

Short Communication

Auxin Action on Proton Influx in Corn Roots and its Correlation with Growth

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Abstract. At concentrations inhibitory to the elongation of corn (*Zea mays* L.) roots, the auxins, indole-3-acetic acid (IAA) and α -naphthaleneacetic acid (α -NAA), cause an increase in the pH of the bathing medium; this increase occurs with an average latent period shorter than the latent period for the inhibitory effect of these auxins on elongation. Indole-2-carboxylic acid, an inactive structural analogue of IAA, and β -naphthaleneacetic acid, an inactive analogue of α -NAA, affect neither growth nor the pH of the medium. Since acid pH is known to promote and basic pH to inhibit root elongation, the data are consistent with the hypothesis that hormone-induced modification of cell-wall pH plays a role in the control of elongation of roots, as has been proposed for elongation of stems and coleoptiles.

Key words: Auxin – Cell wall pH – Growth (roots) – Proton flux and growth – Root growth – *Zea*.

The increasing evidence that auxin regulation of growth in stems and coleoptiles may be mediated by modification of H⁺ efflux (see review by Rayle and Cleland, 1977) raises the question of the role that H⁺ secretion might play in auxin regulation of growth in other types of tissue.

The growth of roots is known to be affected by auxin (Thimann, 1936, 1937). At low concentration (ca. 0.1 nM) auxin is reported to promote root growth

while higher concentrations are inhibitory. Since root growth is promoted at low pH and inhibited at high pH (Edwards and Scott, 1974; Evans, 1976), the possibility arises that auxin action on root growth is mediated by hormone-induced alterations in wall pH. Moloney et al. (1979) have found that the antiauxin *p*-chlorophenoxyisobutyric acid (PCIB) stimulates elongation in corn roots and promotes proton export into the bathing solution. Since PCIB is thought to act by reducing the effective level of auxin in the tissue, this finding lends support to the hypothesis that auxin itself may influence cell-wall pH in roots, as has been proposed for stems. This would be consistent with the long-standing hypothesis that the mechanism of auxin action is similar in roots and stems (Thimann, 1937).

If auxin regulation of root growth is mediated by shifts in wall pH, growth-inhibiting levels of auxin should cause an increase in wall pH. In an earlier study of auxin effects on H⁺ movement in roots (McBride and Evans, 1977), we were able to detect only a weak and transient shift in pH of the external medium upon application of the hormone. We have now used a more sensitive method for detecting H⁺ efflux and influx in roots, and we find that growth inhibitory levels of auxin cause a rapid increase in the pH of the medium while inactive structural analogues do not.

The experiments were performed using roots of 3-d-old seedlings of corn (*Zea mays* L., hybrid WF 9 × 38; Customaize, Moline, Ill., USA). The corn kernels were sown on the surface of moist vermiculite in covered plastic trays and placed in the dark at 26° C.

For measuring H⁺ efflux and influx, 60 1-cm apical sections were cut from the tips of the primary roots of the seedlings and placed in 3.5 ml of 1 mM KPO₄ buffer (initial pH 6.3) in a glass vial (63 mm height, 17 mm diameter, total volume 17 ml). A semi-

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Abbreviations: IAA = indole-3-acetic acid; α -NAA = α -naphthaleneacetic acid; β -NAA = β -naphthaleneacetic acid

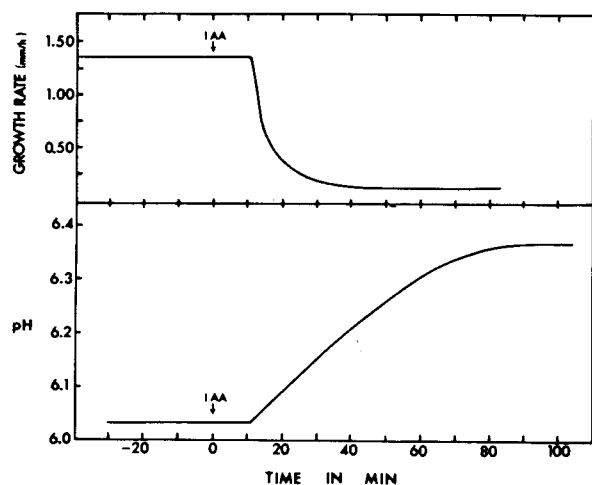


Fig. 1. Effect of IAA on growth and H^+ influx in corn roots. Top: Effect on growth. IAA ($2 \mu M$) added to the medium surrounding an intact corn root. Bottom: Effect on external pH. IAA ($2 \mu M$) added to excised corn root tips in a small volume of phosphate buffer

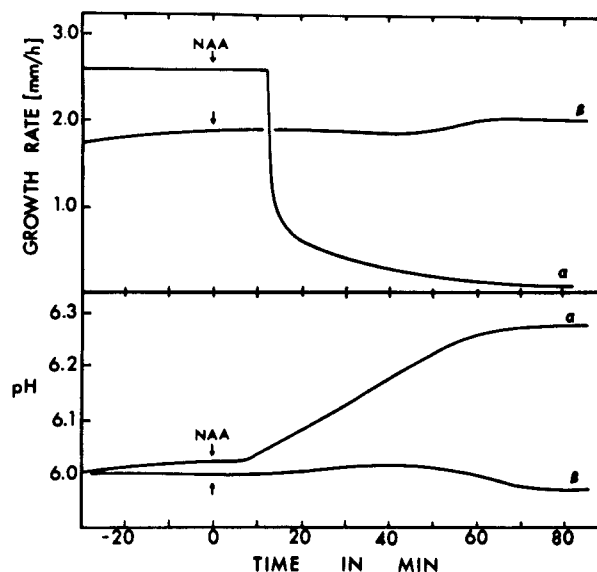


Fig. 2. Effects of α - and β -NAA on growth and H^+ influx in corn roots. Top: Effects on growth. NAA ($2 \mu M$) added to the medium surrounding an intact corn root. Bottom: Effects on external pH. NAA ($2 \mu M$) added to excised corn root tips in a small volume of phosphate buffer

micro combination pH electrode (No. 2885; Markson Science, Del Mar, Cal., USA) with a Corning Model 7 pH meter (Corning Scientific Instruments, Medfield, Mass., USA) was used to measure pH. Output from the pH meter was recorded on an SRLG recorder (Sargent-Welch Scientific Skokie, Ill., USA) at 0.1 mV full-scale sensitivity. The full-scale span on the recorder was adjusted to cover the pH range of 5.7 to 6.4 using an attenuating back voltage from a zero suppress source in combination with the zero adjust capability of the recorder. The medium containing the root segments was continuously oxygenated using pure oxygen introduced at the rate of 26 l/h through a 22-gauge (0.4 mm inner diameter) syringe needle inserted into the vial. In addition to oxygenation the medium was continuously stirred using a small Teflon-coated magnetic spin bar placed at the bottom of the vial and separated from the root segments by a disk of plastic screen mounted 5 mm above the bottom of the vial. Test solutions were added directly to the vial. A micropipet was used to introduce a small volume of stock solution sufficiently concentrated to give the desired final concentration after mixing.

Root growth was measured using whole seedlings mounted in the root auxanometer described by Evans (1976). While the hormone-induced shifts in pH described here are discussed in terms of H^+ uptake, we recognize that our data do not allow us to distinguish between this and some other mechanism, e.g., OH^- efflux.

When IAA ($2 \mu M$) is added to the medium surrounding the root of an intact corn seedling, the growth of the root is inhibited (Fig. 1, top). Inhibition begins after a lag of about 11 min and is maximal within 40 min. Similar effects are seen with $2 \mu M$ α -NAA except that maximum inhibition sometimes sets in more gradually (Fig. 2). When $2 \mu M$ IAA is added to excised root tips there is a lag averaging 8.6 min

Table 1. IAA- and NAA-induced pH increases in media containing corn root tips

Hormone	Latent period (min)	Initial pH	Final pH	Duration (min)
IAA	8.6 ± 2.2	5.95 ± 0.10	6.3 ± 0.12	68.8 ± 14.3
α -NAA	5.8 ± 1.6	5.76 ± 0.14	6.15 ± 0.17	67.7 ± 20

Duration refers to the time between addition of hormone and reaching the final (higher) pH value. Latent period refers to the time between addition of hormone and beginning of the pH increase. All values are averages (with standard deviations, $n-1$) from 12 experiments. Hormone concentration was $2 \mu M$ in all cases

(Table 1) before the pH of the external medium begins to increase (Fig. 1, bottom). The pH increases by an average of 0.36 pH units and then becomes steady at this value. On the average, the rise in pH is complete within 69 min after adding IAA (Table 1). This higher pH value is maintained for about 80 min. Then the pH drops gradually over a 3-h period, reaching a final value at or somewhat below the pH before addition of hormone (data not shown). Similar results are obtained using $2 \mu M$ α -NAA (Fig. 2, Table 1) except that the pH increase in response to α -NAA is maintained for as long as we have continued to record (up to 14 h).

The auxin specificity of the regulation of H^+ movement was examined by determining the effects

of active auxins and inactive auxin analogues on growth inhibition and induction of a pH increase in the external medium. Figure 2 shows the effects of the active auxin, α -NAA, and its inactive position isomer, β -NAA, on growth and apparent H^+ uptake in corn roots. As described above, the active auxin causes rapid inhibition of growth and a rise in the pH of the external medium. The inactive analogue, on the other hand, affects neither growth nor H^+ movement. Similar results were obtained using the active auxin indole-3-acetic acid, and its inactive structural relative indole-2-carboxylic acid (data not shown).

The results of this study indicate that, in roots, auxin inhibition of growth is accompanied by an increase in the pH of the external medium. Our failure to detect strong auxin-induced H^+ uptake in roots in an earlier study (McBride and Evans, 1977) was apparently because we were not oxygenating the segments. Supplementing stirring with oxygenation enhances auxin-induced H^+ efflux in stem tissues by at least two-fold (Evans and Vesper, 1980) and as shown here, allows detection of auxin-induced shifts in external pH in root tissue. Since the hormone-induced increase in external pH occurs with an average latent period shorter than the latent period for hormone-induced growth inhibition and since the effect appears to be specific for active auxins, our data are consistent with the hypothesis that changes in wall pH mediate auxin effects on root growth. We do not know why the pH rise is transient when induced by IAA, and long-lived when induced by α -NAA. However, the large root-to-volume ratio and vigorous oxygenation conditions of the experiment indicate the possibility that the low concentration of IAA applied is being metabolized during the course of the experiment while α -NAA is not.

Because low concentrations of auxin are reported to promote root elongation we also tested the effects of 0.2 nM IAA on root growth and H^+ secretion. Since auxin inhibition of growth is accompanied by an increase in the pH of the medium one might predict that auxin enhancement of growth would be accompanied by acidification of the medium. Using intact corn roots we were able to detect stimulation of elongation by 0.2 nM IAA only occasionally. Similarly, with isolated root tips we are able to detect apparent auxin-induced H^+ efflux only occasionally. It may be that the endogenous concentration of auxin in intact corn roots or apical root sections is high enough to mask the effect of low

concentrations of exogenously applied auxin (Pilet et al., 1979). The report (Moloney et al., 1979) that antiauxin promotes growth and H^+ secretion in roots would be consistent with this interpretation.

It is not clear how the data presented here relate to the recent report by Weisenseel et al. (1979) that H^+ moves into the growing regions and out of the non-growing regions of intact barley (*Hordeum vulgare* L.) roots. We find that inhibition of growth correlates with an apparent inward movement of H^+ while Weisenseel et al. show that non-growing portions of the root tend to pump H^+ outward. Neither our data nor those of Weisenseel et al. permit determination of the quantitative relationship between H^+ movement and growth, and the possibility remains that H^+ movement in roots is a phenomenon accompanying but not playing a causal role in growth.

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References

- Edwards, K.L., Scott, T.K. (1974) Rapid growth responses of corn root segments: Effect of pH on elongation. *Planta* **119**, 27-37
- Evans, M.L. (1976) A new sensitive root auxanometer: Preliminary studies of the interaction of auxin and acid pH in the regulation of intact root elongation. *Plant Physiol.* **58**, 599-601
- Evans, M.L., Vesper, M.J. (in press) An improved method for detecting auxin-induced hydrogen ion efflux from corn coleoptile segments. *Plant Physiol.*
- McBride, R., Evans, M.L. (1977) Auxin inhibition of acid- and fusicoccin-induced elongation in lentil roots. *Planta* **136**, 97-102
- Moloney, M.M., Cleland, R., Elliott, M.C. (1979) The acid-growth theory and the regulation of maize root growth. (Abstract) *Plant Physiol.* [Suppl.] **63**, 23
- Pilet, P.E., Elliott, M.C., Moloney, M.M. (1979) Endogenous and exogenous auxin in the control of root growth. *Planta* **146**, 405-408
- Rayle, D.L., Cleland, R.E. (1977) Control of plant cell enlargement by hydrogen ions. *Curr. Top. Dev. Biol.* **11**, 187-214
- Thimann, K.V. (1936) Auxins and the growth of roots. *Am. J. Bot.* **23**, 561-599
- Thimann, K.V. (1937) On the nature of inhibitions caused by auxin. *Am. J. Bot.* **24**, 407-412
- Weisenseel, M.H., Dorn, A., Jaffe, L. (1979) Natural H^+ currents traverse growing roots and root hairs of barley (*Hordeum vulgare* L.). *Plant Physiol.* **64**, 512-518

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Note added in proof: Applying the method of Weisenseel et al. (1979) we have found that the region of cell elongation in corn roots corresponds to the region of H^+ efflux. This differs from the observations of Weisenseel et al. using barley roots but is consistent with our findings on auxin effects on growth and H^+ influx in corn roots.