Short Communication

Can Lateral Redistribution of Auxin Account for Phototropism of Maize Coleoptiles?1, 2

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ABSTRACT

Elongation growth of intact, red-light grown maize (Zea mays L.) coleoptiles was studied by applying a small spot of an indole acetic acid (IAA)-lanolin mixture to the coleoptile tip. We report that: (a) endogenous auxin is limiting for growth, (b) an approximately linear relation holds between auxin concentration and growth rate over a range which spans those rates occurring in phototropism, and (c) an auxin gradient established at the coleoptile tip is well sustained during its basipetal transport. We argue that the growth differential underlying coleoptile phototropism (first-positive curvature) can be explained by redistribution of auxin at the coleoptile tip.

The Cholodny-Went theory of tropisms states that the tropic bending of plants results from an altered flow of a growth-regulating substance in the tissues of the responding organ (21). This theory has been applied extensively to phototropism of grass coleoptiles (mainly oat and maize). So far as first-positive curvature is concerned, the following features have become apparent. Diffusible auxin levels rise on the shaded side and fall on the illuminated side, while the total auxin level stays constant (2). Lateral redistribution at the coleoptile tip causes the change in auxin levels (2, 5, 14, 15). These results with auxin were in accord with early observations on phototropism: the sensitivity to light is located at the most apical portion of the coleoptile (11), and the onset of tropic bending begins at the tip and moves basipetally (3).

Furthermore, recent studies with maize have shown phototropic growth responses consistent with the reported auxin movement: growth depression on the irradiated side and concomitant stimulation on the shaded side, and basipetal migration of the onset of each response (9). The onsets of these growth responses migrate at the same rate as does the onset of growth stimulation caused by exogenous, apical application of auxin (1). The sensitivity to light has also been shown to be located at the tip (9).

Some questions must still be answered before a lateral redistribution of auxin can be thought to account for coleoptile phototropism. Two outstanding ones are whether the endogenous auxin concentration is limiting for growth of the coleoptile; and if so, whether the small change in auxin concentration that would occur in a process of redistribution is sufficient to cause the observed changes in growth rate, on both illuminated and shaded sides of the coleoptile (4, 19). Additionally, a third problem is whether an auxin gradient established at the coleoptile tip can be sustained during its basipetal transport. Although it has been little appreciated, this gradient must be sustained if apical auxin redistribution can be thought to cause the observed phototropic growth redistribution throughout the long axis of the coleoptile. We present results aimed at answering these questions.

MATERIALS AND METHODS

Seedlings of maize (Zea mays, L. cv T929) were grown as previously described (9). Kernels were soaked in running water for 4 h and then sown on wet, absorbent paper. Two d later, germinated seedlings were transplanted into trays filled with wet vermiculite, 10 to 12 seedlings per tray. A clear Plexiglas cover rested on the vermiculite, enclosing the seedlings. Experiments were done on the day after transplantation, at which time coleoptiles ranged from 2 to 4 cm in height. Experimentation and seedling growth were carried out in a light-tight room. The temperature ranged between 23 and 25°C. Seedlings were exposed from above to continuous, dim red light (0.2 μmol m⁻² s⁻¹) throughout their growth and experimental treatment. This light was sufficient for photography, and was the only light the seedlings received (except, as described below, for inducing phototropism).

Pastes of IAA and lanolin were prepared by adding a known weight of solid IAA (Sigma Chemical Co.) to a known weight of lanolin (anhydrous, J.T. Baker Chemical Co.). Concentrations are referred to as mg IAA per g lanolin, with ‘zero’ referring to lanolin only. These mixtures, including control lanolin, were stirred vigorously for several minutes, and were stored at 4°C. A small amount was applied to coleoptiles by bringing the paste that coated a thin glass rod (about 0.3 mm diameter) into momentary contact with the desired place on the plant; a small spot of the paste remained on the plant surface. In this manner, a tray of 12 plants could be treated in about 2 min. Application on a balance indicated that such a spot weighed approximately 50 μg (range, ±20 μg). Unless otherwise noted, application was made at about 2 mm from the apex, and in the plane of the vascular bundles.

To induce phototropic curvature, a 30 s pulse of unilateral blue light was given by means of a Kodak 600H slide projector, as previously described (9). The direction of the light was parallel to the plane of the vascular bundles. The fluence, 5 μmol m⁻², known to cause optimal first-positive curvature (9) was used.

Curvature was defined as the angle between lines tangent to the coleoptile long axis at tip and node, and was measured from
photocopied images of coleoptiles (9). Growth was measured as follows: plants were marked with India ink (or in some experiments, an aqueous nigrosin solution, with 0.1% Triton X-100) using two threads affixed on a frame. The frame determined the distance from the apex to the first mark. Trays of marked plants were photographed at known times through a 35 mm camera, using red-sensitive film (Kodak Technical Pan 2415), and the lengths of the marked zones were measured as described before (9). Relative growth rates were calculated using:

$$\text{Relative rate} = \frac{1}{\Delta t} \ln \left( \frac{L_f}{L_i} \right)$$

where $L_f$ and $L_i$ are the lengths of a zone at the end and beginning of a time interval equal to $\Delta t$. When expressed as a percentage, the relative rate is multiplied by 100.

RESULTS

Spot application of IAA-lanolin paste to a single side of the tip caused curvature of intact coleoptiles away from the site of application (Fig. 1A). As the dose was increased an increase in response resulted, with the maximum, $-25^\circ$, occurring at about 10 mg g$^{-1}$. No further promotion (or inhibition) of curvature was seen, even at doses as high as 100 mg g$^{-1}$ (not shown). Coleoptiles curving in response to the IAA application were similar in shape to those curving for first-positive phototropic stimulation.

Elongation growth of intact coleoptiles was also studied following spot application of IAA-lanolin paste on two sides of the tip. Figure 1B shows results obtained with a measured zone located between 3 and 10 mm below the apex. Growth of the coleoptiles was stimulated by exogenous IAA, and most of the stimulation occurred over a narrow concentration range in which the sensitivity to IAA was roughly linear. It appeared that curvature increased more gradually with IAA concentration than did growth rate. This difference may be accounted for by the difference in the actual amount of IAA applied for each concentration (the IAA amount in the growth experiment was double that in the curvature experiment) or by the difference in measured zones of the coleoptile (a 7 mm zone versus the entire responding zone for growth and curvature measurements, respectively). For any kind of possibility is less likely because of results presented below.

Next, we investigated the growth occurring at two opposite sides of the coleoptile onto one of which a saturating dose of IAA (30 mg g$^{-1}$) had been applied. Control measurements were obtained on the same occasion from lanolin treated coleoptiles and from ones to which the same IAA dose had been applied bilaterally. The side which received unilateral IAA was stimulated (Fig. 2, A) to about the same extent as observed for bilateral treatment (A). The opposite side (B) was not stimulated above lanolin-treated controls (C). This experiment has been repeated twice with the same results. It has also been ascertained that growth of lanolin-treated plants did not differ from growth of entirely untreated plants (not shown). The result indicates that the growth stimulation by unilaterally applied IAA is largely restricted to the IAA-applied side.

We then examined the effect of auxin applied to phototropically stimulated coleoptiles. If the growth-differential causing bending in these plants is brought about by redistribution of auxin at the extreme tip (most apical half mm) (2), then, since the growth stimulation by IAA is restricted to the side of its application, subapical application of IAA to the irradiated side should effectively counteract phototropic bending. Figure 3 shows results obtained when, immediately following blue light irradiation, spot IAA application was made to the irradiated side of the tip; and growth and curvature measured over the subsequent 100 min interval. Applied auxin markedly reduced curvature in these plants. Curvature was eliminated by roughly 2.5 mg g$^{-1}$, hence at this concentration the irradiated side grew at the same rate as the shaded side. The growth rate observed, 3.5% h$^{-1}$, agrees with prior, direct measurements of the shaded side growth rate in this material, under these conditions (9). At greater IAA doses, plants bent away from the light (by 10°, maximally).

Elongation can be seen to be stimulated in parallel with the reduction of curvature (Fig. 3). Growth rate increases roughly linearly with IAA concentration, from the baseline irradiated-side rate up to, and perhaps a little beyond, the shaded-side rate (point of zero curvature). The growth rate observed for nonirradiated controls (C, Fig. 3), lies in the range of linear sensitivity to IAA concentration, and corresponds to application on the illuminated side of just over 1 mg g$^{-1}$. Application of twice this amount eliminates curvature. This, doubling the exogenous IAA concentration increases the growth rate of coleoptiles to a similar level as occurs on the shaded side during phototropic bending. The results indicate that, if auxin redistribution is responsible for
The growth-rate measurements in Figures 1B and 3 underestimate the magnitude of stimulation because they represent the average of the growth rate over an interval which includes the time lag before the onset of stimulation. The lag observed at the measured zone is about 30 min (30% of total interval) (1) and measurements over shorter intervals do show greater total growth stimulation than presented in Figures 1B and 3 (compare with Fig. 2). Second, the growth enhancement of intact coleoptiles by auxin appears to be a linear function of concentration over a certain range. This range may entirely cover the auxin gradient established by optimal first-positive stimulation (Fig. 3). We have also shown that an auxin gradient established at the coleoptile tip is sustained during its basipetal transport. This follows from the result that unilaterally applied IAA, while being able to induce substantial growth stimulation on the IAA-applied side, does not stimulate growth on the opposite side (Fig. 2). All of the results support the hypothesis that a lateral transport of auxin at the tip accounts for first-positive, coleoptile phototropism.

The effective auxin concentrations used here are quite high compared, for example, to those effective in the classical *Avena* curvature test, but they do agree with those used by others who have reported growth stimulation with similar preparations of IAA in lanolin (17, 20). The need to use very high concentrations of IAA in lanolin may be accounted for by the application of IAA as a mixture of its powder with lanolin rather than as an aqueous solution, and onto an intact epidermis rather than onto a cut surface. It may be pointed out that our application of 1 mg g⁻¹ equals 50 ng of IAA applied per plant.

The saturation of the growth response shown in Figures 1B and 3 does not necessarily reflect saturation for IAA of growth-stimulating reactions. Instead, the saturation could be imposed by the process of IAA uptake into the tissue or of its polar transport within the tissue. Saturation of IAA solubility in lanolin might also account for the apparent saturation of growth stimulation. The possibility that IAA is capable of stimulating the growth of intact coleoptiles beyond the saturation value observed here is open for further study.

It has been argued that the rather small auxin gradients reported with phototropic (or gravitropic) stimulation are not large enough to account for the differential growth underlying these responses (4, 19). This argument has been based on results showing that exogenous auxin stimulates growth as a function of the logarithm of its concentration. This logarithmic nature of the dose-response curve, however, has been found largely in experiments on isolated segments that are incubated in solutions. In agreement with the linear dependence on auxin concentration for growth reported here, experiments in which IAA is applied to the apical cut surface of decapitated plants often show considerable growth stimulation taking place over a narrow concentration range, and having an approximately linear dependence on hormone concentration (7, 8, 18). In an extensive review of *Avena* curvature test experiments, Nissen (13) has demonstrated that most are best fit by a hyperbolic function; however, his analysis considers the entire range of tested auxin concentrations.

An effective system of redistribution only requires a modest range (much less than 10-fold) over which growth has a linear dependence on hormone concentration.

Measurements of the growth of phototropically binding coleoptiles, made under the optimal first-positive stimulus, have shown cessation of growth on the irradiated side and a near doubling on the shaded side (1, 9). Those results indicate that, if the relation between auxin concentration and growth rate is in fact linear, as reported here, then auxin concentrations corresponding to growth rates should be found; namely, small on the irradiated side, and twice as large on the shaded side, as compared to the nonirradiated controls. Measurements of the auxin gradient between irradiated and shaded sides have, in general, only

**DISCUSSION**

The results give answers to the problems posed in the Introduction. First, endogenous auxin is limiting for growth (Fig. 1).
found a ratio of 2:1 (19). However, these measurements have compared auxin levels between coleoptile halves, and therefore average out any gradient of auxin within each half. Experiments designed to show the spatial distribution of auxin in coleoptiles will be required to resolve this point, and to confirm directly the causal involvement of auxin in phototropism argued for here. However, an auxin gradient within each coleoptile half is a reasonable expectation. Investigations on the spatial distribution of labeled IAA during positive gravitropism of dicot shoots have shown the IAA gradient between upper and lower epidermis to be double (12) and quadruple (10) the gradient between upper and lower halves. There is evidence on theoretical (6) and experimental (16) grounds for supposing that the epidermis limits elongation and is the primary target for the growth promoting action of IAA. Thus, the gradient of auxin concentration predicted from the growth measurements may be found in a comparison of the epidermis from irradiated and shaded sides of the coleoptile.

LITERATURE CITED

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