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Physical Restraints Underlying Short-Term Inhibition by Auxin of Root Elongation in Intact Maize Seedlings

Peter M. Neumann and David Leon

Plant Physiology Laboratory, Faculty of Agricultural Engineering, Technion IIT, Haifa 32000, Israel

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Abstract. This report investigates physical changes associated with the short-term inhibition of root elongation in intact maize seedlings (*Zea mays* L. vs. Halamish) by exogenous auxin. Movement of root tips was assayed by video microscopy in control roots, roots grown for 45 min in 10^{-6} M indole-3-acetic acid (IAA), or roots chilled for 3 min at 11°C. IAA and chilling treatments similarly reduced root elongation rates (from 29 ± 6 μm min^{-1} to 6 ± 2 μm min^{-1}). Initial rates of root tip contraction induced by 300 mOsmol mannitol were used to calculate tissue contractibility values. These allowed a comparison of effects of IAA and chilling treatments on apparent rates of water transport out of the root tip tissues. Chilling treatment reduced root tip contractibility by 66%, whereas IAA had much less effect (26% reduction). Roots were also exposed to an osmotic jump treatment; the initial osmotically induced increase in elongation rate was used to determine root tip extensibility values. Both IAA and chilling treatments reduced root tip extensibilities by 57%. Inhibition of wall-yielding properties, rather than hydraulic limitations, appeared to be primarily associated with inhibition of intact root tip elongation by exogenous IAA.

Elongation growth of maize seedling roots is dependent on massive expansion of young cells produced by cell division in the apical meristem. The cell elongation zone extends 5–8 mm behind the tip (Pritchard et al. 1990, Zidan et al. 1990). The rate of cell elongation can be limited by water or solute uptake rates and/or by the yielding properties of the cell walls (Lockhart 1965). Treating maize root tissues with micromolar concentrations of indole-3-acetic acid (IAA) leads to a rapid inhibition of elongation (Mulkey and Evans 1981, Pilet and Saugy 1987, Pilet et al. 1983). Inhibition of root elongation by IAA is accompanied by an inhibition of cell wall acidification in the apical elongation zone, and wall acidification may be one of the biochemical factors required to maintain the yielding properties of expanding cell walls (Cleland 1986, Mulkey and Evans 1981, Pilet et al. 1983, Zidan et al. 1990). The involvement of hydraulic changes and/or changes in cell wall yielding in IAA-stimulated growth of excised shoot tissues has been much investigated (Boyer and Wu 1978, Cosgrove and Cleland 1983; review by Cleland 1986). However, little appears to be known about the physical restraints underlying the inhibition of root elongation by exogenous IAA. We report here on the assay of rapid osmotically induced expansion or contraction in growing root tip tissues of intact maize seedlings. The measurements were used to characterize changes in root tip extensibility and water transport capacity, which might underly the inhibition of root elongation by IAA.

Materials and Methods

Plant Growth

Seeds of *Zea mays* L. cv. Halamish were germinated in the dark for 2 days at 27°C on filter paper wetted with 0.4 mM calcium chloride. The germinated seeds were then transferred to a growth chamber at 27 ± 2°C with relative humidities between 35% by day and 60% at night. Light at plant height was 35 W m^{-2}, provided by mixed incandescent/fluorescent lamps during a 12-h photoperiod. After 2 days the 1- to 2-cm long roots of 15 germinated seeds were inserted through holes in circular polystyrene discs floating on 1 L of aerated 0.1 strength nutrient solution containing extra calcium (Zidan et al. 1990). The water potential of the nutrient solution was 0.11 MPa. Seedlings were assayed 2 days after transfer to nutrient solution.

Root Elongation

Assay of IAA effects on excised tissues can involve artifacts

resulting from wound responses and removal of the growing tissues from endogenous supplies of nutrients and growth regulators. In order to avoid these artifacts we developed methods for assaying IAA-induced changes in growing root tissues of intact maize seedlings. The primary root (±8 cm length and ±0.5 mm radius, 1 cm from root tip) of an intact seedling was firmly taped onto a plastic petri dish (9 cm in diameter) so that a 7-mm section, including the entire growing zone of the root tip, protruded freely from one side of the tape and the remainder of the seedling from the other side; this allowed assay of induced changes in the size of our growing tip tissues independently of the rest of the plant. Under our growth conditions the apical root elongation zone, determined by marking experiments, extended 5.8 ± 1.0 mm from the tip (Zidan et al. 1990). Roots were bathed in 25 ml of fresh nutrient solution within 1 min of transfer to the Petri dish. They were then rapidly transferred to a separate laboratory containing a Nickon SMZ-2T binocular dissection microscope connected to a Panasonic WV-CD 130 video camera and a Panasonic BT-M 1400 PSN color video monitor, all in ambient light at room temperature. The microscope was focused on the horizontally mounted root tip which was illuminated using a cold fiber optic light source. Measurements were completed within 10 min and preceded the onset of any geotropic curvature. A high-power objective lens and maximum enlargement gave a clear screen image of the root tip at 417× magnification. Changes in position of the root tip could be conveniently followed, with a 1-mm shift on the monitor screen equivalent to approximately 2.4 μm of tip movement. After a 2-min equilibration, the root elongation rate was assayed for 1 min, prior to assay of contractibility or extensibility. All solutions were at 27°C unless otherwise stated.

Root Tip Contractibility

For the contraction assay, nutrient solution was aspirated and replaced, within 1 min, by fresh solution containing 300 mOsmol mannitol (equivalent to −0.75 MPa). Recent determinations of turgor pressure in growing apical cells of maize roots gave values in the range of 6–8 MPa (Hsiao and Jing 1987, Pritchard et al. 1990). Thus, the concentration of mannitol was sufficient to counter cell turgor pressure in the maize root tips and prevent early renewal of elongation growth, which can occur when lower concentrations of mannitol are used to induce contraction (see Hsiao and Jing 1987, Kuzmanoff and Evans 1981). Root contact with the hypertonic mannitol rapidly induced water efflux and a contraction of the root elongation zone (Fig. 1). The assay of initial rate of mannitol-induced contraction started within 20 s of mannitol addition and finalized after 20–30 s in order to minimize deviations from initial conditions. We could not detect significant changes in root diameter during the initial longitudinal contraction and assumed that the contraction rate was proportional to rates of water efflux. Contractibility of tip tissues (i.e., the difference between rate of elongation and initial contraction rate) was expressed in μm min^{-1} 0.75 MPa^{-1}. For evaluation of possible contributions of mature nongrowing root tissues to contraction rates, a 2.5-cm apical section, including all the elongating tissues, was excised and discarded. In order to minimize water efflux from the cut surface, it was blotted and sealed with cyanoacrylate adhesive, to a small piece of aluminum foil. The mature root was then taped 7 mm behind the cut apex and assayed as above.

Root Tip Extensibility

Intact plants were mounted as described above and extensibility

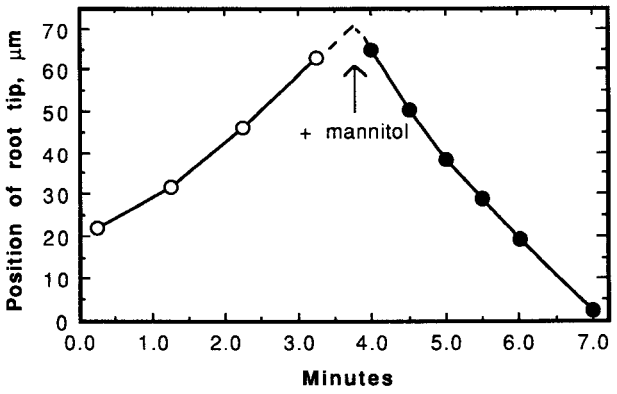

Fig. 1. Typical root growth response during contractibility assay. Root elongation in a control plant was followed microscopically at 1-min intervals by noting the change in position of the root tip (indicated by open circles) relative to a fixed scale (μm). The incubation solution was then aspirated and replaced, at the time indicated by the arrow, with a solution containing 300 mOsmol mannitol. The root tip was out of focus during the solution change. Assay of the subsequent mannitol-induced contraction of the root tip was followed by recording the new position of the tip 20 s after mannitol addition and then at 30–60-s intervals (filled circles).

of the growing tip tissues was then determined by an osmotic jump method. Increases in external osmotic potential ≤0.05 MPa (obtained by appropriate dilution of the nutrient solution) produced only small increases in growth rate of elongating tissues which were at the detection limit of the assay technique. We therefore routinely used an 0.1 MPa increase in external osmotic potential for assaying extensibility. Larger increases were avoided in order to minimize unnecessary perturbation of the system (Okamoto et al. 1989). An immediate swelling response to the osmotic jump, probably associated with elastic expansion of the tip tissues, occurred in the first 20 s. The subsequent positions of the intact root tip, recorded 20 s after the osmotic jump and then 1 min later, were used to calculate the new (accelerated) elongation rate. This initial measurement represented the nearest approach to a new "steady-state" rate, since accelerated elongation subsequently declined due to the onset of an apparent feedback inhibition. The tissue extensibilities calculated from the differences between basal and stimulated elongation rates were expressed in μm min^{-1} 0.1 MPa^{-1}. In order to evaluate possible contributions of mature nonelongating root tissues to extensibility determinations, a 2.5-cm apical section including the elongating tip tissues was excised and discarded. The remaining mature root was then taped 7 mm behind the cut apex and assayed as above.

The measurements reported here are based on following rapidly induced changes in water flux into or out of apical root tissues of equivalent dimensions. The use of hypertonic osmotica to determine root hydraulic characteristics can involve several possible artifacts, such as changing resistance to water transport through the plasmalemma during contraction, the formation of unstirred aqueous boundary layers at the tissue surface, limiting rates of mannitol diffusion through the apoplast, and gradual equilibration between the tissue and external solutes (Boyer 1985, Steudle and Frensch 1989). However, the contraction and expansion assays allowed operational comparisons between

Table 1. Accelerated root elongation rate induced by an osmotic jump.[a]

	Basal elongation rate (μm min^{-1})	Accelerated elongation rate (μm min^{-1})		
		1 min	2 min	3 min
Control	29 ± 6	45 ± 10	41 ± 12	36 ± 13
IAA	6 ± 2 (−79%)	12 ± 4	8 ± 2	7 ± 2
Chilling	6 ± 2 (−79%)	11 ± 2	12 ± 1	14 ± 2

[a] Roots of intact maize seedlings were either chilled for 3 min at 11 °C, or treated for 45 min with 10^{-6} M IAA, prior to assaying extensibility as described in Materials and Methods. Rates of root elongation were measured over 1-min intervals immediately before and 20 s after an 0.1 MPa osmotic jump. The parentheses show treatment-induced reductions in the basal elongation rates on a percent basis. Each value represents mean ±SD for six roots.

treatment effects on water transport and tissue extensibility in the growing root tips.

Assay of IAA and Chilling Effects

For the auxin treatments, single seedlings were transferred to separate pots containing aerated growth solution and 10^{-6} M IAA and incubated for 45 ± 5 min in order to inhibit growth (Mulkey and Evans 1981, Pilet and Saugy 1987). IAA at 10^{-6} M was included in all the exchange solutions used during the subsequent assay of auxin effects.

For the cold treatments, taped roots were first incubated in nutrient solution at 11 ± 2°C for 3 min in order to inhibit their growth. They were then exposed to appropriate levels of either mannitol or diluted nutrient solutions at the same temperature for assay of tip contraction or expansion. Proportional corrections were made for the effects of low temperature on osmolality of treatment solutions. Results are means ± SD for assay of six roots. Experiments were repeated one or more times with similar results.

Results

Root tip elongation was reduced to very low rates after either a 45-min incubation in IAA solution, or a 3-min chilling treatment (Table 1). Similarly, both IAA and chilling treatments caused large reductions (−57%) in root tip extensibility values (Table 2). Thus, reductions in elongation rate caused by IAA and chilling treatments appeared to be associated with similar reductions in root tip extensibility. Table 1 also shows that the accelerated elongation rate, induced by the osmotic jump in IAA-treated roots, declined rapidly after the first minute but did not decline in chilled roots. The gradual onset of these differences is more clearly revealed when accelerated growth rates are expressed on a percent basis, as in Fig. 2.

Table 2. Effects of IAA and chilling on extensibility and contractibility values in root tip tissues.[a]

	Extensibility		Contractibility	
	μm min^{-1} 0.1 MPa^{-1}	% reduction	μm min^{-1} 0.75 MPa^{-1}	% reduction
Control	7 ± 2	0	38 ± 6	0
IAA	3 ± 1	−57	28 ± 6	−26
Chilling	3 ± 1	−57	13 ± 3	−66

[a] Values calculated from differences between rates of tip movement assayed before and 20 s after exposure to an 0.1 MPa osmotic jump, or to 300 mOsmol mannitol (0.75 MPa). Other details as in Table 1.

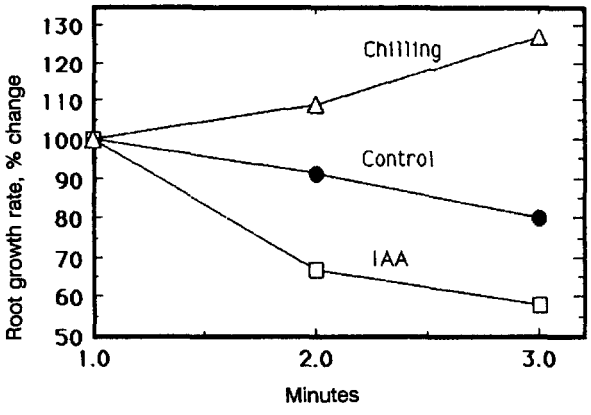

Fig. 2. Kinetics of changes in accelerated rate of root tip elongation obtained after a 0.1 MPa osmotic jump. Means of accelerated elongation rates at 2 and 3 min after the osmotic jump expressed as percentages of 100% values: 100% values are the mean elongation rates assayed 1 min after the osmotic jump for each treatment. Standard deviation of source data shown in Table 1.

The differences between root elongation rates prior to mannitol addition and the initial rate of contraction after mannitol addition (Fig. 1) were used to calculate root contractibility values. These provided a comparative measure of treatment effects on water transport out of the root tip tissues. IAA-treated roots showed only a relatively small reduction in root tissue contractibility (−26%), whereas contractibility of chilled roots was reduced by 66% in comparison with control values (Table 2). Thus, the effects of IAA and chilling treatments on root hydraulic characteristics did not appear to be proportionately related to their inhibitory effects on root elongation.

In order to evaluate possible contributions of mature nonelongating tissues to osmotically induced tip movement, we assayed extensibility and contractibility in mature root tissues from which the apical elongating tissues had been excised. The val-

ues determined for these nonelongating tissues were negligible, presumably because the relative inflexibility of the mature cell walls prevented significant expansion or contraction. Thus, osmotically induced rate changes appeared to be primarily determined by the properties of the elongating root tissues.

Discussion

The results indicated that the chilling treatment had a greater inhibitory effect than IAA on contractibility of the root tip tissues. Osmotically induced root tip contraction presumably involves an efflux of cell water and parallel relaxation of tension in the previously expanding cell walls. Thus, the differences in contractibility might result from treatment-induced differences in capacity for cell wall relaxation or from decreases in the hydraulic conductivity of apoplastic or symplastic pathways used for water transport across the root tissues (Boyer 1985, Steudle and Frensch 1989). Chilling had little effect on the mechanical properties of isolated cell walls from growing tissues, as measured in vitro by the Instron technique (Cleland 1971), so that an effect on wall relaxation seems unlikely. However, several reports show that lowered temperatures decrease water fluxes across root tissues and cell membranes (Ginsburg and Ginsburg 1971, Evlagon et al. 1989, Markhart et al. 1979, Thomas et al. 1989). Thus, it seems probable that the chilling treatment inhibited root tip contractibility primarily via inhibitory effects on water transport. The locus of the comparatively small inhibitory effect of IAA on root contractibility is less clear. However, previous reports indicate that IAA treatments either increase hydraulic conductivities in elongating tissues (Boyer and Wu 1978) or have no effect (Cosgrove and Cleland 1983). Thus, an effect of IAA on cell wall properties may be involved.

Root tip extensibility values were also reduced by chilling and IAA treatments. It might be argued that reductions in water transport capacity rather than cell wall effects were a primary factor in the inhibition of root extensibility by IAA. If so, then the chilling treatment, which appeared to have a greater inhibitory effect on water transport than IAA, should also have had a greater inhibitory effect on extensibility than IAA. However, the inhibitory effects of IAA and chilling treatments on root extensibility values were equal. Thus, IAA did not appear to inhibit root tip extensibility via effects on water transport capacity. Moreover, the gradual feedback inhibition of osmotically accelerated elongation eventually led to a relatively greater inhibition in IAA-treated roots than in chilled roots (Table 1 and Fig. 2). Similar feedback response previously observed in growing roots subjected to osmotic changes were related to alterations in cell wall properties (Hsiao and Jing 1987, Kuzmanoff and Evans 1981).

The experimental approach described here allowed a comparative investigation of physical changes underlying the inhibitory effects of IAA on root tip elongation in intact maize seedlings. Hopefully, future research will reveal more about the physical changes induced by IAA in the tissues and individual cells of the root tip, and their relative contributions to the overall inhibition of root growth. At present, however, direct measurements of wall extensibility or hydraulic conductivity in individual rapidly elongating root cells do not appear to be easily attainable; moreover, there is no model for relating single cell measurements to the behavior of the intact organ.

In conclusion, our results support the hypothesis that the rapid inhibitory effect of exogenous IAA on root elongation in intact maize seedlings involves reductions in the extensibility of the cell walls.

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