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LEAFLET MOVEMENT OF *MIMOSA PUDICA* L. INDICATIVE OF PHYTOCHROME ACTION

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Summary. 1. Closing movements of *Mimosa pudica* pinnae, upon change from light to darkness, depend upon the presence of phytochrome in the far-red-absorbing form.

2. The potentiated control of closing movements by phytochrome can be repeatedly established and reversed by repeated alternations of red and far-red radiation, respectively.

3. Action spectra were measured for the potentiation of closure and for its reversal.

4. The response to phytochrome action is evident in 5 minutes and is fully expressed in 30 minutes.

5. This rapid response and the more rapid potentiation with half times of less than 1 minute for several other responses to phytochrome action indicate that the primary action of phytochrome ist not gene activation, but rather metabolic control at the substrate level.

Introduction

Movements about pulvini of the leaves, pinnae and pinnules of the sensitive plant, *Mimosa pudica*, in response to touch and variations in regimens of light and darkness, have long attracted attention (note ref. [*5*] for literature survey). The movements were thoroughly described by BERT in 1866/70 [*1*] and by PFEFFER in 1873 [*15*]. Significant findings on the responses upon changing from darkness to light were further reported by BURKHOLDER and PRATT in 1936 [*2*]. We now find that the photoconversion of phytochrome, the pigment controlling photomorphogenesis in plants [*9*], plays a determinative role in the photonastic or closing response of *M. pudica* pinnules upon changing from light to darkness. The rapidity of the response, moreover, indicates that gene activation, as currently suggested for phytochrome action by H. MOHR and his coworkers [*10, 12, 13*] is not involved.

A Simple Experiment

The essential finding of this work can readily be seen and repeated. The pinnae (leaflets) of young *M. pudica* plants grown on natural days. which are open or expanded in light, close in darkness by the pinnules

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folding together about the tertiary pulvini. This response, evident in 5 minutes after the beginning of darkness is fully expressed in 30 minutes, depends somewhat on the particular pinnae and the previous treatment of the plants. If the plants are irradiated in the far-red region of the spectrum, >700 mμ, with the usual simple incandescent-light colored-plastic sources at the start of the dark period, the leaflets remain open for many hours when maintained in darkness. If the far-red radiation is followed by the red radiation (fluorescent light), the leaves of the

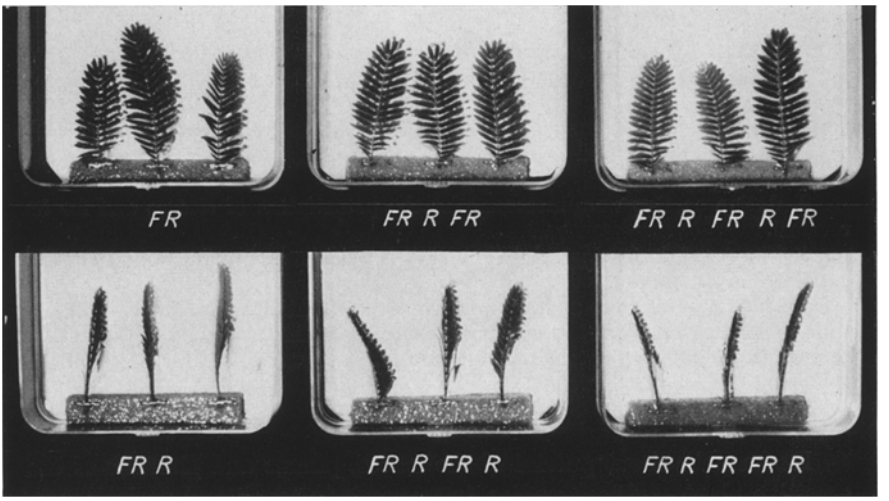

Fig. 1. *M. pudica* pinnae 30 minutes after transition from high-intensity fluorescent light to darkness. At the time of transition they were irradiated in succession for 2 minutes with red (R) or far-red (FR) radiation to establish phytochrome predominantly in the P_{rr} or P_r form. The pinnae remained open if exposure to far red was last, top row, and closed if red radiation was last, bottom row

darkened plants close within 30 minutes. The responses of detached pinnae, floating on water, to several alternations of far-red and red radiations are shown in Fig. 1. Such an alternating change of response is known, in many cases, to arise from photoreversibility of phytochrome [*9*].

Materials and Methods

M. pudica plants were grown in soil until about six leaves had developed. The intermediately developed pinnae were cut from the plants and floated on water under fluorescent illumination (5000 lux) on a 14-hour light — 10-hour dark cycle. They were tested at intervals for sensitivity to touch. Those showing a uniform response were selected and mounted by the base of the petiole as shown in Fig. 1. The mounted pinnae were transferred to the spectrograph or a light interference-filter source and were maintained under fluorescent light until the fully open position was reestablished. They were then either darkened for 30 minutes, after which time the positions of the pinnules were noted under fluorescent light, or they were subjected to far-red radiation for 2 minutes and then darkened.

A dim-green safelight (flashlight) was used in examining the course of movement if desired.

Variability in the movement of the pinnules along the same pinnae and among different pinnae did not justify greater precision than was afforded by an estimate of the degree of opening. We felt that while a more quantitative estimate could probably be worked out, perhaps after the manner of BURKHOLDER and PRATT [*2*] involving the time required for a certain degree of opening, it was not needed for the present work.

Spectral radiation in the regions from 500 mμ to 1 μ was provided by a prism spectrograph [*14*] having a dispersion of 0.8 mμ/mm at 700 mμ and a spectrum height of 8 cm. The intensity at 700 mμ, with a carbon-arc radiation source, was 0.203 mw/cm². Exposures to constant quanta at various wavelengths were made by varying times of exposure from 24 to 256 seconds between 850 and 550 mμ.

Interference filters (Schott, double-band pass) with a carbon-arc source and a 50 cm length of 0.2 M $CuSO_4$ solution in the light path were used in the region of 400 to 500 mμ. These had band-pass widths of about 20 mμ and irradiated an area of 10 × 10 cm at an intensity of 0.33 mw/cm².

Results

Responses were measured in various wavelength regions on the spectrograph, both for maintaining open pinnae following fluorescent radiation, which established phytochrome (P) predominantly in the P_{ir} form (Table 1), and for causing closure after far-red radiation, which established P predominantly in the P_r form (Table 2). Only radiation in the region of 680 to 750 mμ is effective in maintaining open (O) pinnae. The results show that the maximum of action is in the regionof 720 to 750 mμ, with an irradiance of $0.25 \times 3.75 \times 10^{-8}$ Einsteins/cm². Closure of pinnae for wavelengths >800 mμ results from the ineffectiveness of such radiation, as can be seen by comparing the results in Tables 1 and 2.

The wavelength maximum for the closing response (c) following far-red radiation (Table 2) is near 660 mμ where closure is displayed after an irradiance of $0.50 \times 3.75 \times 10^{-8}$ Einsteins/cm². As the irradiance is increased four-fold (n = 2, Table 2), the effective region for closure broadens to 570 to 680 mμ.

Irradiations in four wavelength bands between 405 and 485 mμ were made with the interference-filter sources to obtain high irradiances. In these regions P_{fr} and P_r have similar absorbancies, which are low, however, compared with their maximum values in the red and far-red regions [*3*]. With increase in irradiance, the phytochrome in the pinnules is driven to an equilibrium between the two forms [*3*]. This equilibrium was closely approached by exposures of pinnae to $128 \times 3.75 \times 10^{-8}$ Einsteins/cm², which required 30 minutes. The same final degree of closure was displayed by pinnae having initially predominant P_{fr} (Table 1) or P_r (Table 2). The pinnae remained open under 425-, 440-, and 485-mμ filters with n = 128 and closed under the 405-mμ filter. An irradiance of $8 \times 3.75 \times 10^{-8}$ Einsteins/cm², which required 4 minutes, was close

Table 1. *Closing Responses of Excised M. pudica pinnae to Irradiation in Various Wavelength Bands Following High-Intensity Fluorescent Light (> 5000 lux) which had Established 80% of the Phytochrome in the P_{fr} Form. After Irradiation the Pinnae were Held in Darkness until Examined for Closure. Pinnae Initially Open and 80% Phytochrome in P_{fr} Form*

Wavelength mμ ± 10	Response * to $3.75 \times 10^{-8} \times$ n Einsteins/cm² +			
	$n = 0.25$	$n = 1$	$n = 2$	
850		c	c	
820	c			
800		c	c	
750	O−	O+	O+	
720	O+			
700	c**	O− **	O− ***	
680		O−	O−	
660	c	c	c	
640	c			
625		c	c	
610		c	c	
595		c	c	
570		c	c	
550		c	c	
	$n = 2$	$n = 8$	$n = 32$	$n = 128$
485	c	c	O	O
440	c	c	O	O
425	c	O−	O	O
405	c	c−	c	c

* O = open, c = closed. ** 32-second exposure. *** 64-second exposure. + Einsteins/cm² required for half conversion of isolated P_{fr} to P_r at 730 mμ = 4.3×10^{-8} or $n = 1.15$.

to the threshold value for preventing closure at 425 mμ when the phytochrome was initially in the P_{fr} form (Table 1).

Discussion

Spectral dependencies for pinnae-closure responses of *M. pudica* shown in Tables 1 and 2 are the expected ones for phytochrome action. The wavelength positions for maximum response, namely, 720 to 750 mμ (Table 1) and 660 mμ (Table 2), are at the absorbancy maxima of P_{fr} (725 mμ) and P_r (664 mμ), respectively. The approximately eight-fold lower sensitivity at 425 mμ is in agreement with the *in vitro* photoconversion efficiencies for phytochrome [3]. Ready reversibility, as shown in Fig. 1 is also indicative of phytochrome action.

Results of irradiation at 405 and 425 mμ at adequate energies (n = 128) to attain photoequilibrium between P_r and P_{fr} show that the pinnae are open when 40% of the phytochrome is in the P_{fr} form but are closed with 46% P_{fr}. These particular values are taken from the work on

Table 2. *Spectral Closing Response of Detached M. pudica pinnae Following Far-Red Radiation after High-Intensity Fluorescent Light (> 5000 lux) Pinnae Initially Open before Spectral Irradiation and > 99% Phytochrome in P_r Form*

Wavelength mμ ± 10	Response * to $3.75 \times 10^{-8} \times$ n Einsteins/cm² +		
	$n = 0.50$	$n = 1$	$n = 2$
800	O	O	O
750	O	O	O
700	O	O	O
680	O	O	c—
660	c**	c**	c***
625	c—	c	c
610	O	c—	c—
595	O	c— —	c— —
570	O	c— —	c—
550	O	O	O
	$n = 8$	$n = 128$	
485	O	O	
440	O	O	
425	O	O	
405	O	c	

* O = open, c = closed. ** 32-second exposure. *** 64-second exposure. + Einsteins/cm² required for half conversion of isolated P_r to P_{fr} at 660 mμ = 1.6×10^{-8} $n = 0.43$.

the pigment *in vitro* [3], because at equilibrium with a particular wavelength *in vivo* screening by other pigments or reference to a particular incident energy is not involved. Incident energies of 4.3×10^{-8} and 1.5×10^{-8} Einsteins/cm² are required at 725 and 660 mμ, respectively, for half conversion of P_{fr} to P_r or P_r to P_{fr} *in vitro* [3]. The energies required *in vivo* for half conversion would be greater than these values because of screening by other pigments and scattering. The incident energies for minimal response at maximum absorptions shown in Tables 1 and 2, namely, 1×10^{-8} (n = 0.25) and 2×10^{-8} (n = 0.50) Einsteins/cm², are in approximate agreement with the findings for $P_{fr} \rightleftharpoons P_r$ equilibrium contents at 405 and 425 mμ.

The rapid response of *M. pudica* to phytochrome action is a second example of such a rapid display among the many displays associated with phytochrome. The other example is the control of plastid orientation in the algae *Mougeotia* sp.; HAUPT [8] found that the change in plastid orientation is evident in less than 10 minutes and is fully displayed in 30 minutes. The potentiation of the orientation can be fully reversed by far-red radiation only during the first minute after red irradiation [8].

Potentiation of leaf unrolling in etiolated grasses, associated with phytochrome conversion to P_{fr}, is complete in the order of 60 seconds

as shown by transport of the stimulus for unrolling from the irradiated region [*16*]. Potention of flowering responses as shown by ineffectiveness of photoreversibility is complete in less than 60 seconds for varieties of *Pharbitis nil* [*6*] and *Kalanchoe blossfeldiana* [*7*]. Expressions of the several responses, however, requires periods of a day or days. The slowness of the expression has possibly detracted from an appreciation of the rapidity of the potentiation for which the far-red-absorbing form of phytochrome is essential.

Such striking effects as control of flowering, seed germination, stem lenghtening, and anthocyanin formation accompanying changes in form of phytochrome have invited speculation as to the nature of phytochrome action. These speculations are of two main types. One to which we have held [*9*] is that control of such diverse expressions implies metabolic control through some very basic reaction involved in several metabolic reaction systems or cell permeability. Another advanced by H. MOHR and co-workers [*10*, *12*, *13*], on the basis of changes in nucleic acid contents and effects of inhibitors of protein synthesis in experimental plants, is that phytochrome action might involve gene activation.

The rapidity (minutes) of the *M. pudica* and *Mougeotia* responses and of the leaf unrolling, flowering, and orientation potentiations (seconds) eliminates gene activation as the primary action of phytochrome. In the case of *M. pudica*, and possibly in the other responses, both P_f and P_{fr} were present during the light period preceding darkness. Any phytochrome action accordingly has been going on during the light period, even though the pinnae were open. Upon darkening, according to a concept dependent on change in protein synthesis related to gene action, the mechanism for protein synthesis would have to be quickly destroyed. Gene activation as a control of display involves time not only for desuppression of the gene but also for eventual synthesis of appreciable amounts of protein and their entry into metabolic patterns. Times of the order of hours are expected for expression of such gene-controlled responses in seed plants. Controls of major metabolic pathways, on the other hand, can have time constants of the order of seconds or a few minutes and changes in cell permeabilities can be even faster.

While the primary action of phytochrome is rapid in the several instances noted, the slowness (days) of its expression in many cases is adequate, and probably necessary, for gene activation eventually to be involved. In fact, the early recognition of phytochrome as a non-diffusable factor (protein) capable of changing plants from vegetative to reproductive growth implied that phytochrome controlled a diffusable material playing a part in meristematic differentiation. This duffusable material, or materials, which could well be the postulated florigen, is,

however, a secondary product derived from the primary action of phytochrome.

Other results from the literature are significant in the present context. LÖRCHER [*11*] found that the endogenous rhythm of *Vicia faba* leaf movement was displayed only when phytochrome was in the P_{fr} form at the start of a long dark period. Presence of P_{fr} was also required for display of a rhythmic flowering response of *Chenopodium rubrum* [*4*]. P_{fr} action in these several responses, as well as for pinnae movement of *M. pudica* and for anthocyanin synthesis in various plants [*9*], can be considered as permissive for display.

A rapid protein synthesis in response to light has been found for the enzyme $NaDP^+$-dependent-Glyceraldehyde-3-phosphate dehydrogenase in leaves of several angiosperms [*18*]. Blocking of this synthesis by chloramphenicol, and absence of inhibition by actinomycin-C, led ZIEGLER and ZIEGLER [*18*] to conclude that the light effect was on substrate production for enzyme synthesis rather than on gene activation. In this case, a new level of activity was attained in about 20 minutes with an illumination of 10,000 lux.

The photoresponse under consideration here is one of several displayed by *M. pudica* leaf movements. This particular phytochrome-mediated response is closely associated with another response that maintains open pinnules under high-intensity radiation even though phytochrome is predominantly in the P_{fr} form. Thus, the pinnules held in fluorescent light just prior to use in the several experiments were open. A photoreaction must be counteracting the closing permitted by P_{fr}, which is displayed if the pinnules are darkened without intervening far-red irradiation. We have not studied this photoreaction, which could well be involved in photosynthesis. Also, the pinnae display a photoresponse upon transition from the usual night to light, which was the response studied in detail by BURKHOLDER and PRATT [*2*]. It is probable that more than one photoreaction was involved in the work of BURKHOLDER and PRATT. At this transition, other responses are also displayed, including leaf movements about the primary pulvini.

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