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Light or Ethylene Treatments Induce Transverse Cell Enlargement in Etiolated Maize Mesocotyls

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ABSTRACT

Dark-grown maize seedlings (hybrid WF-9 × 38-11) exposed for 1 or more hours to white light and then returned to darkness developed mesocotyls with enlarged apical diameters. This swelling response was an all-or-none response, and the fraction of the seedling population that showed the response depended on seedling age at irradiation. Irradiation of the coleoptile alone was nearly as effective in causing this response as was irradiation of the nodal region of the epicotyl, but irradiation of the mesocotyl base was ineffective. Removal of the coleoptile prior to irradiation did not prevent the formation of the light-induced swelling. Exogenously applied C_2H_4 (10 microliters per liter) for 24 hours in dark also induced swelling of the mesocotyl. The swelling induced in the intact seedlings was localized in the apical mesocotyl tissues with either light or C_2H_4 treatment, and maximal response to both treatments occurred with 3- to 4-day-old seedlings. Swelling of the mesocotyl was the result of transverse cell enlargement, not increase of cell numbers. The evidence suggests that light and C_2H_4 induce mesocotyl swelling in intact maize shoots by a common mechanism.

Mesocotyls of dark-grown maize seedlings exhibit two photomorphogenic responses following a light interruption of the dark growth: inhibition of mesocotyl elongation (4, 13, 19, 26, 27) and a transverse enlargement of the mesocotyl apical diameter (11, 12). Both responses occur primarily as changes in growth pattern of the tissues in the meristematic and cell enlarging regions of the mesocotyl near the coleoptilar node (4, 12, 13, 21), although some inhibition of elongation can occur in more mature tissues (4, 12). Inhibition of mesocotyl elongation can be induced by short-time exposures (<5 min) to red light of low total fluence, and the induction exhibits some red/far red reversibility (13). The transverse enlargement (swelling) requires white-light exposures of approximately 10-fold greater fluence, but the response can be induced in a small portion of the seedling population by 30-min exposures (12). The swelling response seems to be an all-or-none response and no red/far red reversibility of this light effect has been observed (S. O. Duke, personal communication).

Increase in stem diameter is one response of plants to exogenously applied C_2H_4 (8). Although a report by Malloch and Osborne (22) indicated that maize mesocotyl tissue did not swell when exposed to C_2H_4, other studies indicate that maize mesocotyl swelling does occur in response to exogenous C_2H_4 (9, 24). These observations suggested to us that the swelling which is induced by light in the growing region of maize mesocotyls may be identical to that produced by C_2H_4. We report here evidence that the mesocotyl swellings induced by light and by exogenously applied C_2H_4 are similar, morphologically and anatomically, and that the light and C_2H_4 effects occur during similar stages of seedling development.

MATERIALS AND METHODS

Seeds of *Zea mays* L. hybrid WF-9 × 38-11 (Agricultural Alumni Seed Improvement Association, Inc., West Lafayette, IN) were imbibed 50 to 60 min by alternately flooding and draining with tap water at 25 ± 2 C. Following imbibition, the seeds were placed between vertical pads of absorbent paper and irrigated by capillary flow from below with 2 mM $CaSO_4$ solution. Seeds thus were germinated and seedlings were grown in the dark at 27 ± 1 C and 80 to 100% RH prior to light or C_2H_4 treatment. The growth chamber was provided with flow-through aeration with a stream of water-saturated air. The 24-h dark period that followed the light treatments was under similar conditions. Manipulations, including transport to and from the treatment areas, were conducted in the dark or under dim green light. Any exposure to green light during manipulation was brief and experienced by seedlings of all treatment groups of an experiment.

Light Sources and Methods of Irradiation. Characteristics of the green light source used for manipulations have been described elsewhere (13). Fluence rates from this source at the plane of manipulations were no greater than 50 mJ m^{-2} s^{-1} as measured with a YSI-Kettering model 65 radiometer. Source of white light was provided by cool-white fluorescent lamp equipped with a diffusing filter of tracing Mylar. With this source, seedlings were irradiated from above at a fluence rate of 1.2 J m^{-2} s^{-1} at the plane of the germinated seeds. Lateral irradiation with white light was provided for one set of experiments by unfiltered light from two cool-white fluorescent lamps arranged in parallel and horizontally at 20 cm on either side of a row of seedlings. Each lamp provided 5 J m^{-2} s^{-1} at the plane of the seedlings.

C_2H_4 Treatments. Dark-grown seedlings were placed in a closed, 12-liter glass container and C_2H_4 gas was injected via syringe through the serum vial stopper that closed the single port into the system. A measured amount of C_2H_4 was injected to give an C_2H_4 concentration of 10 μl l^{-1} in the enclosed atmosphere. Subsequent exposure was continued for 24 h in the dark at 26 ± 2 C. Control groups of seedlings were exposed to similar conditions in a similar volume of enclosed air without added C_2H_4.

Assay of Responses. Mesocotyl diameters were measured to the nearest 0.1 mm using a dissection microscope fitted with an ocular micrometer. For those seedlings showing a tranvserve enlargement of the mesocotyl, the diameters were measured 2 mm to the proximal side and 2 mm to the distal side of the point where swelling had initiated. For comparative purposes, the control-seedling mesocotyl diameters were measured approximately 1.5 mm from the coleoptilar node. Cortical cell numbers and diameters were estimated from magnified photomicrographs of tissue

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sections. Mesocotyl segments 6 mm in length and centered on the point of initial swelling or similar segments from comparable regions of control mesocotyls were fixed in formalin-acetic acid-alcohol, dehydrated using *t*-butyl alcohol series (20), embedded in paraffin, and cross-sectioned. The 10- to 12-μm sections were mounted on slides, stained with toluidine blue (6), and then photographed. On each of the photomicrographs four radial lines were drawn from the anatomical center to the epidermis in four randomly chosen sectors of the tissue cross-section image. The number of cells intersected by each transect line gave an estimate of cell number per unit length, and the mean cell diameters then were estimated from the cell number and the line length from the endodermis to epidermis.

RESULTS

Exposure of 3-day-old, dark-grown maize seedlings to 3 h white light induced transverse enlargement (swelling) and inhibition of elongation in the mesocotyl region that developed during a 24-h dark period following the irradiation. Exposure of 3-day-old, dark-grown seedlings to 10 μl l^{-1} C_2H_4 for 24 h in dark produced similar responses (Fig. 1). The fraction of any seedling population that exhibited the swelling response to light depended upon the developmental age (time after imbibition) of the seedlings (Table I). Although seedlings younger than 2.5 days of age at time of irradiation sometimes developed increased mesocotyl diameters, the mesocotyls in these seedlings were too undeveloped at time of light treatment to exhibit an enlarged/nonenlarged transition point (as shown at E in Fig. 1). The age of maximum sensitivity corresponded with the log phase of mesocotyl dark-growth in length (unpublished data). Exposure of dark-grown seedlings of various ages to 10 μl l^{-1} C_2H_4 for 24 h in dark induced transverse enlargement in mesocotyls of all seedlings. However, distinct apical swelling of the mesocotyls occurred only in seedlings 3 to 4 days old, with maximal response in 3.5-day-old seedlings (Table II).

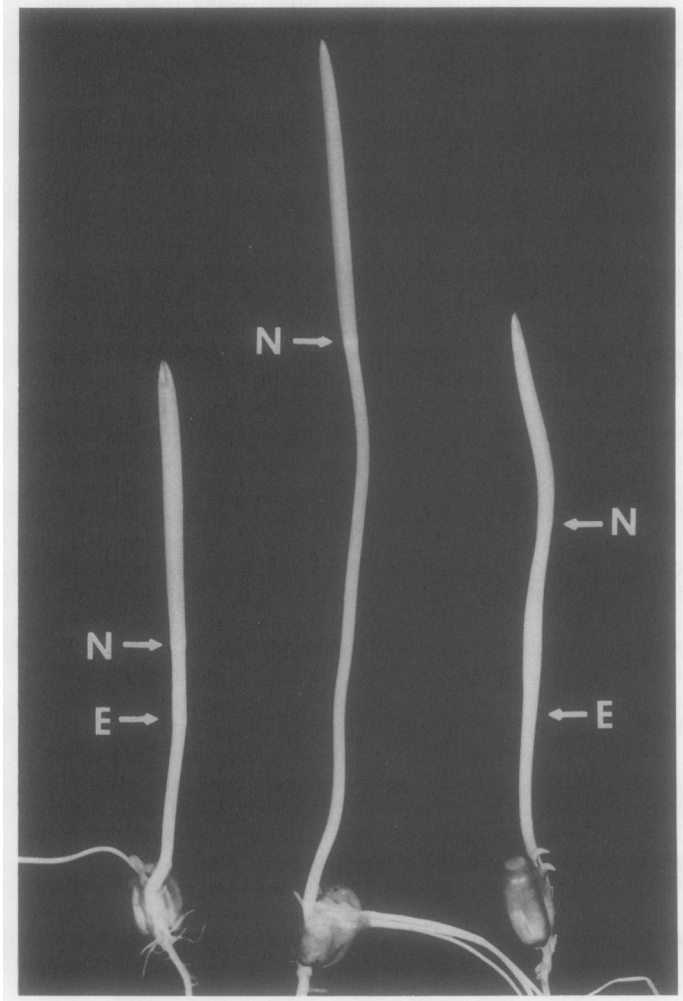

FIG. 1. White light-irradiated (left), dark-grown, untreated (center), and C_2H_4-treated (right) seedlings of similar ages. Transverse enlargement extends between point E and the coleoptilar node (N) of the light-treated and C_2H_4-treated seedlings. All seedlings were dark-grown 3 days prior to the start of treatments. Light-treated seedling was exposed 3 h to 1.2 J m^{-2} s^{-1} and then returned to darkness for 24 h. The C_2H_4-treated seedling was exposed to 10 μl l^{-1} C_2H_4 for 24 h in the dark.

Microscopic examination of tissue sections from enlarged and nonenlarged regions of the mesocotyls of treated and untreated 3-day-old seedlings revealed no apparent differences in tissue patterns or in diameters of the vascular cylinders. There was, however, a significant radial expansion of the cortical tissue (Table III). Light or C_2H_4 exposure induced significant increases in cortical cell lateral dimensions but the number of cells along a transverse dimension was not changed.

In all of the preceding experiments, the entire shoot of the seedlings was exposed to the irradiations. Therefore, selective shading was used to irradiate 1-cm areas laterally at one of three locations: at the coleoptile tip, centered on the coleoptilar node, or at the mesocotyl base. Results of these experiments (Table IV) indicate that the base of 3-day-old, dark-grown maize shoots was least sensitive to light-induction of the mesocotyl swelling response. Irradiation of the coleoptilar tip, however, was nearly as effective as was irradiation of the nodal region for inducing the swelling response.

Removal of the coleoptile (and enclosed undeveloped leaves) from 3-day-old, dark-grown seedlings just prior to irradiation with white light did not alter the light-induced apical enlargement of the mesocotyl (Table V). Although there was increased diameter growth in mesocotyls of seedlings maintained in dark after the coleoptiles were removed, the mesocotyls showed no apical swell-

Table I. *White Light-induced Transverse Enlargement by Maize Seedling Mesocotyls of Various Ages*

Groups of 14 to 22 dark-grown seedlings were irradiated 3 h at 1.2 J m^{-2} s^{-1} and then returned to dark for 24 h prior to assay for response.

Seedling Age	Population with Mesocotyl Enlarged
days	*%*
2.5	37.5
3.0	95.5
3.5	94.7
4.0	20.0

Table II. *C_2H_4-induced Transverse Enlargement in Dark-grown Maize Seedling Mesocotyls of Various Ages*

Groups of 15 to 41 dark-grown seedlings were exposed to 10 μl l^{-1} C_2H_4 for 24 h in dark. Each diameter is the mean response ± SE.

Seedling Age	C_2H_4-treated Mesocotyls		Nontreated Mesocotyl Diameter
	Enlarged diameter	Nonenlarged diameter	
days	*mm*		*mm*
2	2.40 ± 0.03[a]		1.78 ± 0.02
3	2.27 ± 0.04	1.94 ± 0.03	1.84 ± 0.02
3.5	2.76 ± 0.10	2.46 ± 0.02	2.06 ± 0.03
4	2.49 ± 0.02	2.27 ± 0.02	2.00 ± 0.02

[a] Mesocotyls were of uniform diameter throughout their lengths.

Table III. *Mesocotyl Cortical Cell Dimensions of White-light-treated, C_2H_4-treated, and Dark Control Seedlings*

Measurements were made on cross-sections of mesocotyl tissue from 3-day-old, dark-grown seedlings that had been irradiated 3 h with 1.2 J m^{-2} s^{-1} white light and then returned to dark for 24 h. Similar measurements were made on seedlings exposed to 10 $\mu l\ l^{-1}$ C_2H_4 for 24 h in dark. Cells were counted along radial transects between endodermis and epidermis. Values are means ± SD of four measurements on duplicate samples from each treatment.

Treatment	Cell Size	Cell Number/ Transect
	mm	
Dark control	0.036 ± 0.003	17.0 ± 2.4
C_2H_4, enlarged region	0.045 ± 0.002	17.3 ± 1.9
C_2H_4, nonenlarged region	0.039 ± 0.002	16.8 ± 1.3
Irradiated, enlarged region	0.042 ± 0.001	16.3 ± 1.5
Irradiated, nonenlarged region	0.034 ± 0.002	16.8 ± 1.5

Table IV. *Transverse Enlargement of Dark-grown Maize Mesocotyls Induced by White-light Irradiation of Different Shoot Regions*

Tip, nodal, and basal regions of 3-day-old, dark-grown seedling shoots were irradiated with 1 h white light (5 J m^{-2} s^{-1}) from each side by shading appropriate regions of the shoots and then returned to dark for 24 h prior to assay of the response. Mean diameters ± SE were derived from measurements of replicated groups of seedlings in each treatment with 11 to 18 seedlings/group.

Region	Irradiated Seedlings		Dark Control Diameter
	Enlarged diameter	Nonenlarged diameter	
	mm		*mm*
Tip	2.13 ± 0.03	1.87 ± 0.06	2.04 ± 0.02
Node	2.24 ± 0.04	2.00 ± 0.03	2.04 ± 0.02
Base[a]	2.07 ± 0.06	1.93 ± 0.03	2.00 ± 0.04

[a] Diameters of the irradiated seedling mesocotyls are based only on those that developed a transverse enlargement, *i.e.* 73% of the population. All of the irradiated seedlings in other treatments developed enlarged mesocotyls.

Table V. *Effect of Coleoptile Removal on Light-induced Transverse Enlargement Response of Dark-grown Maize Mesocotyls*

All tissues distal to the coleoptilar node were removed just prior to irradiation from above with 1 h white light at 1.2 J m^{-2} s^{-1}. Mean values ± SE were derived from the measurements of replicated groups of seedlings in each treatment with 10 to 27 seedlings/group.

Treatment	Enlarged Diameter	Nonenlarged Diameter
	mm	
Irradiated, coleoptile removed	2.27 ± 0.02	2.07 ± 0.02
Dark, coleoptile removed[a]		2.20 ± 0.02
Irradiated, coleoptile intact	2.24 ± 0.02	2.03 ± 0.01
Dark, coleoptile intact[a]		1.97 ± 0.02

[a] Mesocotyls in these treatments showed no apparent enlargement in the apical region compared to the basal region.

ing, *i.e.* the diameter increase was uniform along the entire length of the mesocotyl.

DISCUSSION

The swelling of maize mesocotyls in response to light or to C_2H_4 treatment is expressed in the apical region (Fig. 1) where cell divisions and early cell enlargement occur (21). Also, the tissues are maximally sensitive to induction during the developmental stage of maximal dark growth rate of the mesocotyl (Tables I and II). These observations indicate that swelling could derive from reoriented cell division to increase the number of cells laterally or from an increase in radial expansion of the individual daughter cells of normal meristematic activity. Reports of several investigations indicate, however, that light inhibition of *Avena* mesocotyl growth is apparently biphasic: at low irradiances, cell division is inhibited (5, 7, 17, 23); at greater irradiances, cell elongation is inhibited (5, 17). It seems more likely that the high-irradiance-induced swelling should result from cell expansion, and not from cell divisions. Results of our study confirm this hypothesis: light-induced swelling was not caused by reoriented cell divisions but by cortical cell enlargement in a radial dimension (Table III).

C_2H_4 inhibition of longitudinal growth and induction of swelling in etiolated stems has been reported for *Pisum* (1, 3, 8, 14, 22, 24), *Avena* (22, 24), and *Zea* (9, 24), although Malloch and Osborne (22) reported that only inhibition of mesocotyl longitudinal growth in *Zea* was caused by C_2H_4. C_2H_4 was also reported to inhibit cell division and to delay cellular differentiation in *Pisum* stems (1, 2, 8), but evidence for similar effects in *Zea* mesocotyls is lacking. In our studies, swelling of dark grown maize mesocotyls was induced by exogenously applied C_2H_4 (Table II). The mesocotyl swelling resulted from increased transverse cell enlargement in the cortical tissues of the mesocotyl similar to the swelling induced by light (Table III). However, the transition from unaffected tissue to affected tissue was not as distinct in C_2H_4-induced swelling as in light-induced swelling (Fig. 1), and the pattern may be an expression of the commonly observed lag period in the development of C_2H_4-induced responses (8). The absence of C_2H_4-induced swelling in the maize cultivar used by Malloch and Osborne (22) suggests that Golden Bantam, the inbred sugary endosperm variety used by these workers, is less sensitive to C_2H_4-induced swelling. In contrast, the hybrid dent starchy endosperm maize that we used showed highly reproducible mesocotyl swelling in response to exogenously applied C_2H_4 gas.

Inasmuch as swelling of the mesocotyl could be induced by irradiation of the coleoptile (Table IV), involvement of a growth effector transfer between site of light reception and site of response is indicated. A similar phenomenon had been suggested for light-induced inhibition of maize mesocotyl longitudinal growth (13, 19). Indeed, light may cause mesocotyl growth inhibition by altering auxin flow from the coleoptilar tip since exogenously applied auxin reversed the light-induced inhibition of elongation in maize mesocotyl segments (26). C_2H_4 also inhibits polar auxin transport in maize coleoptiles and in etiolated pea epicotyls (9), and applied auxin reverses the C_2H_4 growth inhibition in *Pisum* epicotyls (3) and in etiolated *Zea* shoots (22). In our experiments, both light and C_2H_4 inhibited maize mesocotyl longitudinal growth (Fig. 1), suggesting that auxin transport from the coleoptile may have been inhibited by both treatments. However, results of our coleoptile removal experiments (Table V) indicate that interruption of auxin transport from the coleoptile did not alter the light-induced swelling of the mesocotyls. Indeed, coleoptile removal induced mesocotyl diameter increase in absence of light, but the expansion was uniform and not restricted to the apical region of the mesocotyl.

Stimulations of C_2H_4 production in etiolated stem tissues by auxin (8, 15) and by light (10) have been reported. However, light inhibition of C_2H_4 production by etiolated tissues has also been reported (8, 16, 18, 25). The apparently inconsistent effect of light on C_2H_4 production in etiolated plant tissues indicates that there probably is no direct causal relationship between light and C_2H_4 in control of maize mesocotyl growth. Although our results show that light and C_2H_4 treatments produced similar morphological responses in etiolated maize mesocotyls, they provide no evidence

that the light-induced swelling response is mediated through C_2H_4. Our study does suggest, however, that light and C_2H_4 both induce mesocotyl swelling via a common mechanism that may involve inhibition of polar auxin transport as well as inhibition of cell division in the meristem of the mesocotyl.

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