Received: 8 July 2024 | Accepted: 22 October 2024

DOI: 10.1111/php.14046

RESEARCH ARTICLE

Rapid osmocontractile response of motor cells of *Mimosa pudica* pulvini induced by short light signals

Gabriel Roblin[1] | **Christelle Moyen**[2] | **Pierrette Fleurat-Lessard**[1] | **Fabienne Dédaldéchamp**[1]

[1]Laboratoire EBI–Ecologie et Biologie des Interactions, UMR CNRS 7267, Université de Poitiers, Poitiers, France

[2]Laboratoire de Chrono-Environnement, UMR 6249 CNRS/UFC, Campus de la Bouloie, Université de Franche-Comté, Besançon, France

Correspondence
Fabienne Dédaldéchamp, Laboratoire EBI–Ecologie et Biologie des Interactions, UMR CNRS 7267, Université de Poitiers, Poitiers, France.
Email: fabienne.dedaldechamp@univ-poitiers.fr

Abstract

The *Mimosa pudica* leaf has motor organs allowing movements driven by cell osmotic changes in the parenchyma cells in response to various stimuli. Short white light pulses induce rapid and large seismonastic-like movements (denoted "photostimulation") of the primary pulvini in various leaves within 120 s after the onset of light. An early event recorded is a wavelength-related modification of the plasma membrane difference: potential depolarization under white, blue, green, and red wavelengths, and hyperpolarization under far red wavelengths (and also in darkness). The photoreactivity of the pulvini is controlled by a circadian rhythm and modulated by the applied diurnal photoperiod cycle (photophase ranging from 6 to 18 h). The reactivity varied among plants and even between leaves on the same plant. The level of reactivity is related to the photon fluence rate in the range from 10 to 140 $\mu mol\, m^{-2} s^{-1}$ under white light and to the experimental temperature in the range 15°C–35°C. An "accommodation" to light supply is evidenced by a modulation of the reactivity in relation to the schedule of light application under low fluence rates and the introduction of short darkness intervals during the first 30-s light pulse. The blue light-induced photostimulation is under phytochrome control.

KEYWORDS

light signaling, membrane potential, *Mimosa pudica*, osmocontraction, photostimulation, pulvinus

INTRODUCTION

Light is an environmental factor that displays continuous control of many fundamental aspects of plant life. Light energy is converted into chemical energy stored as carbohydrates through the process of photosynthesis. Light also intervenes as a complex signaling input in more specific processes, modulating plant development at many stages: seed dormancy, stem elongation, leaf expansion, transition to flowering, and seed production.[1,2] Besides

Abbreviations: Ψ_0, transmembrane electrical potential; AP, action potential; ATP, adenosine triphosphate; BL, blue light; cry, cryptochrome; D, darkness; f.r, fluence rate; FR, far red radiations; Gr, global reactivity; L, light irradiation; MES, 2-[N-morpholino]ethanesulfonic acid; P1, primary pulvinus; P2, secondary pulvinus; P3, tertiary pulvinus; Phot, phototropins; Phy, phytochrome; PLC, phosphoinositide-specific phospholipase C; r, pulvinar reactivity; R, red radiations; TEM, transmission electron microscopy; WL, white light; Y, Yield.

the irreversible photomorphogenic processes, which develop over long periods of time (hours to days), light also intervenes in faster behavioral responses of plant organs (seconds to minutes), represented by phototropism of stems, heliotropism, and photonasty of leaves.[3,4] Plants sense many light parameters, including intensity, spectral composition, spatial direction, and duration of exposure. This sensing is mediated by specific photoreceptors located throughout the plant organs that accurately detect changes in the spectral composition from UV-B to far-red wavelengths. Five classes of photoreceptors acting in a specific wavelength range have been discovered in the model plant *Arabidopsis thaliana* and characterized in terms of their biochemical features and physiological roles. Thus, light is perceived in the red/far red region (600–750 nm) by phytochromes (phy) A and B, in the blue/UV A region (320–500 nm) by cryptochromes (cry) 1, 2, and 3, phototropins (phot) 1 and 2, and the Zeitlupe family, and in the UV B region (290–320 nm) by UVR8.[1,5,6]

To investigate the rapid light-induced phenomena, the model of the leaf motor organs (pulvini) appears particularly suitable, since the observed responses are not complicated by growth processes. The pulvini in the compound leaf of *Mimosa pudica* act as joints between the different parts of the leaf (primary, P1: stem-petiole, secondary, P2: petiole-rachis, and tertiary P3: rachis-blade), allowing movements driven by osmotic changes in the parenchyma cells of the organs driven by K^+ and Cl^- migrations.[7–9] Various stimuli, such as shock, electrical or thermal stimulation, induce rapid nastic movements within a few seconds.[10,11] In addition, a sudden increase in sunlight intensity can also induce such a rapid response as formerly described.[12,13] Further studies were devoted to this light-induced rapid nastic movement under laboratory conditions.[14,15] In particular, it was observed that flashes of white light (WL) applied for periods as short as 15 s triggered a drooping movement of the leaf (referred to as 'photostimulation') with an amplitude and a rate similar to the shock-induced response. The action spectrum clearly shows that the response is mediated by a photosensitive pigment that is active in the 400–500 nm range. Notably, the movement is triggered 40–120 s after the onset of light application. This latency is a function of light irradiance,[14] indicating that a sequence of metabolic changes occurs between the light perception and the osmocontractile process of the motor cells.

The aim of this work was mainly focused on an analysis of the early events that occur after a short light pulse on the pulvinar motor cell and, in particular, to determine some parameters (namely, light intensity, modalities of light pulse application, energetic implications, and relationship with the circadian clock) that modulate the expression of the subsequent rapid pulvinar cell osmocontraction.

MATERIALS AND METHODS

Plant growth conditions

Seeds of *Mimosa pudica* L. were germinated in an organic compost. Seedlings and older plants were grown in the compost watered daily and were kept in climate-controlled chambers at 28°C ± 0.5°C and 65% ± 5% relative humidity. In standard conditions, illumination was regulated to give 14 h of light (photophase 07.00–21.00 h) provided by fluorescent tubes (mixing Osram fluora and Osram day-light types) giving a photon fluence rate (400–800 nm) of 80 µmol m^{-2} s^{-1} at the plant apex.

Quantitative measurements of pulvinar reactivity

Plants were selected without any prior bias and grouped into sets of 7–8 plants, each having 6 well-developed leaves (Figure 1a1). Photostimulation was applied at regular intervals, usually every 20 min, unless otherwise specified in particular experiments. In the majority of experiments, light stimuli were applied using the fluorescent sources used for plant cultivation. However, in some specific assays, alternative sources were used. Thus, SP Philips sources were used to obtain irradiations with high photon fluence rate (1160 µmol m^{-2} s^{-1}, 40% emitted in the 400–460 nm range). In addition, red irradiation (R) was obtained from fluorescent tubes (Philips TL15) and far red (FR) irradiation was obtained from tungsten lamps filtered through RGN9 Schott glass.

The movements were monitored by time-lapse photography: choosing 5-s intervals allows to count the number of induced rapid leaf downward movements (Figure 1a2) in each interval and to determine the latency of the response. The reactivity (r) corresponded to the total number of pulvini showing such movements in a set of plants consecutively to one light stimulation. When the set was subjected to several stimulations, the global reactivity (Gr) was the sum of r. The yield (%) is the ratio of Gr/N, the number of possible movements (N = number of leaves in the set of plants × number of photostimulations).

Bioelectrical recordings

Intracellular recordings in cortical motor cells were carried out on excised leaves of *M. pudica* maintained in conditions similar to the cultural conditions. The particular anatomy of the P1 of *M. pudica* characterized by many layers of parenchyma motor cells surrounding the central cylinder allows impalement of a microelectrode in a

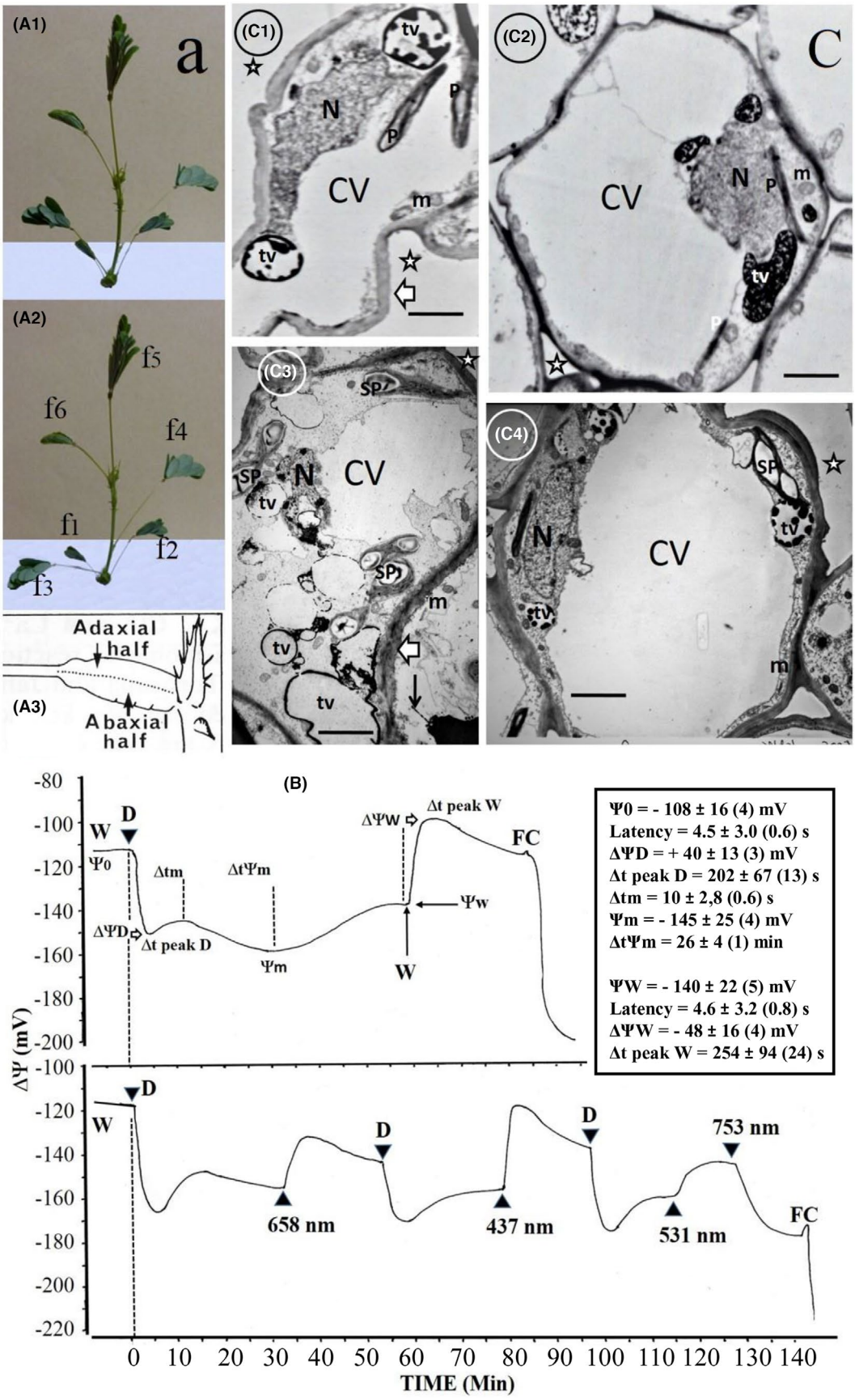


FIGURE 1 Legend on next page

FIGURE 1 (A) The experimental model of *Mimosa pudica* with 6 leaves (f1–f6) in darkness in the unstimulated position (a1) and after a photostimulation applied during the scotophase of the photoperiodic cycle. Photostimulation induced a motile response of some primary pulvini (a2) resulting from differential cell contraction in the motor tissues of the abaxial half (a3). Note the large movement of f1 and f3, the slight movement of f2 and f6, and the absence of movement of f4 and f5. (B) Electrical changes induced by dark and light alternations on the transmembrane potential of a motor cell. Upper panel: Characteristic time courses of membrane potential changes induced by an alternation of dark (D) and white light (W) signals in the primary pulvinus motor cell and, in inset, statistical analyses of the parameters describing the electrical changes. Bottom panel: Time courses of membrane potential changes induced by dark signal (D) and by light signals of different wavelengths (658, 437, 531, and 753 nm, respectively). (C) Ultrastructural changes in motor cells from the abaxial half of the primary pulvinus observed by electron microscopy. Observation on a motor cell of a plant in the photophase of the photoperiodic cycle (c1) after the bending movement induced by a shock and (c2) after recovery. Note that the stimulated cell (c1) has a thin pectocellulose cell wall of irregular shape, a cytoplasm attached to the wall, a nucleus (N) with an irregular profile, mitochondria (m), and plastids (P) without starch or with very small starch granules (s). The large colloidal vacuole (CV) occupies the central part of the cell, whereas small vacuolar tannin (vt) profiles occur adjacent to the nucleus. When turgor is partially restored (c2), the cell has a fairly spherical shape. The cytoplasm forms a narrow layer around the large central colloidal vacuole (CV), while small tannin vacuolar profiles (vt) are distributed around the large nucleus. Note the long starchless plastids (P). Cells from plants photostimulated in the scotophase of the photoperiodic cycle (c3) and after recovery (c4) showed the same features as described in (c1) and (c2), but a major difference lies in the presence of large starchy plastids (SP) in the darkened pulvini. Paraformaldehyde/glutaraldehyde/OsO_4/LRWhite. Ultrathin sections observed in TEM. Scale bars 1 µm.

well-defined kind of cell (motor cell of the abaxial half in our assays). The transmembrane potential was measured by the electrophysiological method using glass microelectrodes (tip diameter <1 µm, tip resistance from 5 to 30 MΩ) drawn from capillaries provided with an internal microfiber (GC 150F15; Clark Electromedical Instruments).[16] Briefly, a leaf was excised from the stem of 2-month-old plants and the pulvinus fixed to the bottom of a 4 mL plexiglass chamber filled with a buffered medium (10 mM MES, pH 5.5) containing 1 mM NaCl, 0.1 mM KCl, and 0.1 mM $CaCl_2$. The microelectrode was impaled into a motor cell of the abaxial half of the organ (Figure 1a3). In these experiments, primary pulvinus was illuminated with a cold light source (Schott 1500) giving a photon flux density of 2500 µmol $m^{-2}s^{-1}$. Under these conditions, the calculated value of the resting transmembrane potential (Ψ_0) from 27 assays was −108 ± 4 mV (SEM).

In a series of assays to observe the influence of the wavelength, interference filters (Schott) were placed in front of the light source to give irradiations in blue (450 nm; 30 µmol $m^{-2}s^{-1}$), green (540 nm; 80 µmol $m^{-2}s^{-1}$), red (630 nm; 70 µmol $m^{-2}s^{-1}$), and far red (753 nm; 7 µmol $m^{-2}s^{-1}$).

Determination of $[Ca^{2+}]c$ in protoplasts of motor cells

The preparation of protoplasts from P1 and the determination of intracellular free Ca^{2+} concentration were largely described in a previous study.[17] Briefly, protoplasts were loaded with 6 µM Indo-1/AM (pentaacetoxymethylester of Indo-1) by incubating them for 30 min at 22°C in the dark. The protoplasts were rinsed twice in the incubation medium (10 mM MES, 0.5 M mannitol, 2 mM $MgCl_2$, pH 5.6) supplemented with 0.1 mM $CaCl_2$ before being irradiated. After a 15 min-time lapse, they were irradiated with a 5 W pulsed argon laser which provided excitation at 351 and 364 nm. The photon fluence rate of the laser beam was fixed at 1 µmol m^{-2} s^{-1}. Calcium measurements were performed using a ratiometric fluorescence method with an ACAS 570 cytometer (Meridian Instr. Inc., USA). Two photomultipliers received emission from two forms of Indo-1: one bound to Ca^{2+} at 405 nm and the other free at 485 nm. The fluorescence ratio R405/485 allowed to determine the $[Ca^{2+}]c$ after correcting for autofluorescence and calibrating for calcium content.

Microscopy

P1 of leaf 1 were fixed in 2% paraformaldehyde/0.5% glutaraldelyde with 0.1 M Sörensen buffer pH 7.3 for 15 min, and were washed in buffer supplemented with 7.5% sucrose for 1 h in 6 baths. Post fixation in 1% osmium tetroxide occurred for 3 min. Dehydration in increasing concentrations of ethanol (20%, 50%, 70%, 95%, and 100%), each for 8 min was followed by P1 embedding in the London White resin. Polymerization was conducted at 60°C for 24 h and sections were obtained using EMUC6 Leica microtome. Ultrathin sections (70 nm thick) of cortical cells in P1 abaxial half were collected on gold grids and stained using uranyl acetate and lead citrate. Transmission electron microscopy (TEM) observation was made with a Jeol 1010 microscope, at 80 kV.

RESULTS

Rapid responsiveness to light of motor cells: early bioelectrical events and cell structure modifications

It was previously observed that the pulvini of *M. pudica* rapidly react by two types of rapid movements when subjected

to white light signals applied during the nyctophase of the photoperiodic cycle. Firstly, P1 can perform a small downward movement of 10–20° from its initial position that starts after a latency of about 1 min and lasts for 12–15 min in accordance with the light stimulus duration (up to 30 min) (Figure 1a2). It has been previously observed that a depolarization of 20–50 mV was monitored in close correlation with the time course of the movement.[18] Secondly, the light signal may induce rapid pulvinar movements ("photostimulation") similar in amplitude (60 ± 8° at 25°C) and time course to that induced after a mechanical shock (downward movement in 1–2 s and recovery in 27 ± 4 min at 25°C)[14,15] (Figure 1a2). At the difference of the instant response observed after a mechanical shock, the rapid movement induced after a light stimulus occurred after a latent period lasting at least half a minute. However, a similar action potential characteristic of the P1 response was recorded in both cases. One of the first aims of this work was to obtain precise information on the early bioelectrical processes in the pulvinar motor cell stimulated by light and dark signals. The use of intracellular microelectrodes implanted in motor cells of the abaxial half of the P1 (Figure 1a3) confirmed the main findings previously measured with extracellular electrodes: hyperpolarization of the membrane potential after darkness and depolarization after light restoration. An extended statistical analysis of the processes allowed the precise definition and quantification of the characteristics describing the process ($\Delta\Psi$, latency and time course). The main remark concerned the latency of a few seconds (4–5 s) observed after either the dark or the light signal (Figure 1B, Upper panel). Furthermore, the application of different light sources showed that blue and red wavelengths induced a depolarization of the membrane potential, whereas far red induced a hyperpolarization similar to darkness. Note that green light induced only a small depolarization (Figure 1B, Lower panel).

In addition to some data described in a previous study,[17] which details the time courses and amplitude of $[Ca^{2+}]$c mobilization in laser-irradiated protoplasts from motor cells, we have determined the latency observed after the onset of UV-A irradiation that induces calcium increase in the cytoplasmic area. A statistical analysis yielded a value of 17 ± 6 s ($n = 15$).

Comparative observations by TME showed some similar cellular structural changes in motor cells localized in the abaxial half of pulvini from leaves taken either in the light or in the dark and subjected to shock or light stimuli (Figure 1C). In both cases, the pectocellulose wall had an irregular shape after the bending movement. The cytoplasm applied against the wall contained a nucleus with an irregular profile, plastids with small starch granules, and numerous mitochondria. The large colloidal vacuole occupied the central part of the cell, while small vacuolar tannin profiles occurred adjacent to the nucleus (Figure 1c1,c3). When turgor was restored, the cell was quite spherical, the nucleus had a regular profile and the colloidal vacuoles were no longer flattened (Figure 1c2,c4). The main difference is the presence of large starchy plastids in cells observed in the dark period compared to cells in the light period (Figure 1c4).

Nycthemeral variation of the photoreactivity of the pulvini

The data given in Figure 2 clearly showed that the motor organs presented a variable photoreactivity depending on the time of application of the light stimulus during the nycthemeral cycle. The P1 were poorly reactive during the photophase of the photoperiodic cycle (about 4% reactivity). The number of induced movements clearly started to increase during the last hour preceding the beginning of the dark period, then it reached a maximum in the middle of the nyctophase (about 50% reactivity) and finally

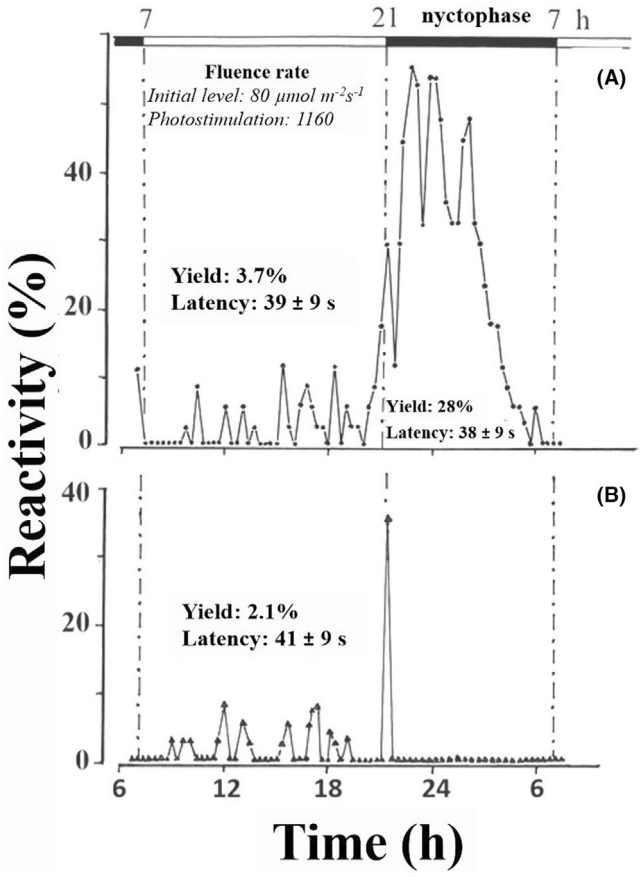
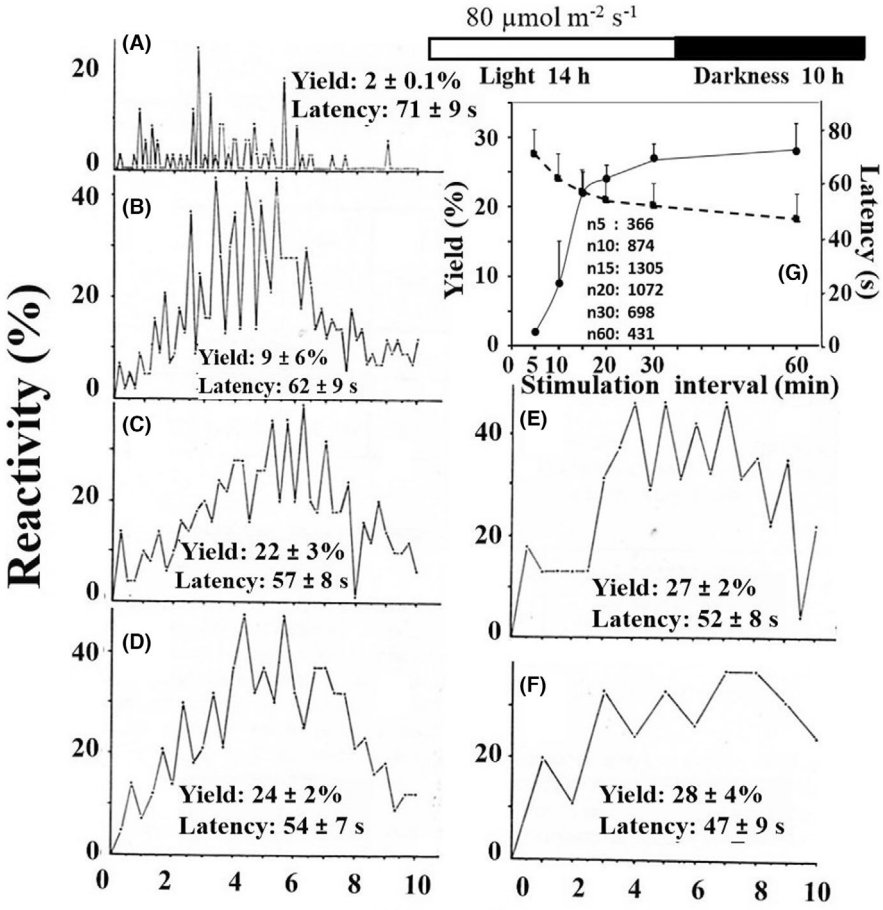


FIGURE 2 Example of the variation in photoreactivity of primary (A) and tertiary pulvini (B) of *Mimosa pudica* leaves in the course of the nycthemeral cycle. The set of 7 plants (with 6 leaves) grown at a fluence rate of 80 µmol m^{-2} s^{-1} was irradiated at a fluence rate of 1160 µmol m^{-2} s^{-1} for 120 s at 20-min intervals. The experiment was repeated three times with the same general result.

decreased about 3 h before the light-on signal (Figure 2A). In comparison, the P3 showed a low yield similar to that of P1 (about 2% reactivity) during the photophase, but a noticeable high reactivity was observed just before the darkening (about 45%). Then, no measurable reactivity can be detected during the nyctophase, as leaflets were closed during this phase (Figure 2B). Similarly, the P2 also showed the same behavior as the P3, as they also joined during the nyctophase (Figure 1A).[19] Statistical calculations showed that the latencies (about 40 s in the experimental conditions) did not differ significantly according to the type of pulvinus. Interestingly, rhythmic variations in photoreactivity were observed, the periodicity of which was determined quantitatively (see below). Taking into account the best photosensitivity during the nyctophase, the following experiments were carried out during this phase of the nycthemeral cycle.

Influence of the frequency of the photostimulations

The frequency of light stimulus induced changes in P1 responsiveness as shown by representative time courses



Plant	1	2	3	4	5	6	7	8	9	10	mean
Leaf n°	1	1	2	2	3	3	3	4	4	4	(min)
b (5 min)	85	81	72	86	85	83	76	68	85	73	79 ± 6
e (30 min)	48	52	56	50	46	55	54	55	62	50	53± 4

FIGURE 3 Examples of variations in the photoreactivity of primary pulvini of *Mimosa* pudica leaves during the nyctophase (10 h) as a function of the time interval between two light stimuli applied at a fluence rate of 80 µmol $m^{-2}s^{-1}$ for 120 s: (A) 5 min, (B) 10 min, (C) 15 min, (D) 20 min, (E) 30 min, and (F) 60 min. (G) Quantitative data on yield variation (± σ) calculated from 3 experiments and mean latency (± σ) calculated from n determinations as indicated in the inset. The sets of 7 plants bearing 6 leaves were grown at a fluence rate of 80 µmol $m^{-2}s^{-}$.[1] (H) Determination of mean latency on pulvini of leaves (n°1, 2, 3 and 4) observed individually and photostimulated at 5 and 30-min intervals.

observed on a series of plants irradiated at respective intervals of 5, 10, 15, 20, 30, and 60 min (Figure 3a–f). Calculations clearly indicated that yields were reduced and mean latencies increased when pulvini were stimulated at 5-, 10-, and 15-min intervals compared to 20-, 30-, and 60-min intervals (Figure 3G and insets). These statistical data on latencies were corroborated by observations on a series of the same leaves stimulated either after 5- or 30-min intervals (Figure 3H), justifying the use of light stimulus applied at 20-min intervals in the following assays as a convenient experimental condition.

Furthermore, the time courses showed rhythmic variations analyzed by the method of mobile means applied in the study of time series.[20] The calculations showed oscillations in reactivity with periods close to 60 min and 120 min in the assay in which the pulvini were stimulated at 15-min intervals.

Differential sensitivity of the pulvini

The variations in photoreactivity described above and a simple visual observation showed that photoreactivity varied from one plant to another and, for the same plant, from one leaf to another. Plant by plant observations made it possible to delineate schematically three distinct profiles of photoreactivity, as summarized in Figure 4A. In some plants where the leaves were very excitable, a "compact" profile was observed (upper panel). In other plants, the pulvini showed a more rhythmic activity, resulting in a "draughtboard" pattern (middle panel). A third type was then found in plants where the pulvini showed a low photoreactivity (lower panel). In these observations on plants with 6 mature leaves, it should be emphasized that the maximum reactivity was observed in leaves numbered 1 and 2 (Figure 4A). The statistical analysis carried out on plants of types "compact" and "draughtboard" confirmed this observation and also showed that the latency decreased in the younger leaves with low reactivity (Figure 4B). The typical example in Figure 4C shows that latencies also varied during the nyctophase, with the lower values observed in the middle of the phase.

Photoreactivity of the pulvini in relation to the characteristics of the light signal

In photostimulation, light can modulate the response through the fluence rate applied, the duration of illumination and its spectral composition. It has been previously determined that the active wavelengths ranged in the UV-A and in the blue parts of the spectrum (350–500 nm).[14] Data in Figure 5A show that there was a fairly linear increase in yield when fluence rates increased from 30 to 120 μmol $m^{-2}s^{-1}$, that slowed down at the higher value (140 μmol $m^{-2}s^{-1}$). Concomitantly, latency decreased linearly over the same range of fluence rates. Giving the lowest yield obtained and a long latency, the fluence rate of 10 μmol $m^{-2}s^{-1}$ can be considered to be the required threshold light level to trigger the pulvinar response (energetic threshold of 300 μmol m^{-2} in 30 s). These data also indicated that some leaves were deprived of reactivity, whatever the intensity applied. Figure 5B shows that the light duration inducing maximum yield decreased when the light fluence rate increased. The Bunsen-Roscoe law (fluence rate × light duration), which is used to achieve a 10% yield, was valid within the range of 40–110 μmol $m^{-2}s^{-1}$ but inadequate for lower (30 μmol $m^{-2}s^{-1}$) or higher (140 μmol $m^{-2}s^{-1}$) values. The data also indicated that, regardless of the fluence rate, the optimal yield was achieved after 30 s, even if the illumination was maintained for a further 30-s period. Additional assays were, therefore, carried out to analyze the modulation of events occurring during this primordial phase (30 s) in the expression of the response. To this end, leaves were irradiated at 20- and 30-min intervals with increasing illumination durations of 5, 10, 15, 18 min and 5, 10, 15, 20, 25, 28 min, respectively. The data in Figure 5C show that dark periods lasting 5 and 10 min did not allow for full recovery of yield and, in this case, responses occurred after high latencies. It is, therefore, concluded that at least a period of 15 min in darkness is required for the cells to recover their full photoreaction capacity. The absence of reaction is linked to photochemical processes and not to the pulvinar machinery as leaves were fully reactive under a shock.

The way in which the light energy is applied is also of importance, as shown by the data obtained when the plants were subjected to a gradual increase in light intensity. Figure 5D shows the different light regimes applied and, in the inset, the quantitative data obtained in each experimental schedule. It appears that the longer the time duration at low fluence rates, the lower the yield of photoreactivity and the longer the latencies. Note that a total inhibition was obtained when low fluence rates were applied for 45 s. When white light stimulus was preceded by red irradiations (15 μmol $m^{-2}s^{-1}$) for 120 s, the yield was not affected (30%), but when far-red irradiations were applied (10 μmol $m^{-2}s^{-1}$) for 90 s, the yield decreased by 17%. Furthermore, when 2 or 3 successive R/FR irradiations were applied, the observed yield was a function of the last type of irradiation: no inhibition when R was the last irradiation, and inhibition when FR was the last irradiation applied (Figure 5E). Consequently, this characteristic result observed in R/FR alternation indicates that the ratios of the two isoforms of phytochrome preceding the white light irradiation determines the efficiency of the photostimulation.

The previous data suggest that two factors influenced the process. The timing of light exposure within the first 60 s was crucial in initiating the motile reaction (Figure 5D), and the implication of darkness played a role in recovery (Figure 5C). Complementary observations showed that applying short dark intervals (of a few seconds) during white light illumination greatly reduced pulvinar reactivity (as shown in Figure 6). This result highlights the primordial role of processes occurring in this step of the reaction. Figure 6A illustrates that, for a given fluence rate (60 µmol $m^{-2}s^{-1}$ in the assay), the yield decreased as the duration of the light/dark treatment increased from 15 to 60 s. In this example, a near complete inhibition was achieved after a 60-s treatment. As shown in Figure 6B, the response was modulated by the fluence rate of the light stimuli as, in this 5 s/5 s alternation, yield inhibition increased as the fluence rate increased in the different pre-stimulation durations. It should be noted that the value of latency indicated that at 30 and 60 µmol $m^{-2}s^{-1}$ the leaves reacted about 50 s after the end of the treatment, but at the higher value (110 µmol $m^{-2}s^{-1}$) the responding leaves were not disturbed by the treatment (latency of 60 s) (Figure 6C). Comparison of the schedules 2 s/13 s and 3 s/17 s versus 2 s/10 s, 2 s/8 s and 4 s/11 s showed that a slight increase in light duration reduced yield, and the responsive leaves were those that were not inhibited by the treatment as indicated by the observed latency in the range of 100 s (duration of the treatment, i.e., 60 s + latency noted on leaves with high reactivity, i.e., 40 s). The importance of the short dark period was highlighted in schedules 7 s/8 s, 8 s/2 s, and 13 s/2 s as yields were significantly decreased under these conditions, especially in the latter case. Observations of the latency of the responding leaves showed that, in the case of 7 s/8 s and 8 s/2 s, two types of responses were triggered; either leaves with high reactivity (60 s) or leaves that were not inhibited by the treatment for 100 s. In the case of 13 s/2 s, it was noted that only the very reactive leaves responded (latency 60 s) (Figure 6D).

Energetic implications in photostimulation

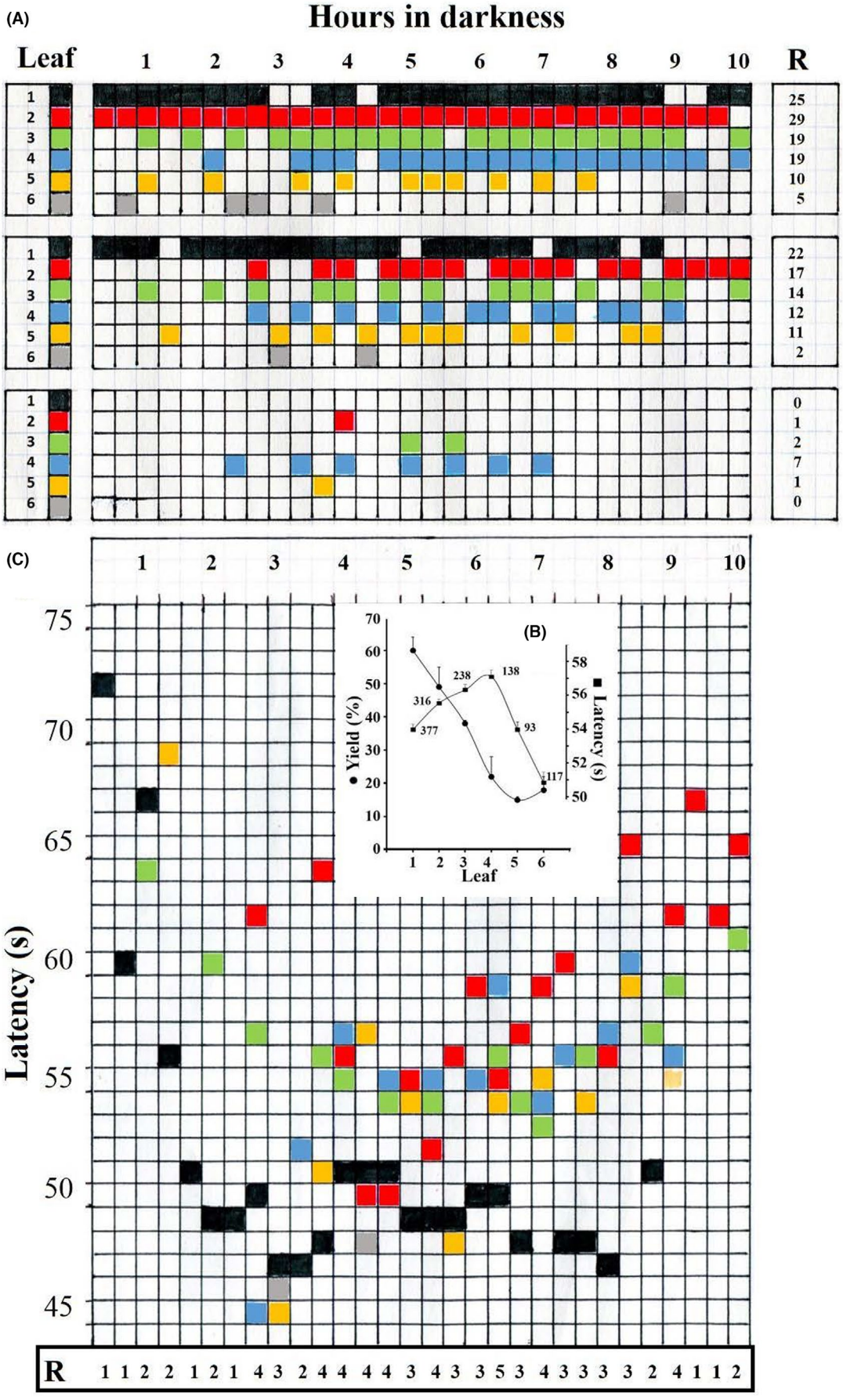
The data on the influence of experimental temperature showed that pulvinar responses were not induced at temperatures lower than 20°C and that the maximum yield was reached at 25°C–30°C, decreasing at 35°C. However, the mean latency decreased continuously as the temperature value increased (Figure 7A). The Q10 value related to yield was 3.67 in the interval 20°C–30°C and the activation energy in this thermal interval was 23.164 kcal mol^{-1}, indicating that the reaction chain triggering cell contractility is under the control of a metabolic biochemical process. Interestingly, we observed that the optimum of reactivity during the 10-h scotophase was modulated by the temperature, occurring namely around 4 h for 35°C, at 5–6 h for 30°C and 25°C, and at 9 h for 20°C after the onset of darkness (Figure 7B).

The influence of light fluence rate in the photophase preceding the period of white light stimulus is shown in Figure 7C. The data clearly show that the light energy accumulated in the preceding photophase affects the yield of reactivity. Under our conditions, the light fluence rate must not be less than 60 µmol $m^{-2}s^{-1}$. It should be noted that the pulvini stimulated at 30 µmol $m^{-2}s^{-1}$ reacted with a similar latency to those stimulated at higher values, indicating that the most sensitive pulvini were reactive in this unfavorable culture condition.

Relation between photoreactivity, endogenous circadian rhythm, and photoperiodic cycle

As seen in Figure 2, the photoreactivity considerably varied throughout the course of day in relation with light and dark periods. To demonstrate a possible endogenous component of the process, plants were maintained in continuous darkness for several days. The data in Figure 8A clearly demonstrated the endogenous nature of the variation in photoreactivity as a circadian rhythmicity was observed under continuous darkness for 8 days. Note that a hindrance of the reactivity was observed on the third and fourth day after the start of constant darkness, followed by a recovery in the following cycles without damping. The mean value of the period calculated from the maximum of reactivity on 3 sets of plants observed over 8 days was 24.1 ± 4.2 h ($n = 24$; mean ± σ). In addition to the circadian variation, the time course was characterized by oscillations of shorter period with a mean value of 130 ± 16 min calculated from 62 oscillations.

FIGURE 4 Response variability of plants and leaves of *Mimosa pudica* photostimulated at 20-min intervals with a fluence rate of 80 µmol $m^{-2}s^{-1}$ for 120 s during the nyctophase. (A) Examples of variation in photoreactivity on 6-leaf plants with different response profiles: Compact (top plot), "draughtboard" (middle plot) and very low reactivity (bottom plot). (B) Variation of yield (± σ; $n = 3$) and mean latency (± σ, n as indicated in the diagram) depending on the number of leaves. (C) Example of the variability of motile response and latency as a function of leaf number of the observed plant.


(A)
Hours in darkness
Leaf
1 2 3 4 5 6 7 8 9 10
R
25 29 19 19 10 5
22 17 14 12 11 2
0 1 2 7 1 0
(C)
Latency (s)
75 70 65 60 55 50 45
R 1 1 2 2 1 2 1 4 3 2 4 4 4 4 3 4 3 3 5 3 4 3 3 3 3 2 4 1 1 2
(B)
Yield (%)
Latency (s)
Leaf
377 316 238 138 93 117

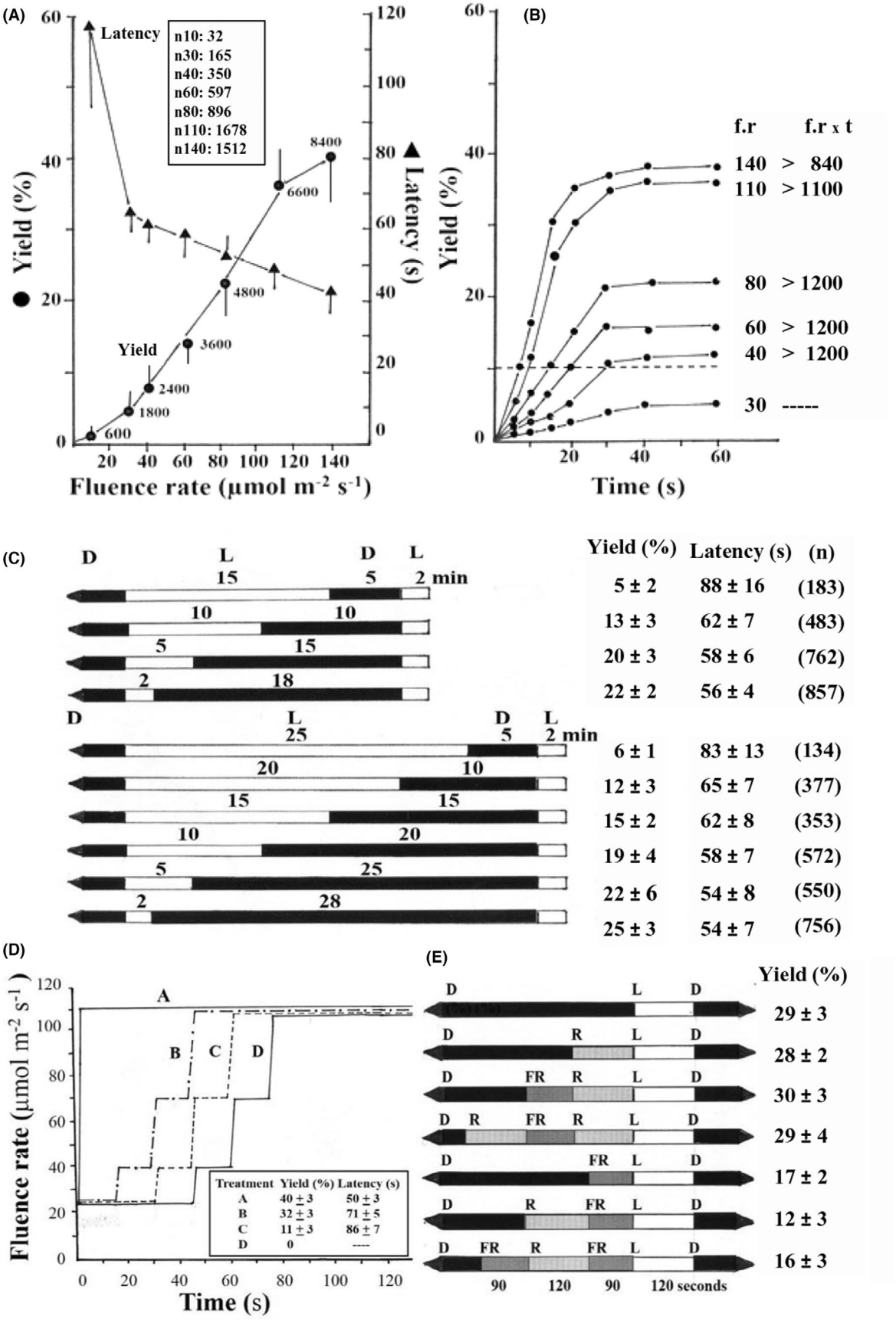

(A)
Latency
n10: 32
n30: 165
n40: 350
n60: 597
n80: 896
n110: 1678
n140: 1512
Yield
Yield (%)
Latency (s)
Fluence rate (µmol m-2 s-1)
600
1800
2400
3600
4800
6600
8400
(B)
Yield (%)
Time (s)
f.r
f.r x t
140 > 840
110 > 1100
80 > 1200
60 > 1200
40 > 1200
30 -----
(C)
D L D L
15 5 2 min
10 10
5 15
2 18
25 5 2 min
20 10
15 15
10 20
5 25
2 28
Yield (%) Latency (s) (n)
5 ± 2 88 ± 16 (183)
13 ± 3 62 ± 7 (483)
20 ± 3 58 ± 6 (762)
22 ± 2 56 ± 4 (857)
6 ± 1 83 ± 13 (134)
12 ± 3 65 ± 7 (377)
15 ± 2 62 ± 8 (353)
19 ± 4 58 ± 7 (572)
22 ± 6 54 ± 8 (550)
25 ± 3 54 ± 7 (756)
(D)
Fluence rate (µmol m-2 s-1)
Time (s)
A
B
C
D
Treatment Yield (%) Latency (s)
A 40 ± 3 50 ± 3
B 32 ± 3 71 ± 5
C 11 ± 3 86 ± 7
D 0 ----
(E)
Yield (%)
D L D
29 ± 3
D R L D
28 ± 2
D FR R L D
30 ± 3
D R FR R L D
29 ± 4
D FR L D
17 ± 2
D R FR L D
12 ± 3
D FR R FR L D
16 ± 3
90 120 90 120 seconds

FIGURE 5 Analysis of characteristic components of the applied light signal during the 10 h-nyctophase that induced the motile response of *Mimosa pudica* primary pulvini from plants grown at a photon fluence rate of 80 μmol m^{-2} s^{-1}. (A) Effect of the photon fluence rate used to stimulate the pulvini on the yield (± σ; $n = 3$) and the mean latency (± σ; n as indicated in the inset) of the induced responses. (B) Effect of the light signal applied at different fluence rates (f.r) and for different durations (t) on the yield of induced responses. (C) Effect on yield (± σ; $n = 3$) and mean latency (± σ; n as indicated in brackets) of the duration of the dark period following the light signal in plants photostimulated at 20 and 30 min intervals, respectively. (D) Effect on yield (± σ; $n = 3$) and mean latency (± σ; n as indicated in the inset) observed on pulvini irradiated for 15 s with progressive fluence rate increases (respectively 24, 40, 70, and 110 μmol m^{-2} s^{-1}). (E) Effect on the yield (± σ; $n = 3$) of the motile responses of pulvini exposed to red (R) and/or far red (FR) irradiations prior to photostimulation.

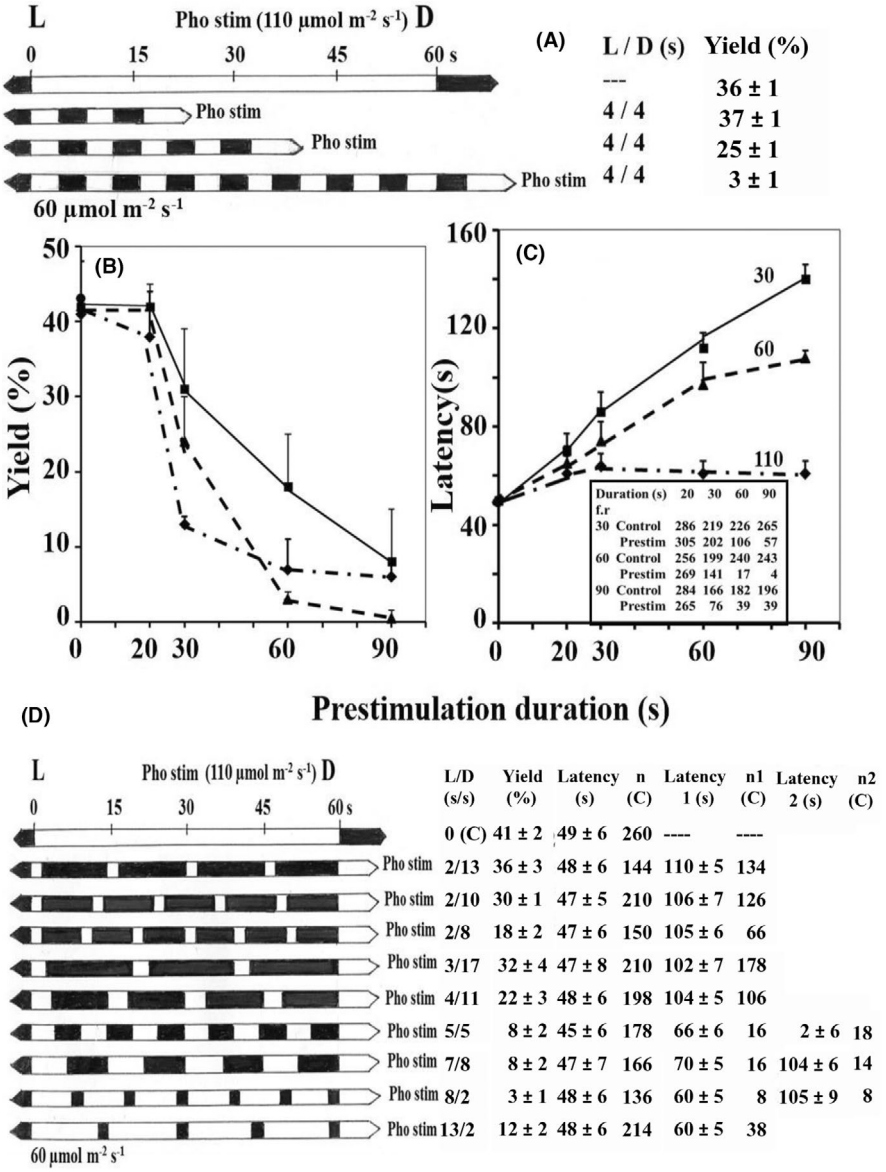


FIGURE 6 Effects of different short light/dark cycles on the yield and mean latency of the motile response of *Mimosa pudica* primary pulvini irradiated during the scotophase of the photoperiodic cycle. The prestimulation treatment was applied 4 h after the onset of darkness (light pulses at 60 μmol m^{-2} s^{-1}) and the photoreactivity was observed for 4 h after 60 s of irradiation at 110 μmol m^{-2} s^{-1} applied at 20-min intervals. (A) Effect of the duration of the light L/dark D treatment (4 s/4 s) on the response yield. Quantitative data on yield (± σ; $n = 3$) (B) Variation of yield (± σ; n = 3) and (C) Variation of mean latency (± σ; n as indicated in the inset), obtained on leaves prestimulated by 5 s/5 s alternations at three fluence rates (f.r) (30, 60 and 110 μmol m^{-2} s^{-1}) applied for 20, 30, 60 and 90 s before 60 s photostimulation at 110 μmol m^{-2} s^{-1}. (D) Effect of different prestimulation schedules of L/D alternations on yield (± σ; n = 3) and mean latency (± σ; n as indicated in the inset).

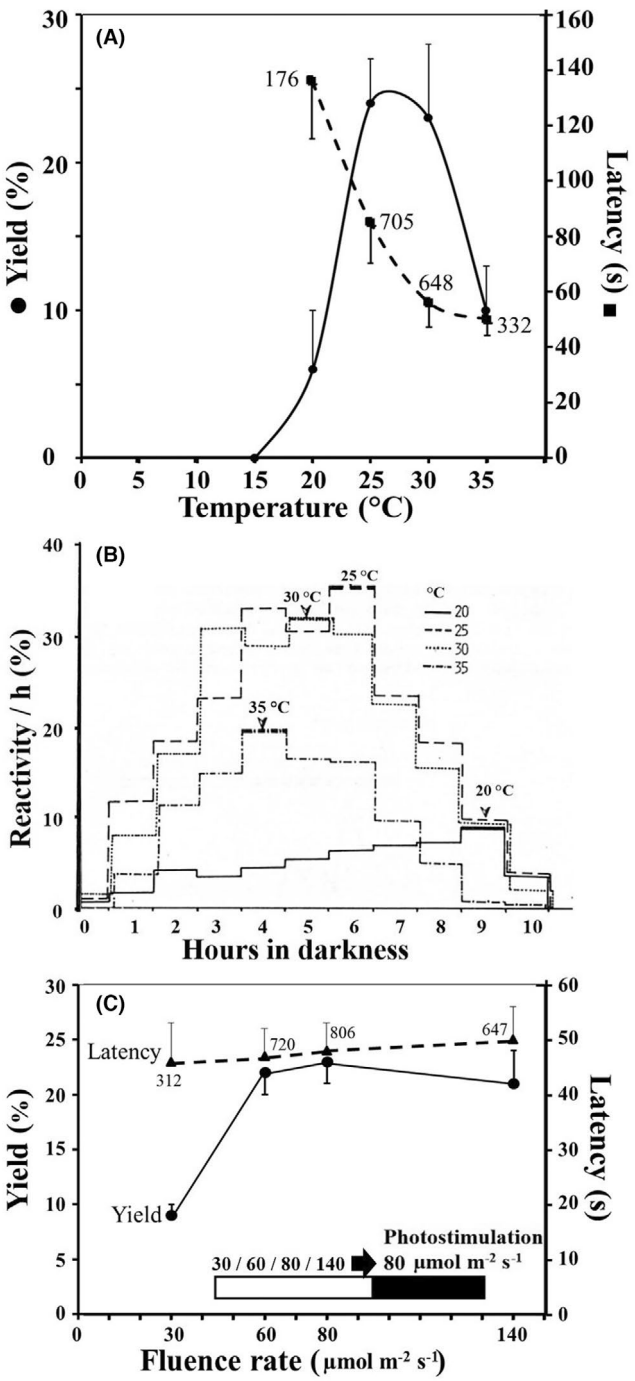


FIGURE 7 Energetic implications in the motile response of *Mimosa pudica* primary pulvini irradiated during the scotophase of the photoperiodic cycle. (A) Yield and mean latency of photostimulated pulvini as a function of experimental temperature (±0.5°C). (B) Effect of experimental temperature on the position of the optimum of photoreactivity in the course of the 10 h nyctophase of the photoperiodic cycle. (C) Effect of the photon fluence rate applied during the photoperiod preceding the photostimulation period on the yield and latency of photostimulated pulvini. Photostimulation on 3 sets of 6-leaf plants illuminated 3 times/h for 10 h under 80 µmol m^{-2} s^{-1}. Yield ± σ, $n = 3$. Latency ± σ; n: Number indicated in the graphs in front of the experimental data.

Data of Figure 8B–E also show that the photoreactivity was modulated by the photoperiodic cycle applied. For each experimental light schedule, the optimum of reactivity was reached about 3 h after the onset of darkness and the decrease of reactivity became visible about 6, 4, and 2 h, respectively, for 18, 12, and 10 h of light duration in photophase. With 6 h of darkness, the pulvini were still highly reactive at the end of the scotophase, as indicated by the responses when darkness was maintained. These experiments show that the photoreactivity was on both influence of the photoperiodic cycle applied and the previously evidenced endogenous component.

DISCUSSION

In most experiments, the effects of ambient light have been observed in long-term developmental processes involving a chain of reactions that trigger the activation of specific genes. In this work, we have focused our main observations on events that occur on plant cells in the first few minutes after the onset of plant illumination, including wavelengths in the blue region of the spectrum. The pulvinus of *Mimosa pudica* leaves was used for this purpose. It is a particularly convenient model because changes in motor cell functioning that modify the turgor status in motor cells are rapidly expressed by macroscopic postural changes of the leaf. This functioning thus avoids any long time lag associated with the establishment of complex growth processes and allows for reversible response.

Differential photoreactivity observed between plants and between leaves

Previous observations have shown that *Mimosa* pulvini exhibit different types of responses when exposed to light and darkness periods.[18] More precisely, a white light pulse applied during the scotophase of the photoperiodic cycle induced two types of responses at the level of P1. Thus, a pulse of light as short as 2 min and up to 10 min induced a slow downward leaf movement of approximately 20° for about 15 min, but some leaves also responded with a rapid drooping movement (1 s) identical to that induced by shock occurring after a 30-s latency, suggesting the synthesis of a putative biochemical effector during this time lapse.

The present data (Figure 2) show that such a rapid response can also be induced on P1 and P3 during the photophase of the photoperiodic cycle, but the plants must be exposed to high light intensity. Therefore, two factors must be considered: the initial light intensity (which is zero during the scotophase) and the time at which the light pulse was applied in the photoperiodic cycle. Observations on a

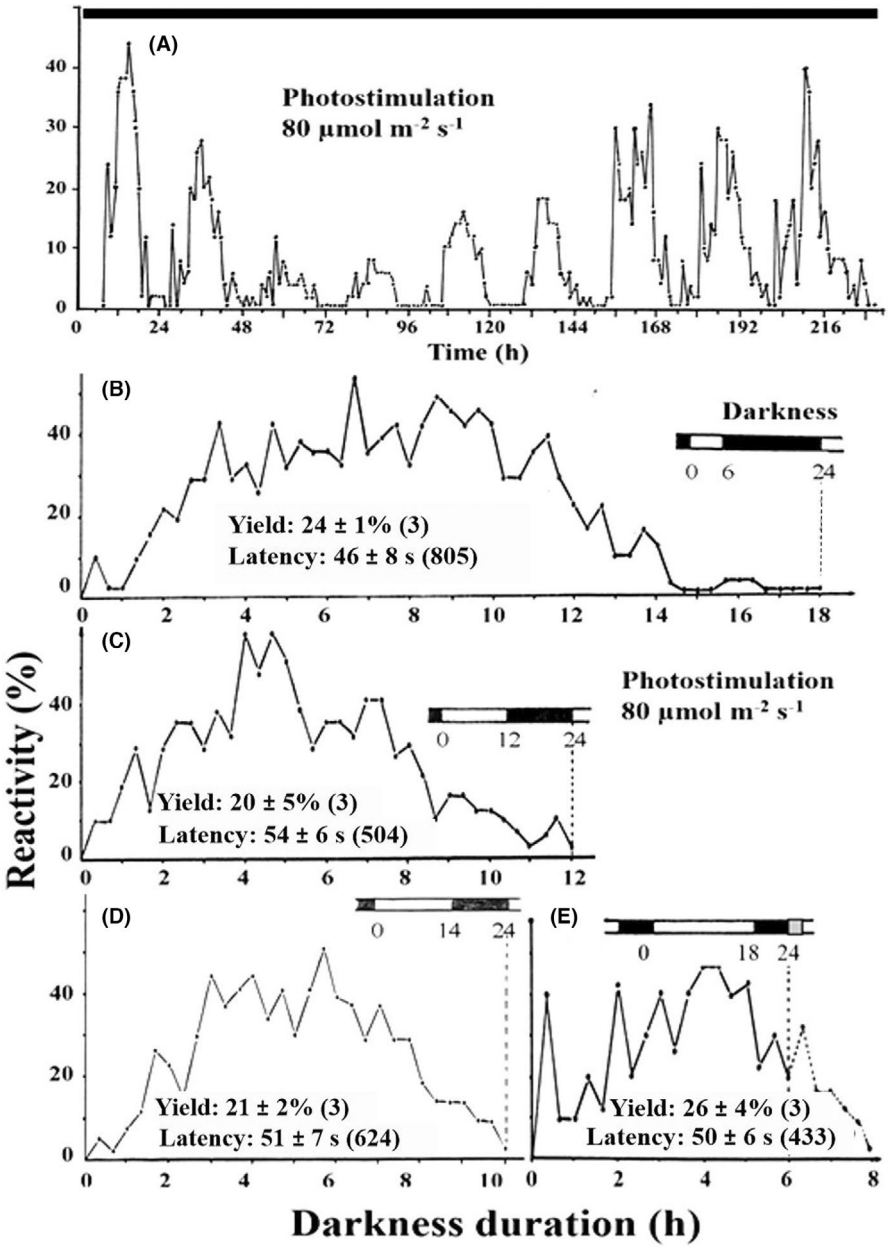


FIGURE 8 Typical example of variations in photoreactivity of primary pulvini of *Mimosa pudica* leaves subjected to (A) continuous darkness for 10 days and to different photoperiodic cycles with scotophase of different durations, (B) 18 h, (C) 12 h, (D) 10 h, and (E) 6 h. Light was applied 3 times/h at a fluence rate of 80 µmol $m^{-2} s^{-1}$.

population of *Mimosa* plants showed that all their leaves did not respond with the same frequency to a sudden pulse of light. We have schematically classified the behavior of the plants into three types of representative profiles (Figure 4), but all intermediate situations were observed. In addition, a variability of the response was also observed on individually observed leaves, as shown in the "draughtboard" "profiles". This is not strictly related to light intensity, as such a behavior was also observed even when light was applied at very high intensities (Figure 3). Therefore, the threshold concept alone is not sufficient to explain the variability in response. The physiological implications must be further considered by looking, in particular, at the evolution of reactivity as a function of the leaf age in relation with the metabolic changes (Figure 4B). Note that the leaf n 1 (preformed in the embryo) is of particular interest for further physiological and biochemical analyses as it appears to statistically show the highest reactivity in a constant manner.

Analysis of factors intervening in the pulvinar photostimulation

The data truly indicated that the light-induced processes governing the leaf drooping are largely controlled by the energetic status of the motor cells since a low light

fluence rate applied over the hours preceding the period of photostimulation reduced the photoreactivity and increased the latency (Figure 7C). The temperature-related photoreactivity and the temperature-dependent latency (Figure 7A) raise the question of the way in which energy can intervene: is it on the photochemical process through a modification of the active form of the photoreceptor[21] or/and on the biochemical reactions leading to the cell response? In the latter hypothesis, the process could be controlled at the level of osmocontraction, which operates through the level of ATP that fuels the motor cell[22] or/and through the rate of light-induced biochemical synthesis of a compound that triggers the cell response. This complex problem will be the subject of further investigations.

There was a direct relationship between light intensity and pulvinar photoreactivity both for the number of responding organs and for the latency of the movement. However, the rapid drooping response was only achieved under two conditions: firstly, when light energy was above a threshold value estimated at 600 µmol m^{-2} (Figure 5A) and, secondly, when the light pulse was applied in such a way that threshold value was reached within the first seconds after light onset. In these conditions, the reaction cannot be stopped by further treatments. In fact, a process of "accommodation" occurred when light was applied by stages below the threshold value, even though the final energy applied was theoretically largely inductive of the response (Figure 5D). This phenomenon is also supported by experiments in which high-intensity light irradiations were interrupted by short dark periods (Figure 6). In this case, "accommodation" is insufficient to fully explain the observed decrease in photoreactivity. Our experiments have demonstrated that this decrease is linked to events occurring early in the latency period (up to 30 s), during which dark periods interrupted light irradiation (Figure 6). This behavior may be related to a photochemical effect linked to the photoreceptor property resulting from photoproduct formation and dark regeneration,[23,24] and/or to autophosphorylation-linked processes,[25,26] and/or to a metabolic disturbance in the synthesis of a biochemical effector.[27]

The data obtained under continuous darkness for several days showed that a mechanism of "habituation" occurred under these conditions. In fact, the total light energy received in a 1 h time-lapse by the plants irradiated 3 times for 2 min was low (28,800 µmol m^{-2}) compared to plants continuously illuminated (288,000 µmol m^2). However, this low energetic status was sufficient to cover the energy consumed by the drooping leaf movement and to maintain the plants alive.

Early events at the cellular level: implication of photoreceptors and induction of electrical potential variation

Given the importance of the first 30 s in the development of the light-induced osmocontractile process in motor cells, as highlighted above, special attention must be paid to particular early events that occur during this time interval at the membrane level.

The first step in the light sensing mechanism involves specific photoreceptors that can detect and respond to changes in fluence rate, direction of light irradiation, and spectral composition ranging from UV B to far red wavelengths. Noteworthy, phototropins, cryptochromes, and Zeitlupe family function in the blue part of the spectrum, whereas phytochromes are activated through a reaction that converts a biologically inactive form (P_R) into an active form (P_{FR}) after exposure to R light, while the P_{FR} form can be inactivated to the P_R ground state by light-dependent photoconversion[1,2,5] or by light-independent thermal reversion, also termed dark reversion.[28]

The phototropins (phot 1 and phot 2), which intervene in BL sensing in many fast light-induced processes, are likely to be the best candidates for inducing the rapid light-induced pulvinar movements described here. The rapidity of the onset of motor cell depolarizations and other membrane-related events, in particular, Ca^{2+} mobilization, may be linked to their localization close to the plasma membrane,[29,30] avoiding the need to involve any immediate implication of genes. Their mode of action through early autophosphorylation fit well with the early time courses of light-induced pulvinar movements, which occur after a short 1-min latency. Comparatively, the phosphorylation levels in guard cells of *Vicia faba* peaked approximately 1 min after the start of the BL pulse and then gradually decreased for several minutes. Upon application of a second pulse, the phototropins were rephosphorylated within 30 s.[25]

The fact that phytochrome modulated the onset of the BL-induced photostimulation was evidenced by the data showing that R activated and FR hindered the reactivity (Figure 5E). The reactivity was controlled by the last irradiation applied, which is the characteristic reaction of phytochrome involvement. Such a control by phytochrome was previously reported in the dark-induced closing of *M. pudica* and *Cassia fasciculata* leaflets[31,32] and in the BL-sensitive shrinkage of pulvinar protoplasts of *Phaseolus vulgaris*.[33] Furthermore, protoplast swelling was observed in plants maintained under R light irradiation.[34] These findings suggest that the cell membrane must be in a P_{FR}-controlled state, ensuring a favorable permeability status of the membrane.

Cryptochromes are mostly involved in the reaction chain that leads to important photomorphogenic responses in plant development. Their direct effect on these long-lasting events is supported by microarray analysis showing that cryptochromes play an important role in the transcriptional regulation of BL-responsive genes.[35] This role does not exclude that cry may exert indirect effects, in particular, by interacting with phytochrome, since both photoreceptors (cry1 and PhyB) colocalize in the cytosol in the dark and also under FR light. In this situation, PhyB exists predominantly in the P_R form and has been shown to interact directly with cry, an interaction that is dissociated by light irradiation.[36] Consequently, P_R can be converted to the active P_{FR} form under R wavelengths, which may represent the first step leading to a favorable state in membrane permeability, allowing facilitated ion migrations implicated in the light-induced motor cell response. A second aspect concerning the impact of cry is related to the endogenous circadian rhythmicity of leaf reactivity (Figure 8A), as its role in the entrainment of the circadian clock is now well documented.[37] Furthermore, the observation that the optimum of reactivity was reached 3–4 h after light onset, regardless of the length of the daily photoperiod, suggests that the circadian clock was reset by the light signal (Figure 8B–E).

A second step to be considered is the early variation in cell membrane potential occurring after a stress is sensed by plants. The bioelectrical modifications induced by WL changes have been well documented in many plant models.[38] The observations carried out in this work from intracellular measurements on pulvinar cell from isolated leaf confirm the previous observations carried out on pulvini in situ using extracellular electrodes.[15,18] Additionally, the data showed that membrane potential varied according to the light wavelength, in particular, BL and R induced depolarization, whereas FR induced a hyperpolarization similar to a darkness application (Figure 1B). The short-term depolarization observed after a BL exposure is a typical response observed on many types of cells: mesophyll cells of growing leaves of *Pisum sativum*,[39] mesophyll cells of leaves of *Arabidopsis thaliana*,[40] mesophyll cells of bean leaves,[41] cucumber hypocotyls,[42] and motor cells of *Phaseolus* pulvini.[43] Depolarization after R irradiation has also been observed in moss *Physcomitrella patens*,[44,45] in internodes of green algae *Nitella*,[46] in bundle sheet cells of *Arabidopsis* leaf.[47] In contrast, changes occurring after FR irradiation are poorly documented. No FR-induced effect has been found on *Phaseolus* pulvinar cells,[48] whereas FR irradiation was found to accelerate the repolarization after R-induced depolarization,[46] and block the R-induced electrical response.[44] The hyperpolarization noted in motor cells after darkness (Figure 1B) has also been monitored in mesophyll cells of leaves of *Arabidopsis thaliana*[40] and *Vicia faba*[49] and in bundle sheet cells of *Arabidopsis* leaf.[47]

Analysis of the ionic mobilizations leading to differential pulvinar responses

A precise determination of the latency that occurs between the onset of the light (and dark) signal and the onset of the ionic migrations is the first parameter that makes it possible to determine the chronology of the events that make up the observed cell response. Rapid changes in membrane potential are always generated by changes in K^+, Cl^-, H^+, and Ca^{2+} near the mesophyll tissue.[41]

An accurate analysis of time courses of osmoregulated pulvinar movements and related bioelectrical events triggered by light and dark signals shows a complex relationship: in fact, potential variations occurred after a short latency (in some s; see Figure 1B), whereas leaf movement was detected only 1–2 min after the onset of the signal.[18] This implies that ionic species intervening in both processes (mainly Cl^- and K^+) are mobilized according to very different mechanisms to participate either in a signaling role or in a functional role.

Thus, we observed that the onset of light irradiation induced a slow depolarization of motor cells triggered after a 5-s latency and lasting for some min in relation with early Cl^- efflux, repolarization being provided by K^+ efflux. The H^+ influx monitored after a latency of 15 s,[18,50] reflecting a H^+ ATPase inhibition as evidenced in motor cells of *Phaseolus*,[51] may account for the sustained depolarization observed (Figure 1B). It has been suggested that calcium influx might directly depolarize the plasma membrane resulting in an activation of voltage-dependent Cl^- and/or K^+ channels.[11] Considering its latency (17 s; this work), the onset of $[Ca^{2+}]c$ mobilization from internal stores described in a previous work cannot be implicated in the triggering of the bioelectrical modifications but may intervene in subsequent biochemical reactions leading to osmocontraction. This $[Ca^{2+}]c$ mobilization through PLC-mediated signaling would be controlled by Phot2.[29] Comparatively, under the dark signal, inverse electrophysiological processes were observed: a long-lasting hyperpolarization of the membrane potential after a 5 s latency, and a transient increase of proton excretion occurring after a latency of 15 s that indicates an H^+ ATPase activation and a sustained hyperpolarization of the membrane (Figure 1B).

Besides these early bioelectrical light-induced signaling characterized by smooth, long-lasting, and low potential variations (about 40 mV in 5 min), motor cells may exhibit AP (about 100 mV in 1 s).[15] It is generally assumed that AP in plant cells results from a sudden exit of Cl^- ions in the phase of depolarization followed by a slower exit of K^+ ions in the phase of repolarization.[38] The outwardly rectified K^+ current, which is activated by membrane depolarization, has been electrophysiologically described in *M. pudica* motor cells.[52] In the case of shock-induced movement, it has been proposed that calcium influx may directly depolarize the plasma membrane, resulting in the activation of voltage-dependent Cl^- and/or K^+ channels.[11] Such a similar implication of Ca ions must be verified in the case of photostimulated rapid movement, considering the model of Ca mobilization through phot1.[29]

Besides their role in signaling stages, K^+ and Cl^- have also been shown to sustain the osmocontractile processes of the motor cells, K^+ ions, in particular, acting as the osmoticum. Thus, concomitantly to the slow downward leaf movement, a bulk exit of Cl^- and K^+ (about 30 mM at the maximum in 1–2 min) was measured in the extracellular medium. In the case of large rapid movement, K^+ exit reached 170 mM in the first s.[50] It has to be remember that this ionic redistribution is only a part of the mechanism leading to the rapid bending movement. Additionally, an active process involving contractile proteins[53,54] is also operating as simply shown by the rapid bending movement of the leaf effected in upward direction when plant was put upside down in the gravitational field (personal observation). Ca ions play an important role at this stage of the reaction chain as $[Ca^{2+}]c$ increase regulates actin depolymerization during pulvinar movement.[11,55]

Differential effect of light

From several comparisons, we highlighted that white light signal induces differential responses in turgor-regulated cell responses. The data presented here showed that the various types of pulvini exhibited different behaviors. In the case of the slow-induced movement (photonasty), P2 responded similarly to P1, whereas P3 showed a very different reaction. Thus, white light induced turgor decrease in motor cells of the lower half of P1, inducing a downward bending, whereas it induced turgor increase in motor cells of P3, inducing an opening of the leaflets. These data contrast with BL-induced photostimulation (similar to shock-induced response) in both type of organs resulting in rapid turgor decrease in motor cells. Note also that the action spectrum of the induced movement also differs. Photostimulation is solely triggered by light wavelengths in the blue part of the spectrum,[14] whereas photonasty of P3 is triggered by both blue and far red wavelengths.[14,56] Note, however, that in both case, light induced early cell membrane depolarization. The comparison with the effect of light on guard cells is questioning. Indeed, the phototropin blue light sensing induces a swelling of the guard cells resulting from a turgor increase, which has been linked to an activation of the H^+ ATPase and, consequently, to membrane hyperpolarization.[57,58] Thus, it is a challenging question to clarify the opposite effect of light on the plasma membrane H^+ ATPase between guard cells and pulvinar cells. Note that the swelling of guard cells is also induced by wavelengths in the red part of the spectrum in correlation with modulation of metabolite variation, in particular, abscisic and jasmonic acids.[59,60] Thus, many physiological light-induced events are known to involve a main photoreceptor while other photoreceptors contribute to fine regulation of the processes.[1,61]

CONCLUSIONS AND PERSPECTIVES

Pulvinar movements contribute not only to optimize the availability of light to the leaves as an energy source for photosynthesis (photonasty) but also to protect them from excessive irradiation (paraheliotropism). We have focused our observations on the important events that occur in the first 1–5 min after the onset of light and darkness in order to tentatively establish their chronology leading to motor cell osmoregulation.

A major point to clarify in the sequence of events is the determination of the cellular mechanism leading either towards slow downward movements of the primary pulvinus or to rapid photostimulation (shock-like). A particular signature is the induction either of slow potential variation or of action potentials.[62] In particular, the occurrence of a latency in the case of photostimulation implies that a chain of reactions is triggered in the first minutes before osmocontraction. The synthesis of a putative metabolite must be considered in response to metabolic changes in photosynthesis (CO_2), photorespiration (glycolic acid), or a specific pathway. In this last direction, the implication of glutamic acid and/or glycine is a conceivable hypothesis to be tested according to their synthesis in stress conditions,[27] and the capacity of these compounds to evoke an action potential.[16,63]

ACKNOWLEDGMENTS

We thank “Cécile Gaillard” for her technical assistance.

FUNDING INFORMATION

European Regional Development Fund (FEDER), and intramural funds from the Center National de la Recherche Scientifique (CNRS) and the University of Poitiers.

ORCID

Fabienne Dédaldéchamp https://orcid.org/0009-0003-2488-5907

REFERENCES

1. Paik I, Huq E. Plant photoreceptors: multi-functional sensory proteins and their signaling networks. *Semin Cell Dev Biol*. 2019;92:114-121. doi:10.1016/j.semcdb.2019.03.007
2. Texeira RT. Distinct responses to light in plants. *Plan Theory*. 2020;9:894. doi:10.3390/plants9070894
3. Satter RL, Galston AW. Mechanism of control of leaf movements. *Annu Rev Plant Physiol*. 1981;32:83-110.
4. Koller D. Light-driven leaf movements. *Plant Cell Environ*. 1990;13(7):615-632.
5. Galvao VC, Fankhauser C. Sensing the light environment in plants: photoreceptors and early signaling steps. *Curr Opin Neurobiol*. 2015;34:46-53.
6. Paradiso R, Proietti S. Light-quality manipulation to control plant growth and photomorphogenesis in greenhouse horticulture: the state of the art and the opportunities of modern LED systems. *J Plant Growth Regul*. 2022;41(2):742-780.
7. Toriyama H. Observational and experimental studies of sensitive plants. VI. The migration of potassium in the primary pulvinus. *Cytologia*. 1955;20(4):367-377.
8. Allen RD. Mechanism of the seismonastic reaction in *Mimosa pudica*. *Plant Physiol*. 1969;44(8):1101-1107.
9. Samejima M, Sibaoka T. Changes in the extracellular ion concentration in the main pulvinus of *Mimosa pudica* during rapid movement and recovery. *Plant Cell Physiol*. 1980;21(3):467-479.
10. Roblin G. *Mimosa pudica*: a model for the study of the excitability in plants. *Biol Rev*. 1979;54(2):135-153.
11. Hagihara T, Toyota M. Mechanical signaling in the sensitive plant *Mimosa pudica*. *Plan Theory*. 2020;9:857. doi:10.3390/plants9050587
12. Bert P. Recherches sur les mouvements de la sensitive (*Mimosa pudica*). *Mém Soc Sci Phys Nat Bordeaux*. 1866;4:11-16.
13. Darwin C. *La faculté motrice chez les plantes*. Reinwald Ed; 1882:599.
14. Fondeville JC, Schneider MJ, Borthwick HA, Hendricks SB. Photocontrol of *Mimosa pudica* leaf movement. *Planta*. 1967;75(3):228-238.
15. Roblin G. Movements and bioelectrical events induced by photostimulation in the primary pulvinus of *Mimosa pudica*. *Z Pflanzenphysiol*. 1982;106(4):299-303.
16. Otsiogo-Oyabi H, Roblin G. Changes in membrane potential related to glycine uptake in the motor cell of the pulvinus of *Mimosa pudica*. *J Plant Physiol*. 1985;119(1):19-24.
17. Moyen C, Cognard C, Fleurat-Lessard P, Raymond G, Roblin G. Calcium mobilization under a UV-A irradiation in protoplasts isolated from photosensitive pulvinar cells of *Mimosa pudica*. *J Photochem Photobiol B Biol*. 1995;29(1):59-63.
18. Roblin G. Movements, bioelectrical events and proton excretion induced in the pulvini of *Mimosa pudica* L. by a period of darkness. *Z Pflanzenphysiol*. 1982;108(4):293-304.
19. Roblin G. Les rythmes endogènes des mouvements foliaires chez *Mimosa pudica* L. *J Interdisc Cycle Res*. 1977;8(2):89-109.
20. Monjallon A. *Introduction à la méthode statistique*. Vuibert Ed; 1966.
21. Hahm J, Kim K, Qiu Y, Chen M. Increasing ambient temperature progressively disassemble *Arabidopsis* phytochrome B from individual photobodies with distinct thermostabilities. *Nat Comm*. 2020;11:1660. doi:10.1038/s41467-020-15526-z
22. Sibaoka T. Rapid plant movements triggered by action potentials. *Bot Mag Tokyo*. 1991;104:73-80.
23. Salomon M, Christie JM, Knieb E, Lempert U, Briggs WS. Photochemical and mutational analysis of the FMN-binding domains of the plant blue light receptor, phototropin. *Biochemistry*. 2000;39(31):9401-9410.
24. Kasahara M, Swartz TE, Olney MA, et al. Photochemical properties of the Flavin mononucleotide-binding domains of the phototropins from Arabidopsis, rice and *Chlamydomonas reinhardtii*. *Plant Physiol*. 2002;129(2):762-773.
25. Kinoshita T, Emi T, Tominoga M, et al. Blue-light- and phosphorylation-dependent binding of a 14-3-3 protein to phototropins in stomatal guard cells of broad bean. *Plant Physiol*. 2003;133(4):1453.
26. Inoue S-I, Kinoshita T, Matsumoto M, Nakayama KI, Doi M, Shimazaki K-I. Blue light-induced autophosphorylation of phototropin is a primary step for signaling. *Proc Natl Acad Sci USA*. 2008;105(14):5626-5631.
27. Choudhury FK, Devireddy AR, Azad RK, Shulaev V, Mittler R. Local and systemic metabolic responses during light-induced rapid systemic signaling. *Plant Physiol*. 2018;178(4):1461-1472.
28. Klose C, Nagy F, Schäfer E. Thermal reversion of plant phytochromes. *Mol Plant*. 2020;13(3):386-397.
29. Sakamoto K, Briggs WS. Cellular and subcellular localization of phototropin 1. *Plant Cell*. 2002;14(8):1723-1735.
30. Harada A, Shimazaki K-I. Phototropins and blue light-dependent calcium signalling in higher plants. *Photochem Photobiol*. 2007;83(1):102-111.
31. Fondeville JC, Borthwick HA, Hendricks SB. Leaflet movement of *Mimosa pudica* L. indicative of phytochrome action. *Planta*. 1966;69(4):357-364.
32. Roblin G, Fleurat-Lessard P, Bonmort J. Effects of compounds affecting calcium channels on phytochrome- and blue pigment-mediated pulvinar movements of *Cassia fasciculata*. *Plant Physiol*. 1989;90(2):697-701.
33. Wang X, Haga K, Nishizaki Y, Iino M. Blue-light-dependent osmoregulation in protoplasts of *Phaseolus vulgaris* pulvini. *Plant Cell Physiol*. 2001;42(12):1363-1372.
34. Amodeo G, Srivastava A, Zeiger E. Vanadate inhibits blue light-stimulated swelling of *Vicia faba* guard cell protoplasts. *Plant Physiol*. 1992;100(3):1567-1570.
35. Ohgishi M, Saji K, Okada K, Sakai T. Functional analysis of each blue light receptor, cry1, cry2, and phot2, by using combinatorial multiple mutants in *Arabidopsis*. *Proc Natl Acad Sci USA*. 2004;101(8):2223-2228.
36. Hughes RM, Vrana JD, Song J, Tucker C. Light-dependent, dark-promoted interaction between *Arabidopsis* cryptochrome 1 and phytochrome B proteins. *J Biol Chem*. 2012;287(26):22165-22172.

37. Lopez L, Fasano C, Perrella G, Facella P. Cryptochromes and the circadian clock: the story of a very complex relationship in a spinning world. *Gen*. 2021;12:672. doi:10.3390/genes120506672
38. Mudrilov M, Ladeynova M, Grinberg M, Balalaeva I, Vodeneev V. Electrical signaling of plants under abiotic stressors: transmission of stimulus-specific information. *Int J Mol Sci*. 2021;22:10715. doi:10.3390/ijms221910715
39. Elzenga JTM, Prins HBA, Van Volkenburgh E. Light-induced membrane potential changes of epidermal and mesophyll cells in growing leaves of *Pisum sativum*. *Planta*. 1995;197(1):127-134.
40. Spalding EP, Slayman CL, Goldsmith MH, Gradmann D, Bertl A. Ion channels in *Arabidopsis* plasma membrane. Transport characteristics and involvement in light-induced voltage changes. *Plant Physiol*. 1992;99(1):96-102.
41. Shabala S, Newman I. Light-induced changes in hydrogen, calcium, potassium, and chloride ion fluxes and concentrations from the mesophyll and epidermal tissues of bean leaves. Understanding the ionic basis of light-induced bioelectrogenesis. *Plant Physiol*. 1999;119(3):1115-1124.
42. Spalding EP, Cosgrove DJ. Mechanism of blue-light-induced plasma-membrane depolarization in etiolated cucumber hypocotyls. *Planta*. 1992;188(2):199-205.
43. Nishizaki Y. Vanadate and dicyclohexylcarbodiimide inhibit the blue light-induced depolarization of the membrane in pulvinar motor cell of *Phaseolus*. *Plant Cell Physiol*. 1994;35(5):841-844.
44. Ermolayeva E, Hohmeyer H, Johannes E, Sanders D. Calcium-dependent membrane depolarization activated by phytochrome in the moss *Physcomitrella patens*. *Planta*. 1996;199(3):352-358.
45. Johannes E, Ermolayeva E, Sanders D. Red light-induced membrane potential transients in the moss *Physcomitrella patens*: ion channel interaction in phytochrome signalling. *J Exp Bot*. 1997;48:599-608.
46. Weisenseel MH, Ruppert HK. Phytochrome and calcium ions are involved in light-induced membrane depolarization in *Nitella*. *Planta*. 1977;137(3):225-229.
47. Szechynska-Hebda M, Kruk J, Gorecka M, Karpinska B, Karpinski S. Evidence for light wavelength-specific photoelectrophysiological signaling and memory of excess light episodes in *Arabidopsis*. *Plant Cell*. 2010;22(7):2201-2218.
48. Nishizaki Y, Kubota M, Yamamina K, Watanabe M. Action spectrum of light pulse-induced membrane depolarization in pulvinar motor cells of *Phaseolus*. *Plant Cell Physiol*. 1997;38(5):526-529.
49. Li J, Yue Y, Wang Z, et al. Illumination/darkness-induced changes in leaf surface potential linked with kinetics of ion fluxes *front*. *Plant Sci*. 2019;10:1407. doi:10.3389/fpls.2019.01407
50. Kumon K, Tsurumi S. Ion efflux from pulvinar cells during slow downward movement of the petiole of *Mimosa pudica* L. induced by photostimulation. *J Plant Physiol*. 1984;115(5):439-443.
51. Okasaki Y. Blue light inactivates plasma membrane H^+-ATPase in pulvinar motor cells of *Phaseolus vulgaris* L. *Plant Cell Physiol*. 2002;43(8):860-868.
52. Stoeckel H, Takeda K. Plasmalemmal, voltage-dependent ionic currents from excitable pulvinar motor cells of *Mimosa pudica* pulvinus. *J Membr Biol*. 1993;131(3):179-192.
53. Fleurat-Lessard P, Roblin G, Bonmort J, Besse C. Effects of colchicine, vinblastine, cytochalasin B and phalloidin on the seismonastic movement of *Mimosa pudica* leaf and on motor cell ultrastructure. *J Exp Bot*. 1988;39(199):209-221.
54. Kanzawa N, Hoshino Y, Chiba M, et al. Changes in the actin cytoskeleton during seismonastic movement of *Mimosa pudica*. *Plant Cell Physiol*. 2006;47(4):531-539.
55. Yao H, Xu Q, Yuan M. Actin mediates the changes of calcium level during the pulvinus movement of *Mimosa pudica*. *Plant Signal Behav*. 2008;3(11):954-960.
56. Evans LT, Allaway WE. Action spectrum for the opening of *Albizia julibrissin* pinnules and the role of the phytochrome in the closing movement of pinnules and stomata of *Vicia faba*. *Austr J Biol Sci*. 1972;25(5):885-893.
57. Roelfsema MRG, Steinmeyer R, Staal M, Hedrich R. Single guard cell recordings in intact plants: light-induced hyperpolarization of the plasma membrane. *Plant J*. 2001;26(1):1-13.
58. Inoué S-I, Takemiya A, Shimazaki KI. Phototropism signalling and stomatal opening as a model case. *Curr Opin Plant Biol*. 2010;13:587-593. doi:10.1016/j.pbi.2010.09.002
59. Yang J, Lli C, Kong D, Guo F, Wei H. Light-mediated signalling and metabolic changes coordinate stomatal opening and closure. *Front Plant Sci*. 2020;11(601):478.
60. Zhu M, Geng S, Chakravorty D, Guan Q, Chen S, Assmann S. Metabolomics of red-light-induced stomatal opening in *Arabidopsis thaliana*: coupling with abscisic and jasmonic acid metabolism. *Plant J*. 2020;101(6):1331-1348.
61. Casal JJ. Phytochromes, cryptochromes, phototropin: photoreceptor interactions in plants. *Photochem Photobiol*. 2000;71(1):1-11.
62. Dziubinska H, Paszewski A, Trebacz K, Zawadski T. Electrical activity of the liverwort *Conocephalum conicum*: the all-or-nothing law, strength-duration relation, refractory periods and intracellular potentials. *Physiol Plant*. 1983;57(2):279-284.
63. Dennison KL, Spalding ERP. Glutamate-gated calcium fluxes in Arabidopsis. *Plant Physiol*. 2000;124(4):1511-1514.

How to cite this article: Roblin G, Moyen C, Fleurat-Lessard P, Dédaldéchamp F. Rapid osmocontractile response of motor cells of *Mimosa pudica* pulvini induced by short light signals. *Photochem Photobiol*. 2025;101:728-745. doi:10.1111/php.14046