Plant Physiol. (1987) 84, 707–710
0032-0889/87/84/0701/04/$01.00/0

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

Received for publication November 5, 1986

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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 replicas, 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.

Since the (presumably) first mention of rhythmic phenomena in plants, made by Androsthenes, a soldier in the expedition army of Alexander the Great (5), this topic has been studied intensively as is obvious from the numerous review papers (*e.g.* 13, 27) and text books (11, 21). Rhythms have been observed in many morphological, physiological, and biochemical parameters of plant development. They illustrate the wide range of possible wavelengths (ultradian, circadian, infradian, circalunar, and circannual cycles). Many of these rhythms were, however, described in plants grown in natural (2) or artificial (17) cycles of alternating environmental conditions. The authors claimed the nonappearance of biological rhythms in plants kept in constant conditions from sowing (3).

Previously, in our laboratory, we have studied rhythmicity in 9 d old etiolated seedlings of *Phaseolus vulgaris* L. cv Limburg, grown in constant conditions in darkness. Oscillations were described in phytochrome mediated leaf expansion and hook opening (29), in stem elongation rate (28) and in enzyme activity of the stem hook (24). Further work showed that these rhythms had a genuine endogenous nature and that the course of the curves was independent of the moment at which imbibition commenced (25).

Subsequently our research has focused on early plant development, close to the stage of the dry seed state. Previous reports have demonstrated ultradian oscillations in seed germination, in the ATP-content of seeds after imbibition (30), and in the rate of seed imbibition (26). In this last paper an effect of the moment of onset of imbibition on the rate of water uptake of bean seeds was demonstrated pointing to an ultradian rhythm with a period length of 6 h.

We now report on the relation between the moment of imbibition onset and the rate of water uptake by the seeds on a longer time scale, searching for the occurrence of circalunar or circannual rhythms.

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 of 25°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 replicas 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 throughs 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 1985 to 1986 and a third summer peak was reached in July 1986, thus establishing a circannual rhythm.

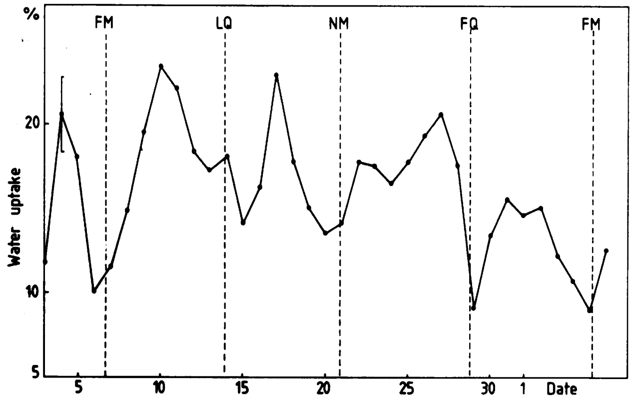

FIG. 1. Mean daily water uptake in percent of initial dry weight by bean seeds after 4 h imbibition at 25°C in darkness (3/1–6/2/85). A representative standard deviation on the mean is indicated. Dotted lines indicate days of local phases of the moon (FM = full moon, LQ = last quarter, NM = new moon, FQ = first quarter).

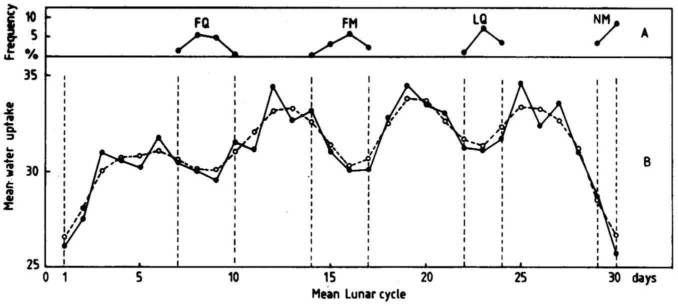

FIG. 2. A, Frequency of the day of appearance of new phases of the moon during the 25 lunar cycles observed considering the day of NM as 1. B, Mean daily water uptake in percent of initial dry weight by bean seeds after 4 h imbibition at 25°C in darkness as a function of the day number in the lunar cycle, considering new moon as day number 1. Mean of 25 lunar cycles from June 1984 through July 1986. Solid line connects real measuring points, dotted line connects points obtained using a three-point moving mean recalculation. Abbreviations as in Figure 1.

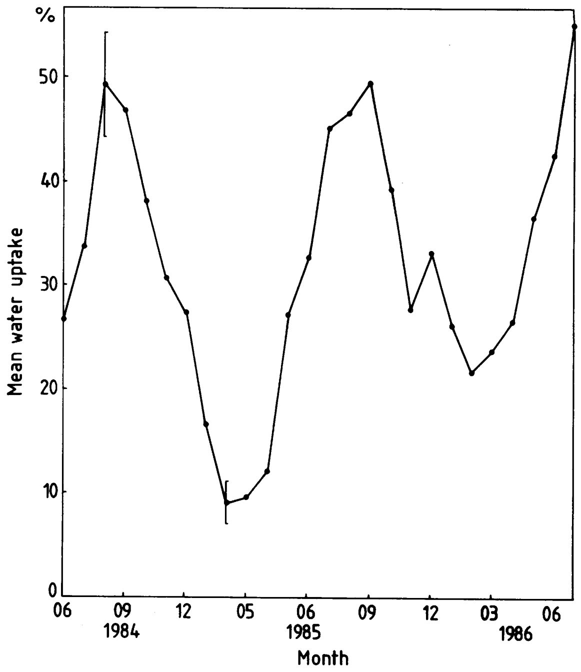

FIG. 3. Mean monthly values of water uptake in percent of initial dry weight by bean seeds after 4 h imbibition at 25°C in darkness from June 1984 until July 1986. Representative standard deviations on the mean are indicated.

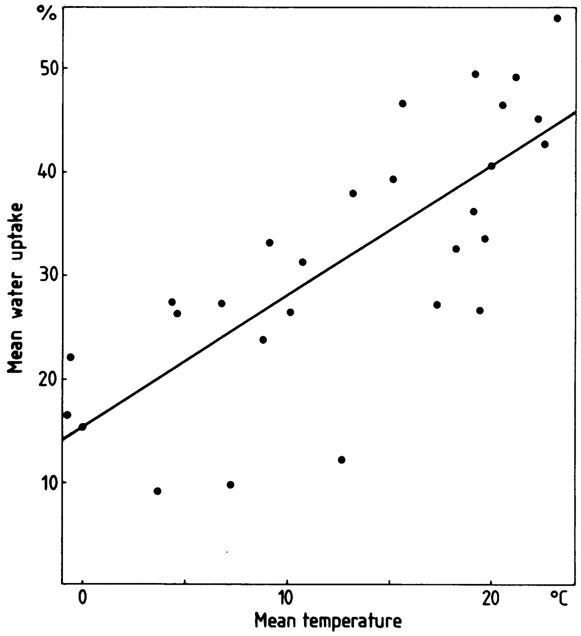

FIG. 4. Mean monthly values of water uptake in percent of initial dry weight by bean seeds after 4 h imbibition at 25°C in darkness from June 1984 till July 1986 *versus* mean monthly maximal outdoor temperatures as obtained from the Royal Meteorological Institute. Best fitting curve for positive linear correlation is $y = 15.42 + 1.27x$ ($r = 0.75$, $n = 26$, significance exceeds 0.999).

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 temperatures 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 Antwerpen 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 perrennnial 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.

Acknowledgment—The authors gratefully acknowledge Dr. B. Thomas (Glasshouse Crops Research Institute, Littlehampton, Great-Britain) for critical reading of the final version of the manuscript.

LITERATURE CITED

1. Abrami G 1972 Correlation between lunar phases and rhythmicities in plant growth under field conditions. Can J Bot 50: 2157–2166
2. Acevedo E, E Fereres, T Hsiao, D Henderson 1979 Diurnal growth trends, water potential and osmotic adjustments of maize and sorgum leaves in the field. Plant Physiol 64: 476–480
3. Ball NG, IJ Dyke 1954 An endogenous 24-hour rhythm in the growth rate of the *Avena* coleoptile. J Exp Bot 5: 421–433
4. Baskin JM, CC Baskin 1983 Seasonal changes in the germination response of buried seeds of *Arabidopsis thaliana* and ecological interpretation. Bot Gaz 144: 540–543
5. Bretzl H 1903 Botanische Forschungen des Alexanderzuges. Teubner, Leipzig
6. Brown FA 1969 A hypothesis for extrinsic timing of circadian rhythms. Can J Bot 47: 287–298
7. Brown FA, CS Chow 1973 Lunar-correlated variations in water uptake by bean seeds. Biol Bull 145: 265–278
8. Brown FA, MF Bennett, HM Webb 1958. Monthly cycles in an organism in constant conditions during 1956 and 1957. Proc Natl Acad Sci USA 44: 290–296
9. Bryant TR 1972 Gas exchange in dry seeds: circadian rhythmicity in the absence of DNA replication, transcription and translation. Science 178: 634–636
10. Bünning E 1949 Zur Physiologie der endogenen Jahresrhythmik in Pflanzen, speziell in Samen. Z Naturforsch 4B: 167–176
11. Bünning E 1977 Die Physiologische Uhr. Springer-Verlag, Berlin
12. Bünning E, EW Bauer 1952 Uber die Ursachen endogener Keimfahigkeitsschwankungen in Samen. Z Bot 40: 67–76
13. Cumming BG, E Wagner 1968 Rhythmic processes in plants. Annu Rev Plant Physiol 19: 381–416
14. Edmunds LN 1983 Chronobiology at the cellular and molecular levels: models and mechanisms for circadian timekeeping. Am J Anat 168: 389–431
15. Graviou E 1978 Analogies between rhythms in plant material, in atmospheric pressure and solar lunar periodicities. Int J Biometeorol 22: 103–111
16. Hegarty TW 1978 The physiology of seed hydration and dehydration, and the relation between water stress and the control of germination: a review. Plant Cell Environ 1: 101–119
17. Jabben M, E Schafer 1976 Rhythmic oscillations of phytochrome and its pelletability in *Curcurbita pepo* L. Nature 259: 114–115

18. Jacobsohn MK, GM Jacobsohn 1976 Annual variation in the sterol content of seedlings of *Digitalis purpurea* L. Plant Physiol 58: 541–543
19. McIntyre GI. AI Hsiao 1985 Seed dormancy in *Avena fatua*. II. Evidence of embryo water content as a limiting factor. Bot Gaz 146: 347–352
20. Oquist G 1982 Seasonally induced changes in acyl lipids and fatty acids of chloroplast thylakoids of *Pinus sylvestris*. A correlation between the level of unsaturation of monogalactosyldiglyceride and the rate of electron transport. Plant Physiol 69: 869–875
21. Palmer JD 1976 An Introduction to Biological Rhythms. Academic Press, New York
22. Rounds HD 1982 A semi-lunar periodicity or neuro-transmittor-like substances from plants. Physiol Plant 54: 495–499
23. Ruge A, D Liedtke 1951 Zur periodischen Keimbereitschaft einiger Malven-Arten. Ber D Bot Ges 64: 141–150
24. Spruyt E, JP Verbelen, JA De Greef 1983 Diurnal rhythms and circannual changes in enzyme activity in *Phaseolus*. Arch Int Physiol Biochim 91: B39–40
25. Spruyt E, JP Verbelen, JA De Greef 1985 About the endogenous nature of the rhythms in photomorphogenetic reactivity and in ATP-content of etiolated *Phaseolus* seedlings. Biol Jaarb 53: 188–202
26. Spruyt E, JP Verbelen, JA De Greef 1986 Imbibition and the moment of its onset. Arch Int Physiol Biochim 94: 31
27. Sweeney BM 1963 Biological clocks in plants. Annu Rev Plant Physiol 14: 411–440
28. Verbelen JP, E Spruyt, E Moereels, JA De Greef 1980 Diurnal changes in stem elongation rate of etiolated bean seedlings. Phytomorphology 30: 407–409
29. Verbelen JP, E Spruyt, E Moereels, JA De Greef 1982 Photomorphogenetic reactions and their relation to time. Photochem Photobiol 35: 247–250
30. Verbelen JP, E Spruyt, JA De Greef 1984 Timing in dry seeds. In N Longdon, O Melita, eds, Life Sciences Research in Space, Sci Publ 212. European Space Agency, Paris, pp 67–72