Correlation between the endogenous circadian rhythmicity in growth rate and fluctuations in oleic acid content in expanding stems of *Chenopodium rubrum* L.

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Abstract. *Chenopodium rubrum* L. plants exhibit an endogenous circadian rhythm in their instantaneous stem extension rate in continuous light (A. Lecharny and E. Wagner, 1984, Physiol. Plant. 60, 447–453). Stem extension rate and fatty-acid composition of two stem parts were measured in plants kept in continuous light for 90 h following a 12-h dark period. Fluctuations in the relative size of the oleic acid pool were evidenced in the stem tissues. The peaks (minima and maxima) of the oleic acid content occurred at the same times after the end of the 12-h dark periods as the peaks of the stem extension rate. This rhythmic behaviour ceased when growth was completed. No significant rhythmic changes were observed in any other fatty acid pools. Lipids in which the oleate content is rhythmically modified were exclusively phosphatidylcholine and phosphatidylethanolamine. Thus, there was a specific correlation between the relative amount of oleic acid in phosphatidylcholine and phosphatidylethanolamine and the rate of instantaneous growth in the same tissue. The rhythmic variations in the oleic acid may be linked to the endomembrane flow in relation to the rate of growth.

Key words: *Chenopodium* – Fatty acid – Rhythmicity (oleic acid content) – Stem extension rate

Introduction

The directional growth of cells in plant stems is modulated by light (Lecharny 1985). This response to light quality and quantity has been studied extensively in *Vigna sinensis* L. (Lecharny and Jacques 1980, 1982). Cellular elongation involves an increase in the cell volume, and synthesis of the plasmalemma is necessary in order to maintain cellular integrity. Direct or indirect photomodulation of the accumulation of lipids in this membrane compartment is a necessary component of the photocontrol of stem growth. In *V. sinensis*, the stem extension rate (SER) is higher in darkness than in white light of 5 μmol·m⁻²·s⁻¹. Fatty-acid accumulation follows two opposing patterns (Lecharny et al. 1981). An accumulation of stearic acid (C 18:0) and oleic acid (C 18:1) is inhibited by light, whereas accumulation of all the other fatty acids is stimulated by light and is probably controlled via the energy state of the chloroplasts (Eastwell and Stumpf 1983). The oleic acid content is specially decreased in two lipid classes, phosphatidylcholine (PC) and phosphatidylethanolamine (PE; De March et al. 1984). These two phospholipids are known to be mainly (for PC) or exclusively (for PE) located in extrachloroplastic compartments implicated in cell growth (Mazliak 1987). Light regulation of oleic acid accumulation into PC concerns mainly the C-1 position of the glycerol backbone (Trémolières et al. 1988). Unfortunately it is not possible from these end-point experiments to decide whether there is a functional link between changes in the sizes of oleic acid and the photocontrol of growth rate, or whether both phenomena are independently controlled by light.

One way to discriminate between these possibilities is to determine if the correlation observed between oleic acid content, in PC and PE, and stem growth is observed in constant environmental conditions. This approach, impossible with *V. sinensis*, which exhibited a constant SER in constant conditions, was attempted using *C. rubrum* L., a plant frequently employed in the course of studies on photoperiodism (Cumming et al. 1965; Lecharny et al. 1985; Wagner 1977). This plant shows an endogenous circadian rhythm in its instantaneous stem extension rate with a period of approx. 23 h (Lecharny and Wagner 1984). It is, therefore, possible to compare...
the stem extension rate, as modulated by the internal clock, with the fatty-acid composition of plant tissues kept under constant temperature and light conditions. The results are discussed in terms of a competition, in rapidly growing organs, between incorporation of the fatty acids into expanding membranes and synthesis of unsaturated fatty acids from oleic acid.

Material and methods

Plant material and growth conditions. Seeds of Chenopodium rubrum L. ecotype 184 (50°10′ N; 150°35′ N) were sown and germinated following the procedures described in Cumming (1963) and then grown under continuous light as in Lecharny and Wagner (1984). After three weeks, plants were transferred to darkness for 12 h and then returned to continuous white light at 85 μmol m⁻² s⁻¹.

Previously (Lecharny and Wagner 1984), an endogenous circadian rhythm in SER of C. rubrum L. was demonstrated under constant temperature and irradiation conditions and this rhythm was reset by such a dark period. Also, the phase of the rhythm is determined by the dark-to-light transition. Thus, in the figures, the time axis is expressed in hours from the end of the dark period. White light was supplied by mercury high-pressure lamps (Osram, HQUI TS 400; München, FRG) filtered through heat-absorbing glass. Temperature was maintained at 24 °C and humidity at 70%.

Sampling was carried out under the growth conditions. For each sample, two segments of the stem (S₁ and S₂) were harvested. The more basal segment (S₁) was that between the second leaf from the base of the stem and the fourth leaf. The more apical segment (S₂) was that between the fourth and sixth leaf. Tissues were weighed quickly, immediately immersed in boiling water for 1 min and then stored in methanol at −20 °C.

Lipid and fatty-acid analysis. Lipids were extracted following Bligh and Dyer (1959), an internal standard — heptadecanoic acid — being added before grinding. Total fatty acids from the lipid extract were trans-esterified as in Carreau and Dubacq (1978) and analysed by capillary gas-liquid chromatography (De March 1984). Protein content was determined as in Lowry et al. (1951). The system used allowed a perfect separation of all fatty acids and particularly of oleic (C 18:1, Δ⁹) and cis-vaccenic (C 18:1, Δ11) acids as shown by comparison with standards. Cis-vaccenic acid was only present in very small amounts in stems of C. rubrum. Separation of different polar lipids and neutral-lipid classes was performed by thin-layer chromatography (Trémolières and Lepage 1971).

Extension-rate recording. Stem extension rate was monitored continuously using linear voltage differential transformers in combination with demodulators (both from Ifelec, Chauvin Arnoux, Paris, France) as in Lecharny and Jacques (1980). To allow a clear detection of the third maximum in SER, slightly younger plants were used for extension-rate recording than for lipid and fresh-weight (FW) measurements.

Results and discussion

Stem growth. At the time of experimentation, whole stem growth was almost exclusively the result of elongation of S₁ and S₂. Using linear voltage differential transformers, the whole-stem SER of five plants was monitored continuously during the experimental period (Fig. 1C). As in previous experiments (Lecharny and Wagner 1984), a strong circadian fluctuation in SER was observed with maxima occurring 33, 58 and 84 h after the end of the dark period. Fluctuations in SER were between 0.05 and 0.25 mm·h⁻¹. Growth was also measured as an increase in FW. While the increase in FW of S₂ was almost constant over the whole 90 h of continuous light (0.69 mg·h⁻¹), the increase in FW of S₁ was completed by the 48th h of light. Therefore, no rhythmic fluctuations in FW were detectable. This lack of fluctuation in FW while SER fluctuated does not mean that SER and FW were not related but may rather be ex-