Temperature profiles for the expression of endogenous rhythmicity and arrhythmicity of CO₂ exchange in the CAM plant
Kalanchoë daigremontiana can be shifted by slow temperature changes

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Received: 18 May 1998 / Accepted: 30 June 1998

Abstract. The crassulacean acid metabolism (CAM) plant Kalanchoë daigremontiana Hamet et Perrier de la Bâthie shows an endogenous circadian rhythm of net CO₂ exchange (\(J_{\text{CO}_2}\)) under constant conditions in continuous light. Previous studies have shown, however, that above a certain threshold temperature \(J_{\text{CO}_2}\) changes from rhythmic to arrhythmic behaviour and that this is reversible when the temperature is lowered again. It is now demonstrated here, that this re-initiation of rhythmic \(J_{\text{CO}_2}\) from arrhythmicity needs a sufficiently strong temperature signal as defined by its abruptness. Rhythmicity reappears only if the temperature is reduced rather rapidly. If the temperature is reduced slowly then arrhythmicity is retained even at a low temperature level which normally would allow rhythmicity. Under these circumstances, however, a distinct temperature increase followed by an abrupt temperature decrease immediately elicits regular oscillations of \(J_{\text{CO}_2}\) at this lower temperature. We suggest that the strong temperature signals function as a definite synchronizer ("zeitgeber") which synchronizes different cells and/or different leaf areas which remain desynchronized after application of only slow temperature changes. This is further supported by Fourier transform analyses, revealing a harmonic structure of the superficially arrhythmic time series of \(J_{\text{CO}_2}\) after application of slow temperature reductions. This conclusion adds a spatial dimension to the otherwise purely time-dependent rhythmicity and arrhythmicity of \(J_{\text{CO}_2}\) in CAM.

Key words: Crassulacean acid metabolism – Circadian rhythm – Kalanchoë – Photosynthesis oscillation – Synchronizer ("zeitgeber") – Temperature gradient

Introduction

The day/night cycle of crassulacean acid metabolism (CAM), with its night-time CO₂ fixation and organic acid accumulation, and its day-time remobilization of CO₂ from the organic acids leading to refixation in the photosynthetic carbon reduction cycle, has long been known to operate also under constant environmental conditions as a free-running endogenous circadian rhythm. Wilkins (1960) first demonstrated that the diurnal rhythm is carried on under continuous darkness and in CO₂-free air. Nuernbergk (1961), Lüttinge and Ball (1978) and Wilkins (1984) showed that the rhythm also persists under constant conditions in continuous light. Later it was observed that endogenous rhythmicity only occurs within certain boundaries of environmental conditions. Light intensity and leaf temperature were found to be the dominant central parameters. Above a certain threshold of these factors, rhythmicity of net CO₂ exchange (\(J_{\text{CO}_2}\)) by the leaves of the CAM plant Kalanchoë daigremontiana reversibly changes to an arrhythmic behaviour (Lüttinge and Beck 1992).

In the arrhythmic mode, \(J_{\text{CO}_2}\) of K. daigremontiana does not turn out to be completely stochastic, because Fourier transform analyses of time series reveal clear structures (Grams et al. 1996; Lüttinge et al. 1996). Theory and computer-based modelling show that a nonlinear feedback system plays the key role in the endogenous CAM rhythm. Both in the model and in vivo, endogenous rhythmicity requires a beat oscillator or hysteresis switch ("endogenous oscillator") for functioning. A particularly precise control parameter of the endogenous CAM rhythm is leaf temperature. It plays a key role in the ability of K. daigremontiana to maintain a stable circadian CAM rhythm. An endogenous rhythm persists only at medium leaf temperatures (Anderson and Wilkins 1989a), while for leaf temperatures above 25–28 °C the time structure becomes arrhythmic (Lüttinge and Beck 1992; Grams et al. 1996). Sudden changes of leaf temperature can either entrain the endogenous rhythm (Lüttinge et al. 1996) re-initiate the rhythm out of the arrhythmic state (Anderson and Wilkins 1989a; Grams
et al. 1996, 1997), or shift the phase of rhythmicity (Anderson and Wilkins 1989b; Wilkins 1962, 1983). Experimental studies of the thermotropic behaviour of the tonoplast membrane in *K. daigremontiana* (see above), rapid changes in leaf temperature were applied. Such fast temperature signals may be considered to act as external synchronizers (“zeitgeber”). For circadian rhythms, light has normally been assumed to be the principal synchronizing factor (Winfree 1990); however, changes in temperature as well as various other synchronizing parameters are also reported in the literature. Moreover, under natural conditions several factors, such as changes in both light and temperature, may occur simultaneously, so that the question of the dominating “zeitgeber” arises (Lennon 1995). Thus, for a more detailed study of the influence of changes in the external control parameters temperature and light on the re-initiation of the rhythm out of the arrhythmicity, in this work, a time series of gas-exchange measurements was recorded which started at high leaf temperatures under continuous light, where gas-exchange of *K. daigremontiana* is arrhythmic. To find out if only abrupt, or also gradual, changes in temperature are effective as a “zeitgeber”, and if this also depends on light intensity, firstly, the slope of the temperature changes was altered, and secondly, experiments were repeated with a gradual temperature slope and different light intensities. It turned out that it is not a given temperature level per se which effects the re-appearance of the rhythm but that the strength of the signal, as defined by its abruptness, plays a major role, and that this is independent of the intensity of continuous light in the range tested. This behaviour, and additional features of the observed CAM rhythm in continuous light and under definite temperature regimes, led us to propose a new explanation for the nature of the rhythmic/arrhythmic gas-exchange pattern of *K. daigremontiana*, where, in addition to the tonoplast membrane functioning as the main beat oscillator, mutual coupling and decoupling of different areas in the leaf is essential. Thus, regulation operates in time and space.

**Materials and methods**

*Plants.* Plants of *Kalanchoë daigremontiana* Hamet et Perrier de la Bâthie were raised from adventitious plantlets obtained from leaves of the plant collection of the Botanical Garden, Darmstadt University of Technology. They were grown in soil culture in a glasshouse until they had produced six to seven pairs of fully developed leaves, and were about 0.4–0.5 m tall. During winter, additional light (HQI-T, 400 W; Philips) was provided to extend the daylight period up to 12 h.

**Gas-exchange measurements.** The measurements of net CO₂ exchange were performed in a climate-regulated chamber of the phytootron at Darmstadt, as previously described (Lüttge and Beck 1992). Net CO₂ exchange was measured using the minicuvette system of Walz (Effeltrich, Germany). A mature leaf of a plant was enclosed in the gas-exchange cuvette while remaining attached to the plant. For a better control of the leaf temperature, the thermistor usually used for measuring the temperature inside the cuvette, was carefully attached to the lower side of the leaf. Thus, leaf temperature rather than air temperature was exactly regulated.

Gas-exchange data were recorded every 5 min using a personal computer and a datalog programme. The relative humidity of the air inside the cuvette was set at 60 ± 5% and was held constant by using the bypass humidity control system [Walz]. Irradiance (photosynthetically active radiation, PAR) was measured in the range of 400–700 nm using a LICOR quantum sensor (LICOR). The conditions inside the phytochamber were adapted to the conditions inside the gas-exchange cuvette.

**Fourier spectra analysis.** To extract the harmonic components of an oscillation, which are present within a time series, fast Fourier transform analysis (FFT) was used. Time series of JCO₂ were subjected to FFT and absolute values of the Fourier coefficients Cn plotted as a function of frequency, giving the power spectrum of JCO₂. The power spectrum gives the series of all harmonic amplitudes of a given frequency which are contained as periodic components in the complex time structure of a signal represented as time series. In this sense, it is complementary to the time series itself, presenting the information contained therein as a frequency spectrum. The higher the frequency coefficient, the more prominent is the corresponding harmonic oscillation present in the complex signal.

If an arrhythmic series is produced only by statistical fluctuations, the power spectrum shows an even distribution of the power coefficients over the range of frequencies (“white noise”). In contrast, if the power spectrum shows discrete frequency peaks, the measured time series is not exclusively due to pure noise. Furthermore, one can obtain some information about the nature of the underlying mechanism, which is present in the superficially arrhythmic structure. Time series of 42.6 h duration were subjected to Fourier analysis.

**Results**

**Variation of the slope of external temperature gradients applied to leaves.** Three time series with continuous recording of net CO₂ exchange (JCO₂) were performed, in which the slope of the leaf temperature gradient from 28 °C (arrhythmic gas exchange pattern) to 21 °C was varied ([slope] = ΔT/C) under continuous illumination. The temperature was reduced in steps of Δ = 0.5 °C with different time intervals between steps in the three experiments, i.e. 0.5 °C per 1 h, per 4 h and per 12 h, respectively (Fig. 1A–C).

The endogenous rhythm reappears only if the slope of the temperature change is steep (0.5 °C per 1 h = steep slope, Fig. 1A). At about 24 °C the endogenous rhythm reappears and persists for at least 3 subsequent days. With a medium slope (0.5 °C per 4 h; Fig. 1B) and a gentle slope (0.5 °C per 12 h; Fig. 1C) the endogenous rhythm of net CO₂ exchange does not reappear even at the lowest temperature of 21 °C. This is in contrast to what would be expected since, according to the behaviour in Fig. 1A and observations of previous studies (Lüttge and Beck 1992; Grams et al. 1996, 1997), at a