Plant Physiol. (1978) 61, 924–928

Phototropic Response to Vectorial Light in Leaves of *Lavatera cretica* L.

Received for publication June 13, 1977 and in revised form January 17, 1978

AMNON SCHWARTZ AND DOV KOLLER
The Hebrew University of Jerusalem, Department of Agricultural Botany, Rehovot, Israel

ABSTRACT

The mechanism by which the mature leaf of certain plants reorients its lamina to face the sun throughout the day was studied in *Lavatera cretica* L. The photoreceptor for this response differs fundamentally from the one involved in the phototropic growth response, by sensing light as a vector, rather than as a difference in luminous flux. The photoreceptor is located in the veins, which radiate in the plane of the lamina from the pulvinus situated at the junction between the lamina and petiole. The integrated response to the messages from the different veins takes place by differential turgor changes in a motor tissue surrounding the central vascular cylinder of the pulvinus, in which the veins coalesce. The differential turgor in the different segments of the motor tissue determines the orientation of the lamina. The photoreceptor reacts only to a parallel light beam striking the vein obliquely (from above). When half of the lamina is shaded, the leaf does not reorient in response to perpendicular illumination and its reorientation in response to an oblique beam is slower and partial, to a greater extend when the half-leaf is centrifugally illuminated than when it is centripetally illuminated. Application of 2,3,5 tri-iodobenzoic acid to the base of the veins in the shaded half-leaf eliminated all restrictions from the response to centrifugal illumination and totally inhibited the response to centripetal illumination. The results are consistent with a hypothesis that centrifugally illuminated veins generate turgor in their associated motor tissue in the pulvinus by activating K^+ uptake, while centripetally illuminated veins cause loss of turgor in their associated motor tissue by deactivating K^+ uptake, which leads to passive leakage of K^+. When the entire lamina is exposed to oblique illumination, the centrifugally illuminated half and the centripetally illuminated half cooperate in the full response. Shaded parts of the lamina apparently interfere with the response by supplying their associated motor tissue with auxin, which presumably causes in it an active export of protons and concomitant uptake of K^+, thereby establishing a static "dark turgor" in it.

In some species, the phototropic response of the leaves and cotyledons to unilateral illumination takes place as in other organs, *i.e.* by unequal growth of the petiole, or hypocotyl, respectively (4, 12, 19). In *Tropaeolum* (6) and *Ipomoea batatas* (11), the petiole apparently acts both as the photoreceptor and site of response, while the lamina is required merely as a source of auxin (5). Leaves of other species are equipped with one or more pulvini and their phototropic responses take place by localized changes in turgor of specialized motor tissues situated in the pulvini (2, 5). Typical cases of such "variation movements" (5) have been described in *Malva* species (23, 25), in *Stylosanthes humilis* (3), and in *Lupinus arizonicus* (24). Outdoors, on clear days, leaves track the sun by continuously facing it perpendicularly, and after sundown they gradually reorient to a predawn East-facing position (24, 25). The leaves show this response from their unfolding until they senesce. In *Malva* the pulvinus joins the petiole to the lamina. By shielding either the lamina or the pulvinus from the sun it was shown that the photoreceptor is localized in the lamina (25). Reviews by Brauner (5) and Ball (2) fail to provide a plausible hypothesis for the mechanism of sun-tracking by leaves.

Sun-tracking obviously maximizes light-harvesting (5) to the over-all advantage of productivity (3, 21), particularly under field conditions, where minima in leaf water stress occur in the morning.

MATERIALS AND METHODS

Mericarps of *Lavatera cretica* L. were pretreated in 70% (v/v) H_2SO_4 for 1 hr and then thoroughly washed in tap water and planted in drained plastic cups (5-cm diameter), containing a 1:1 (v/v) mix of basalt gravel and Vermiculite. The pots were irrigated daily, alternately with tap water, or with half-strength Hoaglund nutrient solution. The plants were grown at 25 ± 2 C in continuous diffuse illumination (from above) from a bank of GRO-Lux fluorescent tubes (Sylvania) providing 150 μE $m^{-2}sec^{-1}$ photosynthetically active radiation. By the 21st day, the first foliage leaf had expanded fully (lamina as well as petiole), and (unless otherwise stated) this leaf was then used to test the phototropic response, in a 25 ± 2 C darkroom. Other leaves, as well as cotyledons, show similar responses. Quantum flux density of photosynthetically active radiation (400–700 nm) was measured with a Lambda Instruments model LI-185 quantum meter. TIBA[1] was supplied by Fluka (Switzerland).

RESULTS

The leaf lamina of *L. cretica* L. is an incomplete, nearly circular disc, in which the junction with the petiole (via the pulvinus) is more or less eccentric (Fig. 1). From this junction, seven primary veins (three in the cotyledons) radiate at nearly equal angular intervals (about 40° in the first leaf, increasing to 50° in subsequent leaves). As they enter the pulvinus, the veins gradually coalesce (Fig. 2). Each sector of this vascular tissue is thus an extension of one of the veins. The vascular tissue is closely associated with a multicellular sheath external to it, composed of large parenchymatous motor cells (MC), in which the differential turgor changes take place that result in the bending of the pulvinus (25). The lamina is normally quite flat and its entire surface is therefore equally illuminated even when light strikes it at an angle. Detailed anatomical studies failed to identify any structure or any ordered arrangement of cells which might be differentially illuminated when light strikes the lamina at an angle, as were postulated in other species (9). The orientation of the lamina was horizontal when exposed to vertical light (diffuse, or a parallel beam) from above, and when the upper (ventral) surface was exposed to an oblique beam of parallel rays, the lamina gradually reoriented to face the beam perpendicularly. Oblique illumination of the lower (dorsal) leaf surface did not elicit any phototropic response. However, no phototropic response took place in vertical

[1] Abbreviation: TIBA: 2,3,5 tri-iodobenzoic acid.

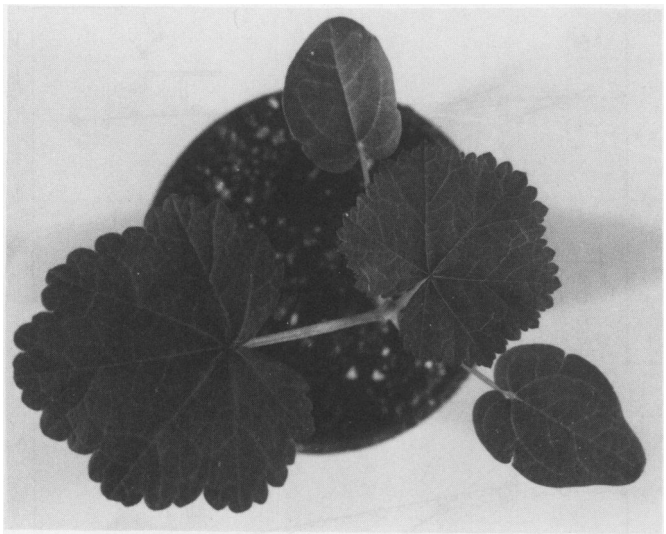

FIG. 1. Leaf lamina of *L. cretica* L. in plane view (× 1).

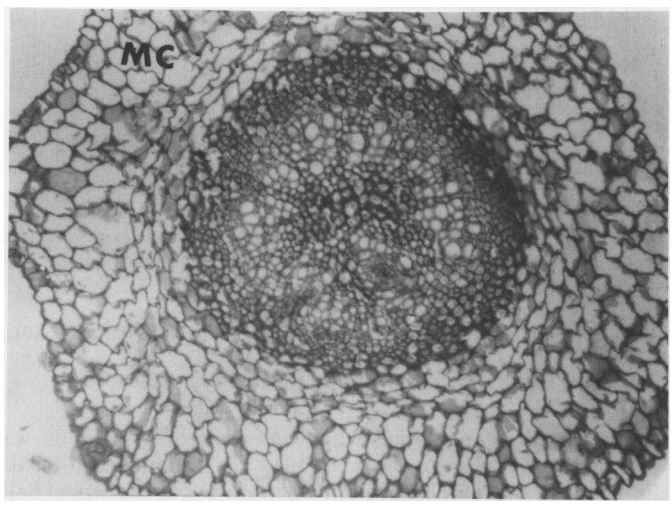

FIG. 2. Pulvinus of *L. cretica* L. in cross-section. MC: motor cells (× 38).

light (from above) when half of the (horizontal) lamina was shaded with aluminum foil. The photoreceptor in *Lavatera* leaves thus does not react phototropically to differences in the irradiance incident on the surface, as described in the leaf of *Sparmannia africana* (2), or in the sunflower cotyledons (12, 19). Instead, it appears to be sensitive to the direction of the light beam.

The phenomenon was studied by means of a beam of white light with near parallel rays from an incandescent source. The beam was passed through a heat-reflecting filter and provided 200 μE $m^{-2}sec^{-1}$ (400–700 nm) at the leaf surface, unless otherwise stated. The beam was directed at the upper surface of the lamina at an angle of incidence of 45°. In order to become normal to the beam, the lamina must therefore rotate 45° around a line intersecting the pulvinus and perpendicular to the beam. The reorientation is quantitatively described as the rate of diminution of the angle between the beam and the line normal to the lamina at the pulvinus.

The kinetic behavior of the response was quantitatively dependent on irradiance. In a typical experiment, increasing the irradiance from 100 to 400 μE $m^{-2}sec^{-1}$ reduced the duration of the lag phase of the phototropic response from 80 to 24 min and reduced the time between the end of lag phase to 50% of the full response from 56 to 32 min. This response is therefore modulative and the action of the phototropically effective light is vectorial.

In looking for the likeliest site of the photoreceptor for such a response, obvious candidates were the veins, which radiate in different directions in the lamina. To test this, a water base aluminum paint was used to cover selected areas of the upper leaf surface, either over all of the major veins or over the entire area between the veins. Foliage leaves were not very suitable for this purpose, as their numerous subsidiary veins could not be effectively painted over selectively. Instead, cotyledons were used, which contain only three major veins and a less extensive system of subsidiary veins. A phototropic response to an oblique light beam was obtained only when the major veins were exposed, whether or not the remainder of the lamina was covered.

When a foliage leaf was covered with aluminum foil, leaving exposed only narrow sectors containing some of the major veins, the phototropic response was obtained when a light beam was directed obliquely at the lamina, along the plane of symmetry of the exposed vein (Fig. 3).

The phototropic response was also obtained in leaves from which the entire lamina was dissected, leaving only one of the major veins (with a minimal amount of adjacent mesophyll) attached to the petiole via the pulvinus. When an oblique light beam was directed along the plane of symmetry of the isolated vein, the latter gradually reoriented to face the beam at right angles.

In the following text, the oblique light beam is referred to as "centrifugal," or "centripetal," with respect to a given segment of the lamina, depending on whether the projection of light beam on the segment is directed from the center of the lamina toward its circumference, or vice versa, respectively. Also, the segment of the lamina closest to the source of the oblique light beam is the "proximal" one, while the opposite segment is the "distal" one. When the entire lamina is exposed to an oblique beam, the proximal segment is illuminated centripetally and the distal one centrifugally.

When the beam was aimed at the intact lamina obliquely along the midvein, the response started earlier if the beam was directed centripetally with respect to the midvein than when it was directed centrifugally to it. However, the rate of reorientation was higher in the centrifugal beam (Fig. 4). In the first expanded leaf the attachment point of the petiole to the lamina is quite eccentric (Fig. 1). It is therefore conceivable that this structural eccentricity facilitates the downward inclination of the lamina in response to an oblique centripetal beam directed along the plane of symmetry of the lamina and delays the start of its upward inclination in

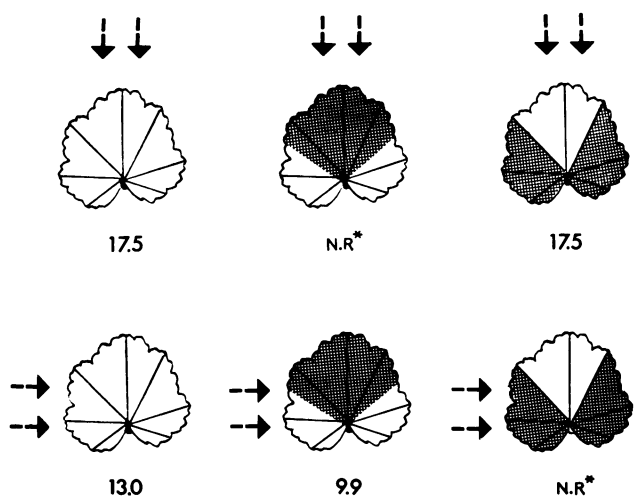

FIG. 3. Rate of reorientation (deg hr^{-1}) of leaf of *L. cretica* L. with unshaded and partially shaded lamina exposed to an oblique light beam (45° from above). Arrows indicate direction of beam. N.R.*: no response: lamina remained within ± 3° of initial position.

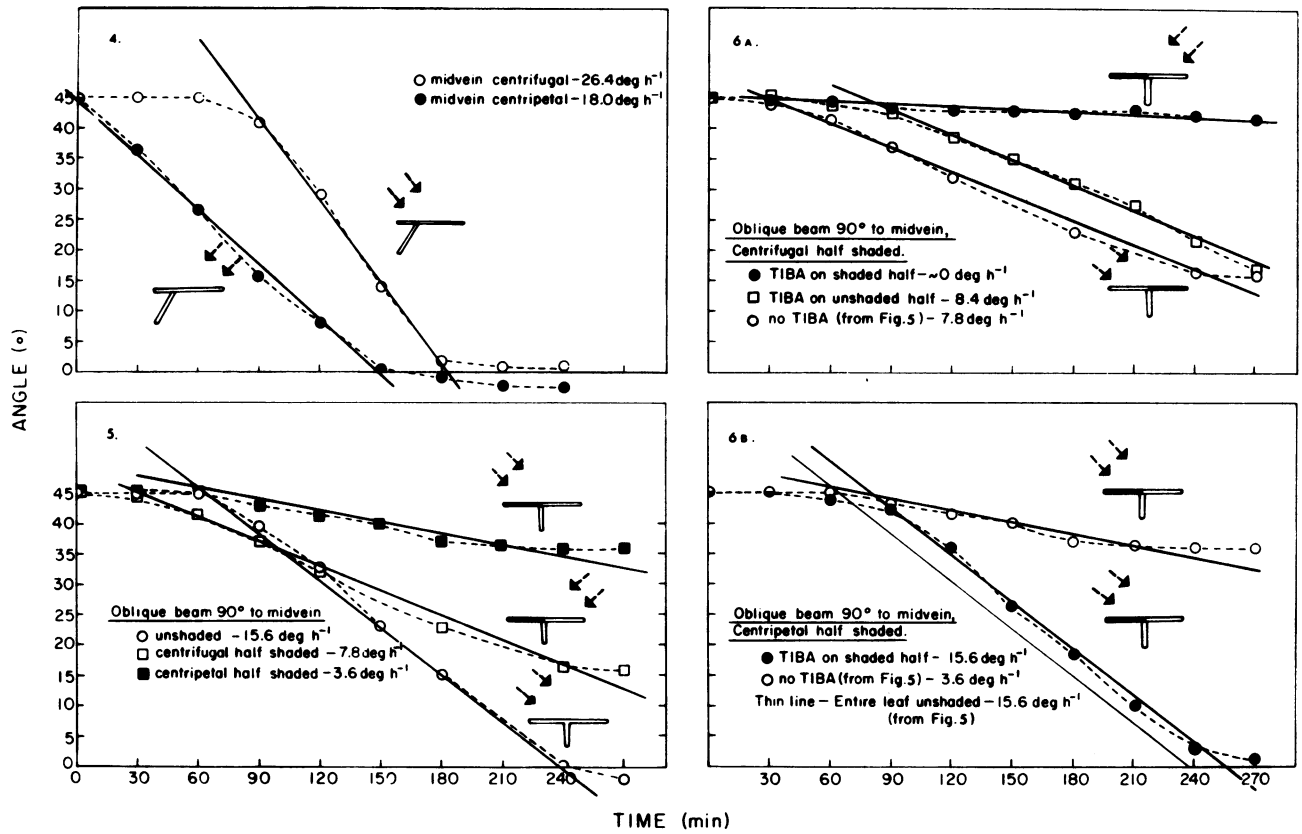

FIG. 4–6. Time course of laminar reorientation in *L. cretica* L. when exposed to oblique (45°) beam of parallel light. Abscissa: exposure time; ordinate: angle of deviation between beam and line perpendicular to lamina, through pulvinus. (——): regression of angle of deviation against time, for linear portion of the curve. Rates of reorientation (from regression coefficients) are shown in figures in degrees hr^{-1}.

FIG. 4. Oblique beam directed along midvein.

FIG. 5. Oblique beam directed across (at right angle) to midvein lamina either unshaded, or with centripetal half (proximal to light source) shaded, or with centrifugal half (distal to light source) shaded.

FIG. 6. Effect of TIBA application to base of veins of shaded half-leaf. A: centrifugal half-leaf shaded; B: centripetal half-leaf shaded.

response to a similarly directed centrifugal beam (Fig. 4). Alternatively, the lamina might be reacting somewhat differently to a centrifugal beam than to a centripetal one. To settle this point, subsequent experiments were done with the oblique beam directed at right angles to the midvein, which is the axis of bilateral symmetry. Thus, in order to reorient, the lamina would rotate around the axis formed by the midvein, where properties on either side are symmetrical. The oblique beam striking the lamina in this direction is thus acting centripetally on the half-leaf proximal to the light source and centrifugally on the symmetrical half-leaf distal to the light source. When the proximal half-leaf is shaded, the response would be to the centrifugal action of the beam on the unshaded distal half-leaf and conversely, when the distal half-leaf is shaded, the response would be the centripetal action of the beam on the unshaded proximal half-leaf.

The rate of reorientation was slower when the lamina was partially shaded than when it was fully exposed to the oblique beam (Fig. 5), suggesting that the strength of the response might be a function of the proportion of the lamina exposed to oblique illumination. However, the rate of reorientation was reduced more when the half-leaf distal to the light source was illuminated (centrifugally) than when the proximal one was illuminated (centripetally). In both cases, the half-shaded lamina never reoriented fully, but stabilized after 3 to 4 hr in an intermediate position (about 35° from the normal in centrifugal light and 15° in centripetal light), while the fully illuminated lamina reoriented fully, to 0° from the normal. It thus appeared that the full response required the cooperation of both the centrifugally illuminated distal half and the centripetally illuminated proximal half of the lamina. Any working hypothesis must account not only for the slower rate of reorientation when only half of the lamina was illuminated, but also for the incomplete reorientation and for the difference in rate of reorientation when the two symmetrical half-leaves were exposed to the same light beam. Since the distal half-leaf rises when it alone is illuminated (centrifugally), while the proximal half-leaf falls when it alone is illuminated (centripetally), it was apparent that the motor tissue associated with the centrifugally illuminated distal half-leaf must be generating higher turgor than exists in the one associated with shaded proximal half-leaf, while the centripetally illuminated proximal half-leaf must be generating less turgor than exists in the one associated with shaded distal half-leaf. The possibility was therefore tested that auxins supplied by the shaded half-leaf were instrumental in establishing dark turgor in the associated motor tissue. This was done by applying a single droplet (3- to 4-mm diameter) of lanolin paste containing 0.5% of the growth regulator TIBA to the base of the veins of the shaded half-leaf, close to the pulvinus. This treatment caused a complete inhibition of the response to centripetal illumination, and removed all inhibition from the response to centrifugal illumination, making it nearly identical with the response of the unshaded lamina (Fig. 6). Application of TIBA in lanolin to the unshaded centripetally illuminated (proximal) half-leaf delayed the start of reorientation, but had no effect on its subsequent rate. The phototropic responses of control leaves, in which the lanolin was applied without TIBA, were the same as without any lanolin. Application of a ring of lanolin with TIBA to the bases of all of the major veins of an unshaded lamina eliminated its capacity to respond phototropically to an oblique light beam. Instead, the lamina reoriented to come to rest approximately at right angles to its petiole.

DISCUSSION

The data indicate that the photoreceptor for light striking the lamina obliquely is closely associated with the veins. The stimulus is then transmitted from the vein, via its extension in the vascular tissue of the pulvinus, to the adjoining sector of motor tissue, where it induces the turgor change which causes reorientation of the lamina (25). Results in Figure 3 indicate that when the intact lamina is exposed to oblique light, all of the veins probably collaborate in the over-all response, but their individual contribution varies as the cosine of the angle between their plane of symmetry and the projection of the beam on the lamina, and may also depend on their size (length).

Increase in turgor in pulvinar motor cells during nastic or rhythmic movements apparently occurs as a result of active uptake of K^+ and the concomitant lowering of solute potential, which causes osmotic water uptake (1, 17, 20), while decrease in turgor takes place as a result of passive efflux of the accumulated K^+ ions, when active uptake is halted (18). This is also the case in the stomatal guard cells (8, 16). Wainwright (24) has shown that La^{3+}, which apparently inactivates the K^+ pump in animal and plant tissues, can cause complete inhibition of sun-tracking by leaves of *Lupinus arizonicus,* thus lending support to the possibility that reorientation of the lamina in response to oblique light is also mediated by a K^+ pump.

The kinetic behavior of laminar reorientation in response to oblique light differs when the proximal half-leaf or the distal one are shaded (Fig. 4). As the two half-leaves are symmetrical, the differences in kinetics of reorientation indicate that while the message from the centrifugally illuminated half-leaf must lead to water uptake by its associated motor cells, which causes this half-leaf to elevate, the message from the centripetally illuminated half-leaf must lead to water loss from its associated motor cells, causing this half-leaf to decline. When both half-leaves are exposed to the same oblique beam, these effects are combined and reorientation is complete. Other evidence showing that the actions of centrifugal and centripetal light differ not only in their kinetics, but also in their dose response, will be reported elsewhere. When one half-leaf is shaded, the segment of motor tissues associated with it presumably has a constant water potential and turgor, by which it resists compression when the opposite segment takes up water and expands as a result of centrifugal illumination, and similarly resists stretching when the opposite segment loses water and contracts as a result of centripetal illumination. The result in both cases is a dynamic equilibrium with incomplete reorientation.

The effects of TIBA (Fig. 6) provide strong support for this hypothesis, assuming that presence of TIBA in the veins of the shaded half-leaf results in loss of the static turgor in the associated motor cells. In this case, when the unshaded half-leaf is centrifugally illuminated, expansion of its associated motor tissue is no longer opposed, and reorientation can proceed to completion. Conversely, when the unshaded half-leaf is centripetally illuminated, both segments of the motor tissue become equally flaccid and the lamina does not reorient at all (Fig. 5).

The hypothesis may also explain the greater inhibitory effect of the shaded half-leaf on the response of the illuminated half-leaf to centrifugal light than to centripetal light. In the first instance, the two segments of the motor tissue tend to expand against each other, while in the second case, the expansion of the segment on the shaded side is virtually unopposed and is therefore limited only by its own water potential.

TIBA inhibits responses to auxin, presumably by interfering with its polar transport (14, 22). Thus, supply of auxin from the shaded half-leaf appears to be implicated in maintaining the constant dark turgor in the associated motor tissue. A similar mechanism has been postulated by Ball (2) to explain the differential response of the leaf of *S. africana* to illumination of its tip or its base. There is strong evidence that auxin activates at the membrane level an energy-dependent, electrogenic mechanism leading to proton extrusion, coupled to uptake of specific cations, with high affinity to K^+ (13). Both centrifugally directed oblique light and darkness cause an increase in turgor of the motor cells, possibly by promoting active uptake of K^+, while centripetally directed oblique light causes reduction of turgor, by promoting passive efflux of K^+. Auxin promotes the rhythmic opening of pinnae in *Albizzia julibrissin* in the dark and inhibits their nyctinastic closure. However, auxin inhibits the opening of pinnae and K^+ flux upon illumination but has no effect at all during other portions of the light period. This implies that changes in endogenous auxin do not control leaflet angle in the light (18).

While the data suggest that the shaded veins supply the auxin that causes dark turgor in their associated motor tissue, there are no indications regarding the nature of the message delivered by the obliquely illuminated veins. Centrifugal illumination apparently causes these veins to deliver a message activating K^+ uptake in the motor cells, while centripetal illumination stops delivery and thus halts active K^+ uptake, causing passive leakage of K^+ and water.

As the veins are relatively transparent, the different response to the direction of the oblique beam indicates that some subcellular, possibly molecular, component of the cells along the vein is anisotropically ordered in a unique orientation with respect to the direction of the vein. Such an organization has been postulated for the photoreceptor responsible for the polarotropic rotation of the chloroplast in *Mougeotia* (10). Two tissues participate in the pulvinar movement of *Samanea saman* pinnae. An extensor tissue is solely responsible for the autonomous rhythmic movements in the dark, while the flexor tissue is responsible for the movement in response to light/dark changes (15). Changes in K^+ levels in the extensor tissue correspond to both autonomous and light-induced movements of the pulvinus (increasing when leaflets open and vice versa). The flexor cells retain a high, constant level of K^+ during autonomous movement in the dark, but exhibit a strong efflux of K^+ upon illumination, concomitantly with a comparable influx into the extensor tissue (17). Leaves of *L. cretica* do not exhibit any conspicuous autonomous movement while leaves of *Stylosanthes humilis* (3) and *L. arizonicus* (24) exhibit diurnal cupping movement which may be associated also with leaf water stress. Tissues analogous to the extensor and flexor cells of *Samanea* have not yet been observed in *L. cretica* or in other sun-tracking leaves. However, all of the phototropic responses of the leaf of *L. cretica* are compatible with the operation of flexor-type cells only, that actively take up K^+ when fed by centripetally illuminated veins and maintain a static level of K^+ when fed by darkened veins. As the lamina remains horizontal when one half is shaded and the other half is illuminated by a vertical beam, vertical illumination apparently acts identically as darkness.

LITERATURE CITED

1. ALLEN RD 1969 Mechanism of the seismonastic reaction in *Mimosa pudica.* Plant Physiol 44: 1101–1107
2. BALL NG 1969 Tropic, nastic and tactic responses. *In* FC Steward, ed, Plant Physiology, Vol VA. Academic Press, New York, pp 119–228
3. BEGG JE, BWR TORSSELL 1974 Diaphotonastic and parahelionastic movements in *Stylosanthes humilis* H. B. K. (Townsville stylo). R Soc NZ Bull 12: 277–283
4. BLACK M, JE SHUTTLEWORTH 1974 The role of the cotyledons in the photocontrol of hypocotyl extension in *Cucumis sativus* L. Planta 117: 56–66
5. BRAUNER L 1959 Phototropismus und Photonastie der Laubblätter. *In* W Ruhland, ed, Handbuch der Pflanzenphysiologie XVII/1: 472–491
6. BRAUNER L, Y VARDAR 1950 Über die Funktion der Lamina bei der phototropischen und geotropischen Reaktion der Tropaeolum-Blattes. Rév Fac Sci Univ Istanbul B Sci Nat 15: 269–299
7. BRUINSMA J, KARSSEN CM, BENSCOP M, JB DORT 1975 Hormonal regulation of phototropism in the light-grown sunflower seedlings, *Helianthus annuus* L.: immobility of endogenous indoleacetic acid and inhibition of hypocotyl growth by illuminated cotyledons. J Exp Bot 26: 411–418
8. DAYANANDAN P, PB KAUFMAN 1975 Stomatal movement associated with potassium fluxes. Am J Bot 62: 221–231
9. HABERLANDT G 1918 Physiologische Pflanzenanatomie. Wilhelm Engelmann, Leipzig, pp 535–591
10. HAUPT W 1972 Short-term phenomena controlled by phytochrome. *In* K Mitrakos, W.

Shropshire Jr, eds, Phytochrome. Academic Press, New York, pp 349–368
11. KUSE G 1954 Bud inhibition and correlative growth of petiole in sweet potato stem. Mem Coll Sci Univ Kyoto Ser B 21: 107–114
12. LAM H, AC LEOPOLD 1966 Role of leaves in phototropism. Plant Physiol 41: 847–851
13. MARRÈ E, P LADO, F RASI-CALDOGNO, R COLOMBO, M COCUCCI, MI DEMICHELIS 1975 Regulation of proton extrusion by plant hormones and cell elongation. Physiol Vég 13: 797–811
14. NIEDERGANG-KAMIEN E, AC LEOPOLD 1975 Inhibitors of polar transport. Physiol Plant 10: 29–38
15. PALMER JH, GF ASPREY 1958 Studies on the nyctinastic movement of the leaf pinnae of *Samanea saman* (Jacq.) Merrill. II. The behaviour of the upper and lower half-pulvini. Planta 51: 770–785
16. RASCHKE K 1975 Stomatal action. Annu Rev Plant Physiol 26: 309–340
17. SATTER RL, GT GEBALLE, PB APPLEWHITE, AW GALSTON 1974 Potassium flux and leaf movement in *Samanea saman*. I. Rhythmic movement. J Gen Physiol 64: 413–430
18. SATTER RL, P MARINOFF, AW GALSTON 1972 Phytochrome controlled nyctinasty in *Albizzia julibrissin*. IV. Auxin effects on leaflet movement and K^+ flux. Plant Physiol 50: 235–241
19. SHIBAOKA H, T YAMAKI 1959 Studies on the growth movement of sunflower plant. Sci Pap Coll Gen Educ Univ Tokyo (Biol Part) 9: 105–126
20. TORIYAMA H 1962 Observational and experimental studies of sensitive plants. XV. The migration of potassium in the petiole of *Mimosa pudica*. Cytologia 27: 431–441
21. TRENBATH BR, JF ANGUS 1975 Leaf inclination and crop production. Field Crops Abstr 28: 231–244
22. VAN OVERBEEK J 1956 Absorption and translocation of plant regulators. Annu Rev Plant Physiol 7: 355–372
23. VÖCHTING H 1888 Über die Lichtstellung der Laubblätter. Botan Z 46: 501–514, 517–527, 533–541, 549–560
24. WAINWRIGHT CM 1977 Sun-tracking and related leaf movements in a desert lupin (*Lupinus arizonicus*). Am J Bot 64: 1032–1041
25. YIN HC 1938 Diaphototropic movement of leaves of *Malva neglecta*. Am J Bot 25: 1–6