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Red-light-regulated growth

Changes in the abundance of indoleacetic acid in the maize (*Zea mays* L.) mesocotyl

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Abstract. The etiolated maize (*Zea mays* L.) shoot has served as a model system to study red light (R)-regulated growth. Previous studies have shown that R inhibition of maize mesocotyl elongation involves a change in the auxin economy. Shown here is that R causes an increased tension in the epidermis relative to the inner tissue indicating that the growth of the epidermis is preferentially inhibited by R irradiation. This observation, taken together with previous indirect estimates of auxin within the epidermis, has prompted the hypothesis that R mediates the inhibition of mesocotyl elongation by preferentially decreasing auxin in the epidermis, a tissue which constrains the growth of the organ. We tested this hypothesis using gas chromatography-selected ion monitoring-mass spectrometry analysis of free indole-3-acetic acid (IAA) levels in both the apical 1 cm of the mesocotyl and the corresponding epidermis of etiolated and 4-h, R-irradiated seedlings. Red light irradiation caused a 1.4-fold reduction in free IAA within the whole section of the apical mesocotyl. However, within the peeled mesocotyl epidermis, R irradiation caused at least a 1.9-fold reduction in free IAA. To determine if the nearly twofold decrease in epidermal auxin occurring after R is physiologically significant, IAA was differentially applied to opposite sides of shoots. A twofold difference in IAA application rate caused asymmetrical growth. Thus, the twofold R-induced decrease in free IAA level in the epidermis, a difference sufficient to affect growth, and the rapid R-induced change in growth rate in the epidermis are consistent with the hypothesis that R causes growth of the mesocotyl to decrease by preferentially regulating the free IAA level in the mesocotyl epidermis.

Key words: Auxin – Growth – Epidermis – Indole-3-acetic acid – Red light – *Zea* (mesocotyl)

Introduction

Several research groups have hypothesized that inner tissues provide the driving force for growth, whereas the peripheral cell layers, most importantly the epidermis, determine the rate of organ elongation (Kraus 1867; Tanimoto and Masuda 1971; Masuda and Yamamoto 1972; Brummel and Hall 1980; Pope 1982; Masuda 1985; Kutschera and Briggs 1987; Kutschera 1992). To illustrate this, consider the constraint upon the movement of a fluid flowing through a cylinder whereby the greatest restrictive force is found along the cylinder wall and has a profound effect on the fluid flow. This example may describe the distribution of forces on walls of cells in a cylindrical, elongating shoot when wall loosening of the epidermal cells proceeds at a slower rate than that of walls of cells in the inner tissues. More specifically, the outer walls of epidermal cells should bear the greatest tensile force (Bret-Harte et al. 1991) and one finds this specialized function illustrated in its unique architecture. Outer epidermal cell walls of a stem or coleoptile are multilayered, of a different structure, and 6–20 times thicker than those of the inner tissues (Anderson 1928; Muhlethaler 1959; Chafe and Wardrop 1972).

In accordance, it is generally agreed that the elongation of the outer epidermal cell wall constrains the overall growth of the organ. But how could epidermal cell elongation be controlled differently from the elongation of cells within the inner tissues? One controversial hypothesis is that the shoot epidermis has a different sensitivity to auxins (Thimann and Schneider 1938; Masuda and Yamamoto 1972; Brummel and Hall 1985; Kutschera et al. 1987; Dietz et al. 1990; Cleland 1991; Hoson et al. 1992; Rayle et al. 1991). This hypothesis assumes that auxin concentrations are the same in epidermal and inner cells. An alternative hypothesis is

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Abbreviations: GC-SIM-MS = gas chromatography-selected ion monitoring-mass spectrometry analysis; R = red light

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that epidermal cells contain a growth-limiting amount of auxin (Jones et al. 1991; Behringer and Davies 1992; Jones 1992) and assumes that cells throughout the shoot are equally sensitive to auxin or at least that the epidermis is no more sensitive to auxin than inner tissues. This latter hypothesis is explored further here using the classical maize mesocotyl which has a well-defined red light (R)-regulation of its elongation.

In 1936, Van Overbeek demonstrated that exposure to R decreased the growth substance content in *Avena* tips. The growth of the mesocotyl is dependent on the coleoptile-derived growth-promoting hormone, indole-3-acetic acid (IAA; Iino and Carr 1982). In addition, it has been repeatedly demonstrated that R decreases the level of diffusible IAA within the mesocotyl and hypothesized that this decrease in IAA causes the decrease in growth even though a strict correlation between auxin levels and growth rates had not been determined (Briggs 1963; Huisinga 1976; Vanderhoef et al. 1979; Iino 1982; Iino and Carr 1982; Behringer and Davies 1992). These workers and others have countered the inhibiting effect of light on internode elongation with applications of exogenous IAA (Inge and Loomis 1937; O'Brien et al. 1985) and have proposed a simple model whereby elongation of the mesocotyl is dependent on a constant supply of IAA from the coleoptile tip.

Jones and coworkers (1991) compared the level of free IAA with growth rates using highly sensitive techniques to measure both parameters in order to determine the robustness of the correlation described above. They noted that the relative decrease in IAA level in the growth zone of the mesocotyl was less than the R-induced decrease in its growth rate. This lack of correlation between IAA level and growth rate disproved the simple model and called for modification and retesting. The new hypothesis is that there is a growth-limiting pool of auxin within the shoot and this was tested by an indirect method. In-situ microautoradiography of sectioned maize shoots was used to show that R-irradiated shoots contain less transported [^{3}H]IAA in the epidermis relative to the dark-grown shoot (Jones et al. 1991), in accord with the new hypothesis and, moreover, assigning the growth-limiting pool of auxin to the epidermis.

Behringer and Davies (1992) used GC-MS to show that R causes a 40% reduction in IAA in the epidermis and an 80% reduction in growth rate of pea epicotyls. The data of Behringer and Davies (1992) are further evidence that the epidermal pool of auxin is both growth-limiting and light-regulated. In similar fashion, we utilize accurate and sensitive techniques to determine if the auxin levels of the epidermis in maize are preferentially light-regulated. We also show for the first time that R causes differential growth rates among shoot tissues and that very small differences in auxin levels between these tissues could establish the different growth rates. Maize was chosen because it is a well-characterized system upon which we can apply these results. The results rekindle the idea that the complexity of tissues within the shoot can not be ignored when trying to understand the mechanisms by which growth of the whole shoot is regulated.

Materials and methods

Plant tissue, chemicals, and light treatments. Maize (*Zea mays* L.) caryopses (hybrid J7710; Jacque Seed Co., Lincoln, Ill., USA) were imbided overnight in tap water, sown on wetted vermiculite, and grown in darkness at 26 °C for 4 d. Continuous R irradiation was provided utilizing a 60-W incandescent bulb mounted in a lamp covered with 1 layer of red acetate (600 nm cutoff, 7.5 mol · $m^{-2} \cdot s^{-1}$). Portions of etiolated seedlings were harvested under dim green light (511 nm peak, 40 nm halfband width, 100 nmol · $m^{-2} \cdot s^{-1}$ at bench level). Through the history of study of maize shoot growth, a number of maize cultivars have been used due to routine discontinuation by seed producers. This slight annoyance to physiologists requires that the growth characteristics of each adopted hybrid be determined because of differences between the cultivars in latent periods and growth rate changes after R irradiation. Figure 1 shows that upon irradiation with continuous R, the growth rate of etiolated maize seedlings, hybrid J7710 used in the present study, rapidly decreases after an approximate 30-min lag, until the growth rate reaches approximately 10% the growth rate of etiolated seedlings. The R-induced decrease in growth rate for this hybrid is complete 4 h after the beginning of irradiation.

Growth measurements. Instantaneous growth rates of the mesocotyl were measured using an angular transducer, as described by Evans (1976). Intact seedlings, planted in vermiculite, were attached to the transducer by means of an insect pin inserted through the coleoptilar node upon which the transducer arm rested. Plants were allowed to equilibrate for a minimum of 1 h before R irradiation as described above. Etiolated shoots were treated in a similar fashion but received no R irradiation.

Analysis of IAA content. The apical 1 cm portions of etiolated or R-irradiated mesocotyls were harvested in dim green light. Epidermal peels were removed manually using forceps, essentially as described by Behringer and Davies (1992). All plant materials were frozen immediately in liquid nitrogen and stored at −80 °C. All extractions and gas chromatography-selected ion monitoring-mass

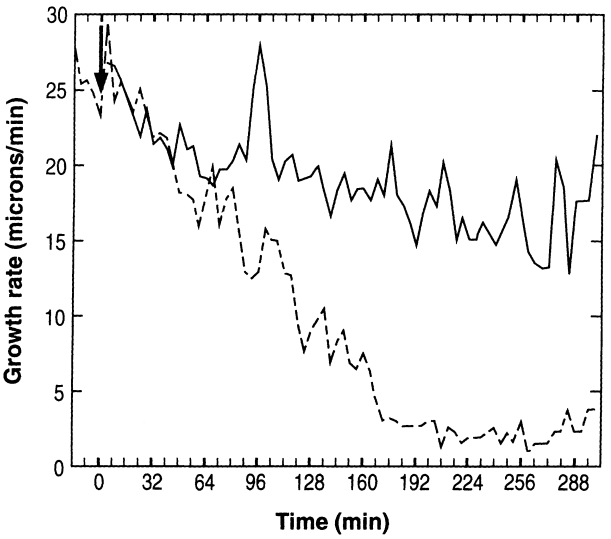

Fig. 1. Growth kinetics of the apical mesocotyl of 4-d-old, etiolated maize seedlings in the dark (*solid line*) or in continuous R (*dashed line*). Growth measurements were performed as described in the *Materials and methods*. Individual seedlings were continuously irradiated with R (660 nm, 7.5 mol · $m^{-2} \cdot s^{-1}$) beginning at the time indicated by the *arrow*. The curves represent the average of five individual experimental data sets. The change in length was calculated from five individual traces at 1-min time intervals and averaged. Standard error of the mean is approx. 2.5 μm · min^{-1}

spectrometry (GC-SIM-MS) analyses were performed under dim incandescent light at USDA-ARS-HCQL, Beltsville, Md., as described by Chen and coworkers (Chen et al. 1988). Each analysis contained at least three replicates and the experiment was repeated twice. Examination of the epidermal peel by bright-field microscopy indicated the thickness to be typically one cell throughout except near the point where the forceps grasped the tissue. However, the inclusion of some subtending cells would tend to decrease the differences in auxin between epidermis and cortex and therefore our values for epidermal auxin concentrations should be considered conservative.

Studies of differential growth. For experiments demonstrating differential growth of mesocotyl tissues, straight, etiolated maize seedlings were harvested after a 4-h exposure to R irradiation or darkness. Mesocotyls were excised 1.8 cm below the node and then longitudinally split from the node down and floated in growth buffer (5 mM potassium phosphate, pH 6; 30 mM sucrose) for 15 min. Differential extension of the split halves was recorded by photography. This experiment was repeated twice with the same result.

For experiments demonstrating asymmetrical growth, straight, etiolated shoots were harvested 2.5 cm below the node and held vertically by inserting the basal 0.5 cm into a 0.5-cm-thick 1% agar slab in the dark within a humid chamber. Nitrocellulose membrane strips (1 mm × 10 mm) were soaked in liquid lanolin containing the indicated concentrations of IAA. The IAA-containing strips were applied to opposite sides of shoots either above or below the node. Lanolin paste on strips was applied alone as a control. After 12 h, the shoots were photographed. This experiment was repeated twice with the same results.

Results and discussion

The instantaneous difference in tension between epidermal and cortical tissues within mesocotyls of R-irradiated (4 h) maize seedlings was shown by longitudinally splitting each mesocotyl and floating the shoot on growth buffer. As shown in Fig. 2, the split halves of mesocotyls of etiolated seedlings were straight, whereas mesocotyls exposed to R-irradiation and longitudinally split showed dramatic outward curvature illustrating the differential tension between outer and inner tissues. Since the overall growth rate of the mesocotyl has decreased (Fig. 1), the outward curvature shown in Fig. 2 reveals increased tension by the epidermis rather than released tension by the inner tissues.

Free IAA levels in the 1-cm growth zone of the mesocotyl and the corresponding epidermis were analyzed by GC-SIM-MS. As shown in Table 1, R decreases the amount of free IAA in the whole apical mesocotyl and in the epidermis by 32% and 46%, respectively. While there is variability in the absolute amount of free IAA between experiments, the ratios of free IAA between tissues and between light treatments calculated for individual experiments is highly reproducible as shown in Table 2. The largest variation in the ratio of free IAA between experiments was less than 0.8%. By this comparison, R is shown to cause a 1.4-fold decrease in free IAA in the entire apical mesocotyl. However, R causes a significantly greater decrease in the epidermis (at least 1.9-fold). This difference in the R-induced change in the epidermis as compared with the entire apical mesocotyl can also be shown by comparing the distribution of free IAA between the epidermis and the entire mesocotyl of R-irradiated and dark-grown seedlings (Table 2). These comparisons are internally consistent with the preferential decrease in auxin within the epidermis.

In etiolated mesocotyl sections, the epidermis has a 40% lower concentration of free IAA than the entire apical mesocotyl. This direct analysis conflicts with the previous conclusion, based on an indirect auxin measurement, that the epidermis contains twice as much

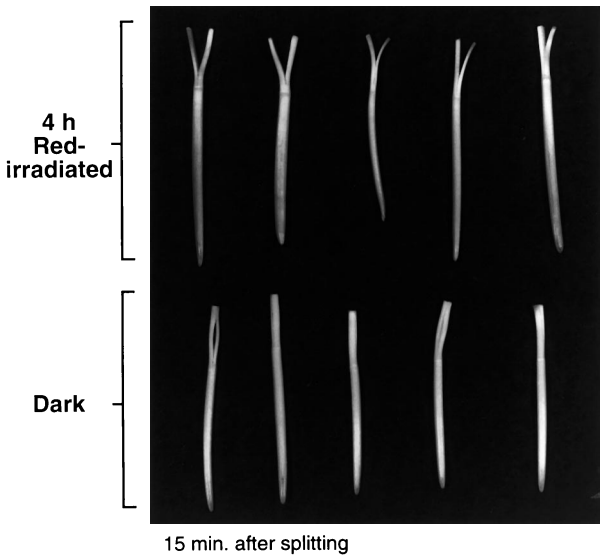

Fig. 2. Differential growth rate of epidermal and cortical tissues within shoots of dark-grown and R-irradiated (4 h) etiolated maize seedlings. Four-day-old seedlings were either irradiated with R (660 nm, 7.5 mol · m^{-2} · s^{-1}) for 4 h or kept in darkness; then the coleoptile plus the apical 1.8 cm of the mesocotyl were harvested. The mesocotyl was longitudinally split from the node down and each shoot floated on growth buffer. After 15 min, the shoots, arranged upside down, were photographed

Table 1. Free IAA in epidermal peels and the whole apical 1 cm of mesocotyl of dark-grown and R-irradiated maize seedlings

Tissue	Experiment #	DW (mg)	IAA (ng · g DW)
R epidermis			
	1	38.7	237.1
	2	33.1	96.4
	3	36.2	157.5
	Ave ± SE	36.0 ± 0.002	163.7 ± 41.5
D epidermis			
	1	24.7	437.8
	2	23.0	179.6
	3	32.1	294.3
	Ave ± SE	26.6 ± 0.003	303.9 ± 76.1
R whole			
	1	44.5	519.3
	2	39.2	212.1
	3	41.2	346.6
	Ave ± SE	41.6 ± 0.002	359.3 ± 90.6
D whole			
	1	39.8	724.8
	2	40.1	298.9
	3	38.3	500.3
	Ave ± SE	39.4 ± 0.006	508.0 ± 125.3

Table 2. The relative distribution of IAA between maize mesocotyl tissues and in tissues with different light treatments. Ratios were calculated from the data shown in Table 1

Comparison	Experiment #	Ratio
D:R epidermis	1	1.85
	2	1.86
	3	1.86
	Average ± SE	1.86 ± 0.005
D:R whole	1	1.40
	2	1.41
	3	1.44
	Average ± SE	1.42 ± 0.01
Whole:Epidermis R	1	2.19
	2	2.20
	3	2.20
	Average ± SE	2.20 ± 0.00004
Whole:Epidermis D	1	1.66
	2	1.66
	3	1.70
	Average ± SE	1.67 ± 0.013

auxin as the subtending cortical cells (Jones 1990). However, there is no conflict regarding the relative change in auxin. The auxin determination here using GC-SIM-MS is consistent with the previous conclusion that R causes a preferential decrease in auxin within the epidermis (Jones et al. 1992).

The key question is whether this difference in auxin between the epidermis and cortex is sufficient to cause the growth assymetries shown in Fig. 2 and to control the growth rate of the shoot. Figure 3 shows that with maize shoots, a ratio of IAA of two, regardless of whether it is applied above or below the node, caused a differential growth response, indicating that the small differences in auxin distribution established after R (Table 2) are sufficient to cause the observed differential growth (Fig. 2). This observation is consistent with previous work on dicots. Harrison and Pickard (1989) showed that auxin and growth asymmetries in tomato begin to develop at closely comparable times. In addition, ratios of IAA asymmetry as low as 1.4 were sufficient to generate differential growth. Migliaccio and Rayle (1989) applied IAA asymmetrically to *Helianthus* hypocotyls using gradients as small as 1.3 and produced curvature.

It is interesting to note that auxin levels in these mesocotyls are quite variable (Table 1) but that the ratio of auxin in dark-grown vs. R-irradiated sections as well as between the epidermis and the whole sections are precise and reproducible (Table 2). This shows that the absolute levels of auxin are less important than the change in its level indicating that tissues utilize a sensory adaptation mechanism. Smith et al. (1994) have shown that tomato plants which have abnormally high or lower auxin levels established by transgene functions still behave normally with respect to the fluence of R required to alter growth rates. Thus, despite the auxin levels in these pre-irradiated plants, the R-induced change in auxin level rather than the absolute level is probably the parameter utilized to establish a new growth rate.

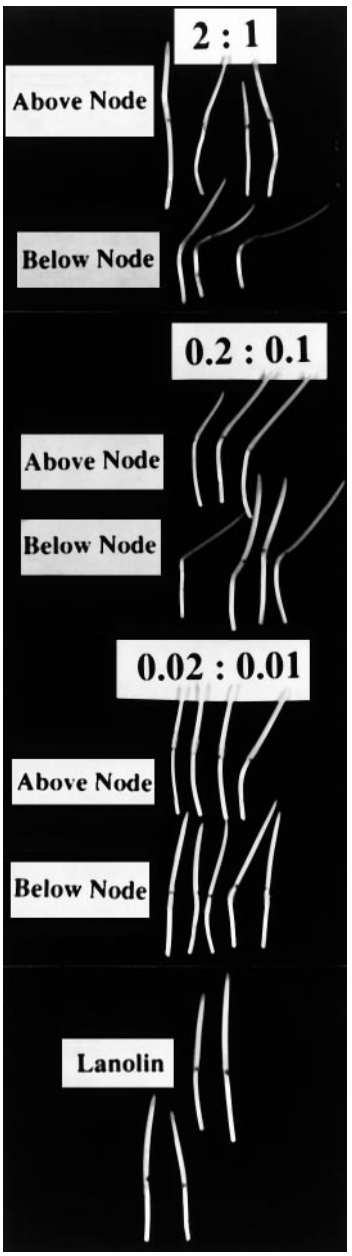

Fig. 3. Asymmetrical growth of maize mesocotyl induced by an asymmetrical application of IAA. The indicated concentrations of IAA (mg IAA per g of lanolin paste) were applied via a thin nitrocellulose patch shown on the left and right sides of 4-d-old, etiolated, maize shoots harvested 2.5 cm below the node. Strips were placed either above or below the node. Each shoot was held vertically with its base in an agar slab in a humid chamber for 12 h and photographed. Lanolin (*bottom panel*) represents the no-IAA control. An ink mark was placed at the node before photography for visualization

Taken together, the data support the notion that tissues differ with respect to auxin levels and that this difference is used to control overall organ growth rates, at least in part. This idea is similar to that proposed for a mechanism of gravitropism whereby auxin levels between cortical and epidermal tissues change in opposing fashion on opposite sides of gravistimulated shoots prior to assymetrical growth (MacDonald and Hart 1987),

except in this model the concept of differential responsiveness to auxin in inner and outer tissues, as discussed above, is included as well.

Our measurements of the relative levels of IAA in the epidermis compared with the mesocotyl quantified a profound R-induced change in IAA level that was demonstrated as physiologically significant. Our data are consistent with the hypothesis that the auxin within the epidermis represents a special pool of auxin that is preferentially light-regulated and growth-limiting.

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