, we noticed a net influence of the lunar cycle. We have no explanation for these findings. The influence of the seasons seems easier to accept, although it is not clear how the seeds respond to the seasonal rhythmicity occurring in the natural environment.

The uptake of water by the seeds was positively and linearly correlated with the monthly mean maximal outdoor temperature (Fig. 4). This could be expected from the curve of the circannual rhythm itself (Fig. 3) but the question remains open as to how the seeds perceive this environmental cue. Indeed, the shoulder in the curve in December 1985 coincides with an exceptionally cold November, followed by December with a higher mean temperature (4.4 versus 9.1°C as mean maximal temperature). This is difficult to reconcile with the idea that the seeds have a built-in awareness of the general course of the seasons.

In older work (10), rhythms (in the circannual range) in water uptake of seeds have been described. They were reported to parallel rhythms in germination (23). Brown and Chow (7) have reported a rhythm in water uptake rate with a period length of a week, as in our results, but they correlated high water uptake with the new phases of the moon, in contrast to our findings.

Circannual rhythms have mostly been studied on perrennal plants grown in the field (e.g. 20), although some authors have studied annual plants in constant conditions (18) using the same approach as we have done. Circalunar rhythms on the other hand are well known in species living in the intertidal zone (e.g. 21). Rounds (22) reported on a semilunar rhythm in the concentration of a neurotransmitter-like substance in plants grown in the laboratory, while Abrami (1) mentioned a correlation between lunar phases and plants grown in the field. Graviou (15) proposed a lunar modulation of plant function and Brown et al. (8) reported on monthly cycles in an organism held in constant conditions.

Some of these papers gave explicit support to the idea of an exogenous origin for biological rhythms, as proposed by Brown (6) when reporting observations of rhythmicity in material kept for a long time in constant, noninducing conditions. The exogenous idea requires the existence of an extraordinarily specialized receptive capacity for the natural geoelectromagnetic field, causing biological rhythms through autophasing (6).

Nevertheless most authors endorse the endogenous nature of rhythmicity, as put forward by, e.g. Bünning (11) and Edmunds (14). In previous work we showed that the rhythms observed in 9 d old etiolated bean plants met the conditions for demonstrating the endogenous character of a circadian rhythm (25). The present results were obtained in the first hours of imbibition of bean seeds, previously stored dry in constant conditions. Other authors have previously reported that circadian (9) and circalunar (15) rhythms can be observed even in gas exchange of dry seeds. To hold the endogenous view in these cases it is necessary to postulate very fine perception mechanisms, active in 'dry' (about 8% water content) seeds and able to modulate phase-shifts in the presumed endogenous clock through environmental signals other than light, humidity, and temperature.

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