Phytochrome-controlled Nyctinasty in *Albizia julibrissin*

IV. AUXIN EFFECTS ON LEAFLET MOVEMENT AND K FLUX

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ABSTRACT

Indole-3-acetic acid, a-naphthylacetic acid, and 2,4-dichlorophenoxyacetic acid (0.001 to 1.0 m) inhibit the nyctinastic closure of excised *Albizia* leaflet pairs; antiauxins and auxin analogs are ineffective, and the auxin effects seem not to be mediated by ethylene. Indoleacetic acid (0.001 to 0.1 m) also promotes rhythmic opening in the dark, but is ineffective during that phase of rhythmic closure ("leaky phase") which is insensitive to azide. At these concentrations, all of the indoleacetic acid effects are reversible upon transfer of the tissue to water and are linked to alteration of potassium flux in pulvinule motor cells.

A supraoptimal concentration of indoleacetic acid (1 m) inhibits rhythmic opening as well as nyctinastic closure, although it has little or no effect on potassium flux in motor cells. These inhibitions cannot be completely reversed upon transferring the leaflets to water.

Although indoleacetic acid (0.01 to 1.0 m) inhibits leaflet opening and potassium flux in dorsal and ventral motor cells when leaflets are transferred from darkness to light, it has no effect during other portions of the light period, implying that changes in endogenous auxin do not control leaflet angle in the light. Neither does auxin seem to be involved in the phytochrome-regulated process, since it does not alter phytochrome control of leaflet movement or potassium flux. However, endogenous auxin probably plays an important role in controlling potassium flux into ventral motor cells during the opening phase of rhythmic leaflet movement in the dark.

*Albizia julibrissin* leaflets open and close when the relative turgidity of dorsal and ventral pulvinule motor cells is altered (21). Previous studies in our laboratory (8, 18–20) have shown that such turgor changes are accompanied by K flux whether leaflet movement is controlled by light-dark transition, phytochrome, endogenous rhythm, or exogenous salts of organic acids.

Our search for metabolic changes that might precede K flux has led us to investigate the possible role of plant hormones. Other investigators have reported that exogenous auxin and gibberellic acid inhibit leaflet closure in *Albizia* (15) and that auxin (1, 3, 11, 26, 27) and ethylene (16) alter leaf and leaflet movement in other species. In addition, recent studies have shown correlations between the level of endogenous IAA and ABA and the turgor of stomatal guard cells (23). K is the osmotically active cation in guard cells (4, 22) as well as in *Albizia* motor cells.

This paper reports our study of the effect of several auxins, auxin analogs, ethylene, and ABA on *Albizia* leaflet movement, and of IAA on K flux. It was our hope that this investigation would indicate whether alterations in the endogenous level and distribution of these hormones could play a role in phytochrome-controlled (12, 13, 20) or endogenously rhythmic (18, 19) leaflet movement and K flux.

MATERIALS AND METHODS

*Albizia julibrissin* plants were grown from seed in a greenhouse and were transferred to controlled growth chambers a few days before experiments, as previously described (20). Growing conditions, light sources for R and FR irradiation, and experimental procedures were the same as previously used. Pairs of leaflets excised just prior to experimental use were floated on test solutions in Petri dishes (Figs. 1, 2, 5, and Tables I, II, III) or were floated on H$_2$O supplemented by test solutions added 2.5 hr before leaflet angles were measured (Fig. 4). In the latter experiment 2 ml of 1 mm IAA or 10 mm Nap$_2$ was added to 18 ml of H$_2$O. Leaflet movement studies utilized six leaflet pairs per treatment and were repeated three or more times.

Hormone Solutions. One mm stock solutions of IAA and auxin analogs were prepared as follows: 1 meq of IAA was dissolved in 10 ml of 0.1 m KHCO$_3$, while 1 meq of each auxin analog (2,4-D, NAA, CPB, DCPIB, and benzoic acid) was dissolved in 7 ml of 0.1 m KOH; H$_2$O was added to make 1 liter of each solution. The pH was adjusted to 5.0 ± 0.5 with HCl or KOH. Leaflet movement is independent of pH values in this range (20).

One liter of 0.1 mm ABA was prepared by dissolving 0.1 meq ABA (racemic, gift from Hoffmann-LaRoche) in 1 ml of DMSO, then adding H$_2$O. DMSO at this concentration had no effect on leaflet movement.

Mercuric perchlorate was prepared just prior to experimental use from mercuric oxide and perchloric acid (28). Ethylene was injected into closed chambers with a hypodermic needle.

Elemental Analysis. Pinnae were excised, their four basal pinnae pairs were removed, and their cut ends were placed in test solutions. After they had received the light and dark treatments described in the text, the six lowest pulvinule pairs were excised and frozen for analysis of K and Ca with an

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Abbreviations: NAA: a-naphthylacetic acid; CPBA: 4-chlorophenoxyisobutyric acid; DCPIBA: 2,4-dichlorophenoxyisobutyric acid; DMSO: dimethyl sulfoxide; R: red light; FR: far red light.
Acton electron microprobe (20). Each point of our graphs is an average of 36 measurements; each experiment was performed at least twice.

RESULTS

Effect of IAA and Auxin Analogs on Nyctinastic Closure. Interaction of phytochrome and auxins on nyctinastic closure was tested 1 to 2 hr after the start of the photoperiod, when phytochrome is known to exert optimal control of leaflet closure (19). Open leaflet pairs excised from a plant in white light were floated on test solutions, irradiated with R or FR and then darkened. Preliminary experiments revealed that auxin effects were most evident if leaflets were preirradiated with R; thus FR pretreatment was omitted in some experiments to reduce the requirement for an unduly large number of replicate leaflet pairs. Leaflet angles were measured after 120 to 160 min of darkness.

Figs. 1, 2. Nyctinastic closure of leaflet pairs floating on IAA, 2,4-D or NAA (Fig. 1) or on IAA, CPIBA, DCPIBA, or benzoic acid (Fig. 2). Open leaflet pairs, excised from a plant that had been in white light for 90 min, were floated on test solutions, irradiated briefly with R or FR, then darkened. Leaflet angles were measured 2 hr (Fig. 1) or 2 hr 40 min (Fig. 2) later.
IAA, NAA, and 2,4-D (0.01–1.0 mm) inhibited nyctinastic closure (Fig. 1). Threshold effects were obtained at 1 μM IAA, the most effective of the auxins. Inhibition by IAA and Pr appear to be additive, confirming recent results of others (15). Benzoic acid, CPIBA and DCPIBA, which show anti-auxin or weak auxin activity in cell elongation bioassays (7), were sometimes mildly inhibitory but usually ineffective (Fig. 2).

Reversibility of IAA Effects. Leaflets floated on IAA solutions for 90 min were transferred to H2O and their response to light and darkness compared to that of H2O controls. Three hours later, leaflets that had been on 0.1 mm IAA moved almost as rapidly as did controls, indicating a complete reversal of the IAA-induced inhibition. On the other hand, leaflets that had been on a 1.0 mm solution moved sluggishly and never regained complete mobility. In additional experiments, leaflets from leaves of different age or from different plants were tested and varied somewhat in sensitivity to IAA; the effects of 1 mm IAA on some leaflets were completely reversible.

K Flux in Pulvinate Motor Cells of Leaflets Exposed to IAA. K flux into dorsal and out of ventral motor cells appears to be the basis for the closure of leaflets on an intact plant (18) and on excised pinnae (8, 19, 20). To determine the effect of IAA on K flux during nyctinastic closure, open pinnae whose cut ends were in IAA solutions were irradiated with R or FR and then darkened. After 2 hr of darkness, leaflet angles were measured, and pulvinules were excised and frozen in preparation for electron microprobe analysis (20) of the K content of motor cells. K flux is indicated by changes in K in pulvinate motor cells (Fig. 3); similar trends were apparent if K flux was expressed by changes in K/Ca or in K/P (20).

K preirradiation promotes nyctinastic closure and K movement into dorsal and out of ventral motor cells (Fig. 3; also refs. 8, 18–20). External IAA increased K in ventral motor cells, and to a lesser extent in dorsal motor cells, but did not alter the effects of phytochrome. Although the inhibition of leaflet closure increased progressively as the IAA concentration increased from 0.01 to 1.0 mm, a median IAA concentration (0.1 mm) had the greatest effect on K flux in this experiment. A slightly higher concentration was maximally effective in a replicate experiment.

Phytochrome Control of K Flux during Rhythmic Leaflet Movement. Darkened leaflets that have been closed for about 12 hr will open in response to circadian rhythmic control (18, 19). The rate and extent of leaflet opening are enhanced if the Pfr level is lowered by a brief exposure to FR, while the movement of R preirradiated leaflets is indistinguishable from that of dark controls (19). To determine phytochrome effects on K flux during rhythmic opening, we used the electron microprobe to measure K flux in motor cells of leaflets that had opened 50° after R or 115° after FR irradiation (Table I). The K content of the ventral motor cells, expressed as K/Ca to correct for cellular volume changes (20), increased 52% (R) compared to 145% (FR), while that of the dorsal motor cells decreased 10% (R) compared to 24% (FR). Similar results are obtained if the data are considered as absolute changes in K, although the fluxes are smaller.

IAA Alteration of Phytochrome-controlled Rhythmic Leaflet Movement. Intact leaflets or excised leaflets floating on H2O begin to open between the 7th and 10th hr of the dark period, but opening will start several hours earlier if leaflets are treated with IAA (Table II). This premature opening is partially controlled by phytochrome. IAA also increases the rate of movement during the usual opening period; 0.001 to

![Fig. 3. Effect of IAA on K flux in motor cells during nyctinastic closure.](image)

Table 1. Effect of Phytochrome on K Flux in Motor Cells During Rhythmic Opening in the Dark

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (hr)</th>
<th>Angle (°)</th>
<th>K (mm)</th>
<th>K/Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>2.5</td>
<td>0</td>
<td>189 ± 34</td>
<td>0.65 ± 0.28 ± 0.50</td>
</tr>
<tr>
<td>FR</td>
<td>2.5</td>
<td>115</td>
<td>284 ± 32</td>
<td>1.59 ± 0.19 ± 0.73 ± 0.42</td>
</tr>
</tbody>
</table>

1 After excision.
2 Values are scintillations ± SE.
Table II. Opening in the Dark of R and FR Preirradiated Leaflets
Floating on IAA

Closed leaflet pairs excised from a plant that had been darkened for 5 hr were floated on IAA, irradiated briefly with R or FR, then returned to darkness for 5.5 additional hr.

<table>
<thead>
<tr>
<th>Time</th>
<th>Preirradiation</th>
<th>H2O</th>
<th>IAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>hr</td>
<td></td>
<td>mM</td>
<td>mM</td>
</tr>
<tr>
<td>1.7</td>
<td>R</td>
<td>0</td>
<td>6 ± 4(^2)</td>
</tr>
<tr>
<td>5.5</td>
<td>FR</td>
<td>1</td>
<td>10 ± 8</td>
</tr>
</tbody>
</table>

1 After excision.
2 Degrees opening ± SD.

Fig. 4. Leaflets on a plant that had been darkened for 8 hr were excised just as they were beginning endogenously rhythmic opening. They were transferred to Petri dishes containing 18 ml of H2O and were irradiated briefly with R (4 min) or FR (90 sec), then returned to darkness for 13 additional hr. Two ml of 1 mM IAA or 10 mM NaN\(_3\) was added to water in a Petri dish, 2.5 hr before angles were measured. Leaflet pairs were measured once, then discarded. Each point on the graph is the average angle of six leaflet pairs. Data for hr 8 to 13.5 and 13.5 to 21 were obtained in separate experiments.

0.01 mM was threshold, and 0.1 mM was the most effective concentration in the majority of our experiments.

Leaflets that open in the dark remain open for several hours and then begin to close (18, 19). We tested the effect of IAA on both the opening and the closure phases of rhythmic leaflet movement as follows: a large number of replicate leaflet pairs were excised from a darkened plant just as they were beginning to open, and were floated on H2O, exposed briefly to R or FR light, then returned to darkness for 13 additional hr. Two and a half hr before leaflet angles were measured, the H2O bathing a group of leaflets was supplemented with IAA (0.1 mM) or NaN\(_3\) (1.0 mM). The latter compound helped us determine which phases of rhythmic leaflet movement require metabolic energy. Previous experiments had shown that NaN\(_3\) prevents opening (19); thus it was added during the closure phase only in these experiments.

Leaflets preexposed to FR opened more rapidly and completely and maintained their maximum angles longer than those exposed to R (Fig. 4). IAA promoted a wide angle during the energy-requiring phases of leaflet movement (i.e., opening of all leaflets and closure between hr 16 and 21 of R leaflets) but had no effect during the azide-insensitive phases (hr 16 to 21 for FR leaflets and hr 13 to 16 for R leaflets).

Effect of Auxins on Leaflet Opening in White Light. Closed leaflet pairs were excised from a darkened plant, floated on solutions of IAA, NAA, or 2,4-D and irradiated with white light. Opening was inhibited by 0.01 mM and higher concentrations of all three compounds (Fig. 5). The concentration required for threshold response in the light is 1 to 2 log units higher than that required for activity in the dark, even if leaflets for both experiments are taken from the same leaf (Table III).

We analyzed K flux in pulvinule motor cells of IAA-treated leaflets and found that the K content of the ventral motor cells was lower and that of the dorsal cells was higher than that in H2O controls (Fig. 6). Thus IAA appears to inhibit leaflet opening in the light by interfering with requisite K fluxes in both groups of motor cells.

However, IAA had no effect on leaflet angle during other portions of the light period. Open leaflets floating on H2O remain wide open for 10 to 12 hr, then begin a slow, rhythmically controlled closure (18): IAA solutions (≤0.1 mM) did not alter either of these phases of leaflet movement.

Since IAA action in the light: (a) is confined to the opening phase, (b) is always inhibitory, and (c) often occurs only at supraphysiological levels (Table III), it is unlikely that alterations in IAA are part of the endogenous regulatory mechanism.

Activity of Ethylene and ABA. We considered the possibility that auxin might control *Albizzia* leaflet closure by promoting ethylene formation, since ethylene inhibits leaf movement in other species (16) and ethylene production is stimulated by auxin (17). However, leaflets in a chamber with 10 or 100 µl/l ethylene closed at the same rate as did controls. Also the addition of a vial containing 0.25 M HgCl\(_2\), an effective ethylene absorber (28), to a chamber enclosing leaflets floating on IAA, did not reduce the inhibitory effect of IAA. Thus it is unlikely that auxin effects are mediated by ethylene.

We also studied the movement of leaflets floating on ABA solutions, since stomatal movement is extremely sensitive to ABA, and turgor changes in *Albizzia* motor cells (20) and stomatal guard cells (22) are accompanied by K fluxes of similar magnitude. We used the same procedures and environmental conditions described for IAA experiments and found that ABA (≤0.1 mM) had no effect on leaflet movement in the dark in most experiments, but inhibited rhythmic opening and promoted both rhythmic and nyctinastic closure in leaflets from some leaves (about 10% of those tested). It did not alter leaflet angle in the light in any of our experiments.

**DISCUSSION**

Potassium flux in pulvinule motor cells provides an osmotic rationale for the turgor changes underlying phytochrome-con-
trolled, endogenously rhythmic, and acetate-promoted leaflet movement (8, 18–20). The present data (Figs. 3, 6) permit the conclusion that exogenous IAA (≤0.1 mM) can also control leaflet movement by control of K flux. This recalls Commoner and Mazia’s (2) 30-year-old suggestion that auxin control of salt absorption is a general phenomenon and is consistent with reports that external auxin alters bioelectric potentials in roots (14) and solute uptake in endocarp (9) and in isolated protoplasts (10, 25). However, another explanation is required for the failure of leaflets floating on 1.0 mM IAA to close when darkened (Fig. 3), since K flux in these leaflets is not very different from that in H₂O controls. Some other mechanism, such as an altered plasticity and elasticity of motor cell walls may be involved here (1). This suggestion is consistent with the incomplete mobility of leaflets transferred from 1.0 mM IAA to H₂O and would explain paradoxes such as the inhibition of rhythmic opening in the dark by 1.0 mM IAA solutions despite promotion by less concentrated solutions (Table III).

**K Flux in Darkened Leaflets.** There are two alternative explanations for the high K content (Fig. 3) of the ventral motor cells of leaflets treated with 0.1 mM IAA during nyctinastic closure: auxin might have inhibited the passage of K out of or promoted the movement of K into the ventral motor cells. There is considerable evidence suggesting bidirectional K movement in the ventral motor cells of darkened leaflets, i.e., K moves into ventral motor cells of darkened leaflets preirradiated with R or FR, but this flux can be masked either by phytochrome-controlled active secretion or by rhythmic leakage of K ions out of these same cells (19).

The second interpretation is supported by experiments showing promotion by IAA of rhythmic opening in the dark (Table II, Fig. 4), since opening under these conditions is closely correlated with K movement into the ventral motor cells (Table I and refs. 18, 19). The many parallels between phytochrome control of nyctinasty and of rhythmic movement during a long dark period suggest these are different manifestations of the same phenomenon (19). Phytochrome has the same effect on leaflet angle and the K content of both groups of motor cells during opening in the dark (Table I) and nyctinastic closure (Fig. 3), i.e., a high Pfr level favors a small leaflet angle, low K in ventral and high K in dorsal motor cells. Also, the angle of IAA-treated leaflets exceeds that of H₂O controls following nyctinastic closure (Figs. 1–3) or rhythmic opening (Fig. 4), but IAA does not alter phytochrome control of either process.

### Table III. Effects of IAA on Leaflet Opening in the Light and in the Dark

<table>
<thead>
<tr>
<th></th>
<th>H₂O</th>
<th>IAA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>.01</td>
<td>.1</td>
</tr>
<tr>
<td>Light</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>160 ± 5</td>
<td>161 ± 10</td>
</tr>
<tr>
<td>Dark</td>
<td>47 ± 21</td>
<td>58 ± 29</td>
</tr>
</tbody>
</table>

1 Degrees opening ± sd.

Closed leaflet pairs excised from a plant that had been darkened for 8 hr were floated on IAA solutions and kept in the dark for several additional hours or illuminated with white light. Rhythmic opening in the dark (18, 19) began 2 hr after excision and was measured 110 min later. The angle of leaflets in the light was measured 150 min after excision.
interpretation is correct, the differential effect of IAA on R compared to FR preirradiated leaflets is an indirect consequence of phytochrome action and does not implicate IAA in the Pfr process.

Several investigators have reported that IAA is required for a measurable response to phytochrome in other systems (5, 6, 24). In our experiments, Pfr control of leaflet movement is evident early in the dark period only if leaflets are treated with IAA; R and FR preirradiated H2O controls have 0° angles (Table II). However, this does not mean that the K content of the motor cells of control leaflets is not influenced by phytochrome at this time, since a large K flux can precede measurable leaflet opening. (In one experiment, for example, leaflet angles were 0° at hr 0 and 6 of the dark period, but the K content of the ventral motor cells differed by 70% [18]). Thus, our data do not indicate an interaction between IAA and phytochrome despite earlier evidence of Pfr control when leaflets are supplied with IAA (Table II).

Role of Endogenous IAA in Control of Leaflet Movement.

Previous workers studying leaf movement in Phaseolus vulgaris (1, 11) and in Carica papaya (27) reported that rhythmic variations in diffusible IAA were correlated with rhythmic leaf movement and postulated that these changes in endogenous auxin were the basis for leaf movement. Our data indicating promotion of leaflet opening in the dark by physiological levels of IAA support this hypothesis and show that IAA exerts its effect via K flux in the motor cells (Fig. 3).

However, endogenous regulation of leaflet movement is undoubtedly more complex than this. Diurnal variations in leaflet angle persist even if leaflets are supplied with optimal IAA; e.g., auxin is ineffective in preventing the azide-independent phase of rhythmic closure. Endogenous regulation probably involves the interplay of several hormones and their interaction with a Pfr controlled system. McEvoy and Koukkari (15) found that GA has a small inhibitory effect on nystatic closure in Albizia, whereas Williams and Raghavan (26) reported a synergistic response when leaflets of Mimosa pudica, a close relative of Albizia, were supplied with both GA and IAA. Although ABA and ethylene were ineffective in our experiments, they too might show activity if applied together with IAA or GA.

Further experiments involving hormonal interactions and measurements of endogenous hormonal levels are clearly necessary, although it is unlikely that these will reveal the nature of the basic oscillator system producing rhythmic behavior. For even if rhythmic changes in hormone levels should be found, it will be difficult to decide whether these are causes or results of the basic oscillation. Our conjecture (19) that the rhythm involves alternating phases of integrity and "leakiness" of membranes provides one framework for understanding the action of hormones involved in the nystatic or rhythmic leaflet movements of Albizia.

LITERATURE CITED


