Untitled Essay, Research Paper

Involvement of K+ in Leaf Movements During SuntrackingIntroduction

Many plants orient their leaves in response to directional light

signals. Heliotropic movements, or movements that are affected by the sun, are common

among plants belonging to the families Malvaceae, Fabaceae, Nyctaginaceae, and

Oxalidaceae. The leaves of many plants, including Crotalaria pallida, exhibit

diaheliotropic movement. C. pallida is a woody shrub native to South Africa. Its

trifoliate leaves are connected to the petiole by 3-4 mm long pulvinules (Schmalstig). In

diaheliotropic movement, the plant’s leaves are oriented perpendicular to the

sun’s rays, thereby maximizing the interception of photosynthetically active

radiation (PAR). In some plants, but not all, his response occurs particularly during the

morning and late afternoon, when the light is coming at more of an angle and the water

stress is not as severe (Donahue and Vogelmann). Under these conditions the lamina of the

leaf is within less than 15? from the normal to the sun. Many plants that exhibit

diaheliotropic movements also show paraheliotropic response as well. Paraheliotropism

minimizes water loss by reducing the amount of light absorbed by the leaves; the leaves

orient themselves parallel to the sun’s rays. Plants that exhibit paraheliotropic

behavior usually do so at midday, when the sun’s rays are perpendicular to the

ground. This reorientation takes place only in leaves of plants that are capable of nastic

light-driven movements, such as the trifoliate leaf of Erythrina spp. (Herbert 1984).

However, this phenomenon has been observed in other legume species that exhibit

diaheliotropic leaf movement as well. Their movement is temporarily transformed from

diaheliotropic to paraheliotropic. In doing so, the interception of solar radiation is

maximized during the morning and late afternoon, and minimized during midday. The leaves

of Crotalaria pallida also exhibit nyctinastic, or sleep, movements, in which the leaves

fold down at night. The solar tracking may also provide a competitive advantage during

early growth, since there is little shading, and also by intercepting more radiant heat in

the early morning, thus raising leaf temperature nearer the optimum for photosynthesis.

Integral to understanding the heliotropic movements of a plant is

determining how the leaf detects the angle at which the light is incident upon it, how

this perception is transduced to the pulvinus, and finally, how this signal can effect a

physiological response (Donahue and Vogelmann).

In the species Crotalaria pallida, blue light seems to be the

wavelength that stimulates these leaf movements (Scmalstig). It has been implicated in the

photonastic unfolding of leaves and in the diaheliotropic response in Mactroptilium

atropurpureum and Lupinus succulentus (Schwartz, Gilboa, and Koller 1987). However, the

light receptor involved can not be determined from the data. The site of light perception

for Crotalaria pallida is the proximal portion of the lamina. No leaflet movement occurs

when the lamina is shaded and only the pulvinule is exposed to light. However, in many

other plant species, including Phaseolus vulgaris and Glycine max, the site of light

perception is the pulvinule, although these plants are not true suntracking plants. The

compound lamina of Lupinus succulentus does not respond to a directional light signal if

its pulvini are shaded, but it does respond if only the pulvini was exposed. That the

pulvinus is the site for light perception was the accepted theory for many years. However,

experiments with L. palaestinus showed that the proximal 3-4 mm of the lamina needed to be

exposed for a diaheliotropic response to occur. If the light is detected by photoreceptors

in the laminae, somehow this light signal must be transmitted to the cells of the

pulvinus. There are three possible ways this may be done. One is that the light is

channeled to the pulvinus from the lamina. However, this is unlikely since an experiment

with oblique light on the lamina and vertical light on the pulvinus resulted in the lamina

responding to the oblique light. Otherwise, the light coming from the lamina would be

drowned out by the light shining on the pulvinus. Another possibility is that some

electrical signal is transmitted from the lamina to the pulvinus as in Mimosa. It is also

possible that some chemical is transported from the lamina to the pulvinus via the phloem.

These chemicals can be defined as naturally occuring molecules that affect some

physiological process of the plant. They may be active in concentrations as low as 10-5 to

10-7 M solution. Whatchemical, if any, is used by C. pallida to transmit the light signal

from the lamina of the leaflet to its pulvinule is unknown. Periodic leaf movement factor

1 (PLMF 1) has been isolated from Acacia karroo, a plant with pinnate leaves that exhibits

nychinastic sleep movements, as well as other species of Acacia, Oxalis, and Samanea. PLNF

1 has also been isolated from Mimosa pudica, as has the molecule M-LMF 5 (Schildknecht).

The movement of the leaflets is effected by the swelling and shrinking

of cells on opposite sides of the pulvinus (Kim, et al.) In nyctinastic plants, cells that

take up water when a leaf rises and lose water when the leaf lowers are called extensor

cells. The opposite occurs in the flexor cells (Satter and Galston). When the extensor

cells on one side of the pulvinus take up water and swell, the flexor cells on the other

side release water and shrink. The opposite of this movement can also occur. However, the

terms extensor and flexor are not rigidly defined. Rather, the regions are defined

according to function, not position. Basically, the pulvini cells that are on the adaxial

(facing the light) side of the pulvinus are the flexor cells, and the cells on the abaxial

side are the extensor cells. Therefore, the terms can mean different cells in the same

pulvinus at varying times of the day. By coordinating these swellings and shrinkings, the

leaves are able to orient themselves perpendicular to the sunlight in diaheliotropic

plants.

Leaf movements are the result of changes in turgor pressure in the

pulvinus. The pulvinus is a small group of cells at the base of the lamina of each

leaflet. The reversible axial expansion and contraction of the extensor and flexor cells

take place by reversible changes in the volume of their motor cells. These result from

massive fluxes of osmotically active solutes across the cell membrane. K+ is the ion that

is usually implicated in this process, and is balanced by the co-transport of Cl- and

other organic and inorganic anions.

While the mechanisms of diaheliotropic leaf movements have not been

studied extensively, much data exists detailing nyctinastic movements. Several ions are

believed to be involved in leaf movment. These include K+, H+, Cl-, malate, and other

small organic anions. K+ is the most abundant ion in pulvini cells. Evidence suggests that

electrogenic ion secretion is responsible for K+ uptake in nyctinastic plants. The

transition from light to darkness activates the H+/ATPase in the flexor cells of the

pulvinus. This leads to the release of bound K+ from the apoplast and movement of the K+

into the cells by way of an ion channel. This increase in K+ in the cell decreases the

osmotic potential of the cells, and water than influxes into the flexor cells, increasing

their volume. In Samanea, K+ levels changed four-fold in flexor cells during the

transition from light to darkness. In a similar experiment, during hour four of a

photoperiod, the extensor apoplast of Samanea had 14mM and the flexor apoplast had 23 mM

of K+. After the lights were turned off, inducing nyctinastic movements, the K+ level in

the apoplast rose to 72 mM in the extensor cells and declined to 10mM in the flexor cells.

Therefore, it appears that swelling cells take up K+ from the apoplast and shrinking cells

release K+ into the apoplast.

In the pulvinus of Samanea saman, depolarization of the plasma membrane

opens K+ channels (Kim et al). The driving force for the transport of K+ across the cell

membranes is apparently derived from activity of an electrogenic proton pump. This creates

an electrochemical gradient that allows for K+ movement. From concentration measurements

in pulvini, K+ seems to be the most important ion involved in the volume changes of these

cells. How then, is K+ allowed to be at higher concentrations inside a cell than out of

it? Studies indicate that the K+ channels are not always open. In protoplasts of Samanea

saman, K+ channels were closed when the membrane potential was below -40mV and open when

the membrane potential was depolarized to above -40mV. A voltage-gated K+ channel that is

opened upon depolarization has been observed in every patch clamp study of the plasma

membranes of higher plants, including Samanea motor cells and Mimosa pulviner cells.

It is proposed that electrogenic H+ secretion results in a proton

motive force, a gradient in pH and in membrane potential, that facilitates the uptake of

K+, Cl-, sucrose, and other anions. External sodium acetate promotes closure and inhibits

opening in Albizzia. This effect could be caused by a decrease in transmembrane pH

gradients. The promotion of opening and inhibition of closure of leaves by fusicoccin and

auxin in Cassia, Mimosa, and Albizzia also implicate H+ in the solute uptake of motor

cells, since both chemicals are H+/ATPase activators, stimulating H+ secretion from the

plant cells into the apoplast. Vanadate, an H+/ATPase inhibitor, inhibits rhythmic leaflet

closure in Albizzia. Although this conflicts with the movement effected by fusicoccin and

auxin, it is believed that vanadate affects different cells, acting upon flexor rather

than extensor cells. The model indicates that there are two possible types of H+ pumps.

One is the electrogenic pump that creates the pmf mentioned above and opens the K+

channels. The other pump is a H+/K+ exchanger, in which K+ is pumped into the cell as H+

is pumped out of the cell in a type of antiport. The presence of this typ of pump is only

hypothetical, however, since at present there is no evidence to support it. Thus there are

two possible ways for K+ to enter the pulvini cells. The buildup of the pH gradient may

also promote Cl- entry into the cell via a H+/Cl- cotransporter as the H+ trickles back

into the cell. Cl- ions may also be driven by the electrochemical gradient for Cl- via Cl-

channels, as with K+. A large Cl- channel was observed in the membrane of Samanea flexor

protoplasts. The channel closed at membrane potentials above 50mV and opened at potentials

as low as -100mV.

Light-driven changes in membrane potential may be involved in the

activation of these proton pumps. This may be mediated by effects on cytoplasmic Ca2+.

Ca2+-chelators inhibit the nyctinastic folding as well as the photonastic unfolding

responses in Cassia. Thus Ca2+ may act as a second messenger in a calmodulin-dependent

reaction. The Ca2+ may be what turns on the electrogenic proton pumps, causing changes in

membrane potential. However, there is no direct evidence to support this hypothesis,

although chemicals that are known to change calcium levels have been shown to alter the

leaf movement of Cassia fasciculata and other nyctinastic plants. One study involving

Samanea postulates that Ca2+ channels are also present in the plasma membrane of pulvini

cells, and inositol triphoshate, a second messenger in the signal transduction pathway in

animals, stimulates the opening of these channels. This insinuates that some light signal

binds to a receptor on the outside of the cell and stimulates this transduction pathway.

However, whether this hypothesis is true is unclear. It has also been proposed that an

outwardly directed Ca2+ pump functions as a transport mechanism to restore homeostasis

after Ca2+ uptake through channels.

The changes in Cl- levels in the apoplast are less then that for K+.

The Cl- levels are 75% that of K+ in Albizzia, 40-80% in Samanea, and 40% in Phaseolus.

Therefore, other negatively charged ions must be used to compensate for the positive

charges of the K+. Malate concentrations vary, and it is lower in shrunken cells than in

swollen cells. It is believed that malate is synthesized when there is not enough Cl-

present to counteract the charges of the K+.

An experiment with soybeans (Cronland) examined the role of K+ channels

and H+/ATPase in the plasma membrane in paraheliotropic movement. This was done by

treating the pulvini with the K+ channel blocker tetraethylammonium chloride (TEA), the

H+/ATPase activator fusicoccin, and the H+/ATPase inhibitors vanadate and erythrosin-B. In

all cases the leaf movements of the plant were inhibited, leading to the hypothesis that

the directional light results in an influx of K+ into the flexor cells from the apoplast

and an efflux of K+ from the extensor cells into the apoplast, and these movements are

driven by H+/ATPase pumps. This combined reaction results in the elevation of the leaflet

towards the light.

In this study, the diheliotropic movements of C. pallida are examined.

The purpose of this experiment is to determine which ions, if any, are used by pulvini

cells of Crotalaria pallida Aiton to control the uptake of water, thereby affecting

diheliotropic movement. As mentioned before, most studies investigating the mechanisms of

leaf movement have been performed on nyctinastic plants. These plants respond to light and

dark changes, not direction or intensity of a light stimulus. Therefore, it is of interest

to learn whether the same principles can be applied to diheliotropic movement.

Different inhibitors at varying concentrations will be injected

individually into the pulvinus of C. pallida, and the suntracking ability of the plant

will then be measured. Tetraethylammonium (TEA), a K+ channel blocker will be added to

test whether K+ is involved in suntracking. Likewise, , a Cl- channel blocker will be

added to determine if Cl- is used. Vanadate, a H+/ATPase inhibitor, will determine if

hydrogen ions are pumped across the plasma membrane, causing a hyperpolarization of the

membrane. Fusicoccin, a H+/ATPase activator will also be tested .