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COMPARATIVE EFFECTS OF AUXIN AND ABSCISIC ACID
ON GROWTH, HYDROGEN ION EFFLUX AND GRAVITROPISM IN
PRIMARY ROOTS OF MAIZE

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1. INTRODUCTION

According to the acid growth hypothesis of auxin action, promotion of cell elongation by auxin is due, at least in part, to stimulation of hydrogen ion (H^+) efflux from the cytoplasm into the cell wall. Acidification of the wall is thought to enhance wall loosening and allow rapid growth (Rayle and Cleland, 1977). There is now considerable evidence that acid efflux plays a role in auxin action on cell elongation in stems and coleoptiles. However, relatively little study has been made of the role of H^+ movement in auxin action on roots. This is in spite of the fact that auxin can either promote (at low concentrations) or inhibit (at higher concentrations) elongation in both shoot and root cells. Based on this bimodal concentration dependence, Thimann (1937) proposed that the molecular basis of auxin action is fundamentally the same in shoots and roots. This proposal is strengthened by the finding that root growth is strongly stimulated by acid pH just as is shoot growth (Edwards and Scott, 1974; Evans, 1976).

In order to test the idea that auxin action on root growth may be mediated by H^+ movement, we have examined the correlation of auxin action on growth and H^+ movement in roots and looked for changes in H^+ efflux patterns associated with the asymmetric growth which occurs during gravitropism. We have also compared the effects of IAA and ABA on growth, H^+ secretion, and gravitropism in roots.

2. MATERIALS AND METHODS

The experiments were done using the following cultivars of maize (Zea mays L.): Bear hybrid WF9 x 38MS, Orla 231, and LG 11. Unless otherwise indicated, the experiments were done with WF9 x 38MS seedlings. Dark grown seedlings were raised with a brief red light treatment as described by

Mulkey et al. (1981) or raised in total darkness. The seedlings were used when they were 2.5 to 3.5 d old, when the primary root was about 1.5 cm long.

Root elongation was measured using the root auxanometer described by Evans (1976). The time course of gravitropism was determined from time-lapse Super-8 mm films of seedlings mounted horizontally in a Plexiglas chamber. The chamber was maintained at 100% relative humidity and $22^{\circ}\pm 2^{\circ}$ C.

The pattern of acid efflux from roots of intact seedlings was determined using the modification of the Weissenfeld method described by Mulkey and Evans (1981). Seedlings are placed on a plate of agar containing the pH indicator dye, bromocresol purple. Acid efflux from the root causes the indicator to become yellow. In regions of apparent acid entry into the root, the pH of the medium adjacent to the root increases and the dye in that region turns red.

To obtain a quantitative measurement of hormone effects on H^{+} efflux from maize roots, 1-cm apical root segments were used, and acid efflux was measured as a decrease in the pH of 3.5 ml of 1 mM KPO_4 buffer containing 60 segments (Mulkey et al., 1982a). Since treatment of apical root segments with IAA or ABA enhances their ethylene biosynthesis, the effects of IAA and ABA on H^{+} efflux in root segments were measured using segments pretreated with a solution containing 1 μ M aminoethoxyvinylglycine plus 0.1 mM cobalt nitrate (AVG/Co^{2+}) to minimize the enhancement of ethylene formation (Mulkey et al., 1982a).

Abscisic acid (+/- cis,trans) was purchased from Sigma Chemical Co., St. Louis, MO, USA and from Calbiochem-Behring Corp., La Jolla, CA, USA. AVG was provided by Dr. Richard Gladon, Dept. Horticulture, Iowa State University, Ames, IA, USA.

3. RESULTS

Auxin Action on Growth and H^{+} Movement in Roots

Using intact seedlings of maize, we obtain stimulation of root elongation by low (e.g. < 1 nM) concentrations of IAA only occasionally. In most experiments, IAA in concentrations < 1 nM has no effect on root elongation. However, if the roots are pretreated with AVG/Co^{2+} (Mulkey et al., 1982b), we obtain consistent promotion of elongation using IAA in concentrations from 0.1 nM to 10 nM (Fig. 1).

The ability to obtain consistent promotion of root growth with low concentrations of auxin and inhibition with high concentrations, has allowed us to compare auxin action on H^{+} efflux and growth over both concentration ranges. When a growth promoting concentration of auxin is added to isolated

corn root tips, there is strong enhancement of H^+ efflux from the segments. This parallels the promotion of growth in intact roots using the same concentration of auxin (Fig. 2). Conversely, treatment of isolated root tips with a growth inhibiting concentration of IAA causes an apparent uptake of H^+ from the medium, and this is paralleled by inhibition of growth (Fig. 2).

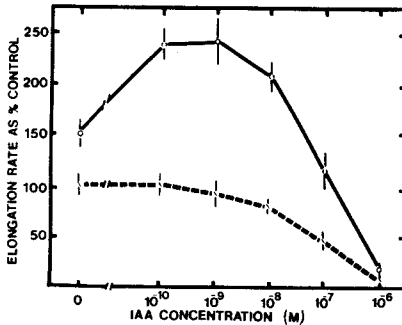


Fig. 1. Concentration dependence of IAA action on elongation of maize roots pretreated with AVG/ Co^{2+} . Solid lines: roots pretreated with AVG/ Co^{2+} 1 h and then treated with the indicated concentration of IAA (plus AVG/ Co^{2+}). The growth rate was measured 2 h after transfer to the IAA-containing solutions. Dashed line: control roots held in buffer 1 h and then treated with IAA. Means, \pm S.E. are shown.

Visualization of H^+ Efflux Patterns During Straight Growth and Gravitropism

The above results indicate a close relationship between H^+ movement and growth rate in maize roots. However, since the H^+ efflux experiments were done on apical root segments and the growth experiments were done using intact roots, we also wanted to look at H^+ efflux in intact, relatively undisturbed roots. This was done by placing whole seedlings on a plate of agar containing the pH indicator dye, bromocresol purple. With the seedling oriented vertically, the pH pattern shown in Figure 3A develops around the root within 8 min and intensifies thereafter. The medium adjacent to the elongation zone and the medium along the root hair zone become yellow, indicating acid efflux. The medium adjacent to the non-growing region behind the elongation zone and the medium around the tip of the root become red, indicating acid uptake. When 2 μ M IAA is included in the medium (Fig. 3B) the medium along the elongation zone becomes red instead of yellow, indicating H^+

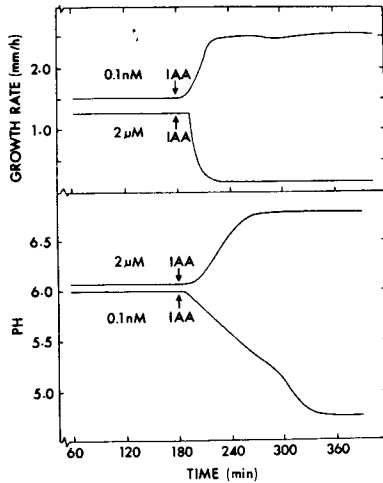


Fig. 2. Effect of IAA on growth and H^+ movement in maize roots. Top: Effect of IAA on elongation. A single intact root was treated with the indicated concentration of IAA. The root receiving 0.1 nM IAA was pretreated for 1 h with AVG/ Co^{2+} prior to exposure to auxin. Bottom: Effect of IAA on H^+ efflux. The indicated concentrations of IAA were added to sixty 1-cm apical root segments in 3.5 ml of medium. The segments receiving 0.1 nM IAA were pretreated for 3 h with AVG/ Co^{2+} prior to exposure to auxin.

uptake into the elongation zone. These data show that intact roots secrete acid from the elongation zone and that growth inhibiting concentrations of IAA reverse the direction of acid movement in that region.

When a seedling in contact with an agar/dye plate is oriented horizontally there is, of course, a shift in the growth pattern of the root as positive gravitropism occurs. The rapid growth rate on the top relative to that on the bottom is paralleled by intense acid efflux on the top and diminished acid efflux on the bottom (Fig. 4).

The correlation between the H^+ efflux pattern and gravitropism suggests the possibility that asymmetric acid efflux may cause the asymmetric growth which leads to positive gravitropism. If so, the manner of development of asymmetric acid efflux becomes important in considering models of gravitropism. According to the Cholodny/Went hypothesis (Went and Thimann, 1937), root gravitropism results from movement of auxin toward the lower side of the root where it accumulates to growth-inhibiting levels. Since we find that high concentrations of auxin inhibit H^+

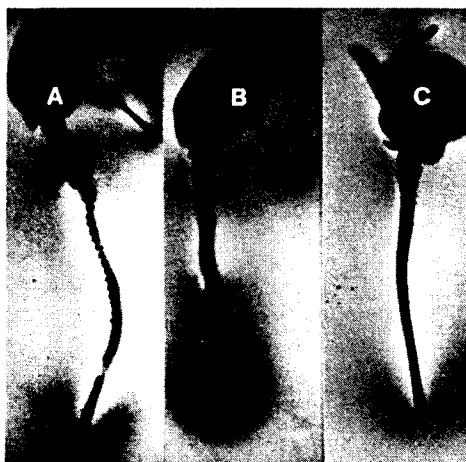


Fig. 3. H⁺ efflux pattern of maize roots on agar plates containing the pH indicator dye, bromocresol purple. The light regions represent yellow zones indicating acid efflux. The dark regions represent red zones indicating acid uptake. A: control. B: 2 μM IAA. C: 0.1 mM ABA. Photos taken 2 h after placing seedlings on the medium.

efflux from roots while low concentrations promote H⁺ efflux, it is possible that the H⁺ efflux asymmetry associated with gravitropism is caused by auxin redistribution. We find that inhibitors of auxin transport which prevent root gravitropism (e.g. naphthylphthalamic acid) also prevent the development of acid efflux asymmetry in gravistimulated roots (Fig. 4).

Comparative Effects of ABA and IAA on Root Growth and Gravitropism

A second major hypothesis of root gravitropism is the root cap inhibitor hypothesis. According to this model, root gravitropism results from the movement of a growth inhibitor from the root cap to the elongation zone where it accumulates on the lower side (Keeble et al., 1931; Pilet, 1974). ABA is suggested to be a major component of the cap inhibitor (Pilet and Rivier, 1980). With this model in mind, we have examined the effects of ABA on H⁺ efflux, growth and gravitropism in several cultivars of maize.

Using the root auxanometer, we find that the initial effect of a wide concentration range of ABA is to promote

growth (Fig. 5). With low concentrations (e.g. 0.1 μM) there is a transient stimulation of elongation. Using higher concentrations (e.g. 0.1 mM) there is a period of stimulation followed by long term inhibition of root elongation, so that the net effect on growth is inhibitory.

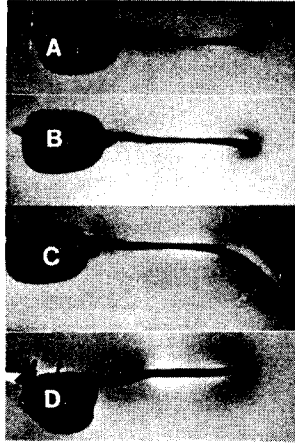


Fig. 4. Sequence of changes in surface pH patterns along the primary root of a maize seedling exposed to a gravitropic stimulus. Regions of high pH (reddish purple in color) appear as dark areas. Regions of low pH (yellow in color) appear as light areas. Elapsed time after placing the root in a horizontal position on the agar/dye plate are (A) 2 min, (B) 20 min, and (C) 120 min. The seedling shown in (D) was treated in the same manner but placed on an agar/dye plate containing 4.86 mM naphthylphthalamic acid (NPA) and photographed after 18 h.

However, with both low and high concentrations of AbA, the initial stimulatory phase lasts several hours. Data very similar to these have also been reported by Smith and Ho (1982).

The initial stimulatory effect of even high concentrations of AbA is reflected in the inability of AbA to inhibit or reverse H^+ efflux from the elongation zone of intact roots of maize (Fig. 3C). In fact we find that treatment of apical root sections with AbA causes stimulation of H^+ efflux (Fig. 6), an observation consistent with the initial growth promoting effect of AbA.

The fact that the initial effect of applied AbA on intact roots is stimulatory, conflicts with the proposed differential inhibitory action of AbA in root gravitropism. We therefore looked at the ability of unilateral application of

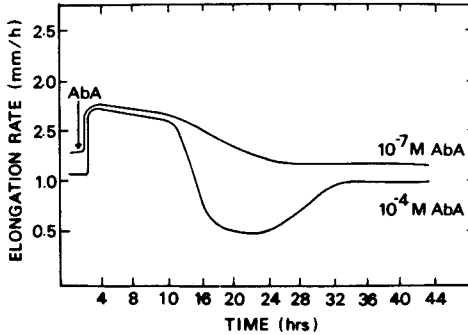


Fig. 5. Time course of ABA effects on primary root elongation in intact seedlings of maize. The indicated concentrations of ABA were added at the arrow. Qualitatively similar data were obtained with the three cultivars of maize tested.

ABA to modify root gravitropism in several cultivars of maize (Fig. 7). We found that application of an agar blocklet containing 0.1 mM ABA to the upper surface of horizontal roots, accelerated gravitropism (Fig. 7, column 1) while application of ABA to the bottom of the root retarded gravitropism (Fig. 7, column 2). These results are contradictory to the proposed role of ABA as a growth inhibitor in gravitropism.

4. DISCUSSION

Our results show a close correlation between H^+ efflux and growth in maize roots. In intact roots there is strong H^+ efflux from the elongation zone and apparent H^+ influx on both sides of the elongation zone. Concentrations of IAA which inhibit root growth also inhibit H^+ efflux, while growth promoting concentrations of IAA stimulate H^+ efflux.

During gravitropism the H^+ efflux from the elongation zone becomes asymmetric with greater H^+ efflux on the top relative to that on the bottom. This parallels the asymmetric growth which causes gravitropism. The possibility that auxin redistribution contributes to the development of acid efflux asymmetry and hence asymmetric growth, is indicated by the observation that inhibitors of auxin transport prevent both the development of asymmetric acid efflux and gravitropism.

The effects of applied ABA on root growth and gravitropism are inconsistent with its suggested role as an endogenous growth inhibitor mediating gravitropism. We find

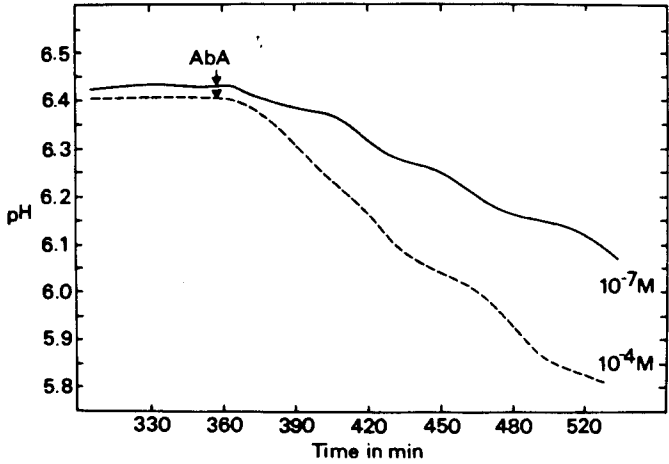


Fig. 6. Stimulation of H^+ efflux from maize root segments by AbA. The indicated concentrations of AbA were added to sixty 1-cm apical root segments in 3.5 ml of medium.

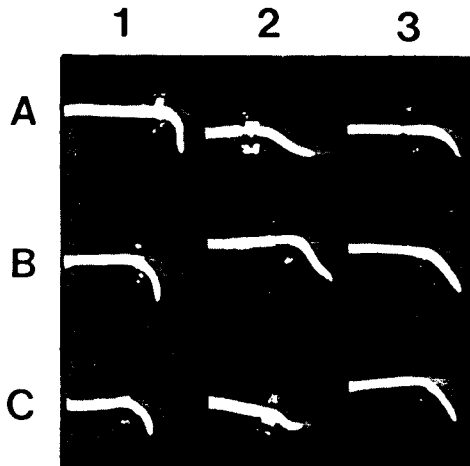


Fig. 7. Effect of unilateral application of AbA on gravitropism in maize roots. Column 1: AbA (0.1 mM) applied to the top (bottom block, agar only). Column 2: AbA applied to the bottom (top block, agar only). Column 3: control with agar blocks only. The roots were oriented horizontally immediately after applying the agar (1%) blocks. Photos taken 1 h after gravistimulation. Row A: cv WF9 x 38MS. Row B: cv Orla 231. Row C: cv LG 11.

that the initial effect of applied AbA is to promote root growth and that the period of growth promotion is long lived compared with the time required for completion of gravitropism. The ability of AbA to stimulate root growth is reflected in its ability to stimulate H^+ efflux from apical root segments. It is also manifested in the ability of asymmetric application of AbA to enhance gravitropism when the hormone is applied to the top of a horizontal root and its ability to inhibit gravitropism when applied to the bottom. This aspect of our results is in agreement with other reports of the ability of AbA to promote root growth (Gaither et al., 1975; Yamaguchi and Street, 1977; Abou-Mandour and Hartung, 1980; Smith and Ho, 1982). In light of these findings, it would seem that, if AbA mediates gravitropism in roots, its redistribution would need to be toward the top of the root, not the bottom. Ironically, Suzuki et al. (1979) reported accumulation of AbA on the upper side of graviresponding roots, and Hartung et al. (1976) noted a preferential short term upward movement of labelled AbA in horizontal maize roots.

Our results confirm other reports of long term inhibition of root growth by high concentrations of AbA (Pilet and Chanson, 1981). We see long term inhibition of elongation with concentrations of AbA equal to or greater than about 0.1 mM. However, the inhibition is slow to develop and occurs at such high concentrations that it is unlikely to be of physiological significance.

Although our data are consistent with a possible role for IAA in mediating root gravitropism, the results do not address a number of objections which have been raised to the Cholodny/Went model of auxin mediation of gravitropism (Wilkins, 1979). The possibility remains that root gravitropism may not be mediated by changes in hormone concentration (Digby and Firn, 1980; Trewavas, 1981) or that it may be mediated by redistribution of some, as yet, unidentified inhibitor from the root cap (Suzuki et al., 1979; Feldman, 1981).

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