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IN MAIZE AND ARABIDOPSIS Final
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ROOT GRAVITROPISM IN MAIZE AND ARABIDOPSIS

FINAL REPORT
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PRINCIPAL INVESTIGATOR
Michael L. Evans
Ohio State University

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Accomplishments March 1, 1992 -- November 30, 1993

Research during this period focused on the following: 1) improvements in a video digitizer system designed to automate the recording of surface extension in plants responding to gravistimulation. The improvements included modification of software to allow detailed analysis of localized extension patterns in roots of Arabidopsis. We used the system to analyze the role of the postmitotic isodiametric growth zone (a region between the meristem and the elongation zone) in the response of maize roots to auxin, calcium, touch and gravity. We also used the system to analyze short-term auxin and gravitropic responses in mutants of Arabidopsis with reduced auxin sensitivity. In a related project, we studied the relationship between growth rate and surface electrical currents in roots by examining the effects of gravity and thigmostimulation on surface potentials in maize roots.

Improvements in the video digitizer system. We spent considerable effort modifying our current software to allow more precise automated analysis of time-dependent tip displacement during root growth. The improvements are important to our research on hormone and gravity response kinetics in roots of Arabidopsis. Because of the small size of these roots, it is more difficult to get sharp contrast between the root and the background and this results in errors in edge analysis. This was corrected by improved software allowing multiple point sampling and averaging. The new software is working well and we are collecting data on the kinetics of auxin and gravity responses in auxin-resistant mutants of Arabidopsis as described below. Further improvements in the software are being made so that we can analyze localized curvature patterns in the tiny roots of Arabidopsis. This information will be crucial to our plans to focus on the electrical properties of the earliest responding cells as outlined in our plans for research for the coming year.

The role of the postmitotic isodiametric growth zone in the response of maize roots to auxin, calcium, touch and gravity. Our earlier studies showed that the region of cells between the root meristem and the elongation zone (a region referred to as the postmitotic isodiametric growth zone, or PIG zone) plays an especially important role in the response of maize roots to gravity. These are the cells which show the earliest growth response to gravity. There is a large increase in the elongation rate of PIG zone cells along the top of a horizontally oriented root. We have extended these studies to analyze the role of these cells in the response of roots to calcium, touch, and auxin. Briefly, we find that the PIG zone cells are most

responsive to applied calcium and that effects on the PIG region account for curvature induction by applied gradients of calcium. The root cap is highly sensitive to touch. Tactile stimulation of the cap induces negative curvature and we conclude that tactile stimulation of the root cap during experimental treatments accounts for the reports of negative curvature induced by calcium application to the cap.

We examined the role of the PIG zone in the apparent ability of roots to adapt to inhibitory levels of auxin. We found that resumption of elongation in the presence of a strongly inhibitory concentration of auxin is due to time-dependent auxin-induced enhancement of cell elongation in the PIG zone. In related experiments we found that roots treated with a concentration of auxin high enough to inhibit elongation 100%, exhibit rapid and strong gravitropism upon gravistimulation. The curvature is accounted for by gravity-induced activation of elongation in PIG zone cells along the top of the root. The pattern of gravistimulated enhancement of elongation along the top of the root in the presence of a high concentration of auxin is nearly identical to that in gravistimulated controls. This has important implications for assessment of the validity of the Cholodny-Went theory. In particular, it seems necessary to conclude that the enhancement of elongation along the tops of gravistimulated roots occurs by an auxin-independent mechanism.

Analysis of short-term auxin and gravitropic responses in mutants of Arabidopsis with reduced auxin sensitivity. With the modifications of the digitizer system described above, we have been able to do high resolution growth studies of the auxin response in roots of wild type and auxin resistant mutants of Arabidopsis. The short term goals of these experiments are 1) to compare the kinetics of the auxin response in these very small (rapid auxin uptake and equilibration) roots with known data from larger roots, 2) to compare the kinetics of the response in the mutants with that of the wild type in order to gain more information on the nature of the mutants (e.g. will differences in the kinetics indicate impaired hormone uptake, enhanced adaptation, ...), and 3) to compare the kinetics of the gravity response in wild type and auxin resistant roots in order to assess the role of auxin in various phases of the response. The longer term goal of these experiments (will require further enhancement of digitizer capability) is to determine which cells show the earliest growth responses following gravistimulation so that we can focus our attention on these cells for the electrophysiology experiments proposed in this continuation application.

Briefly, our Arabidopsis studies have shown that 1) the kinetics of the auxin response are similar in roots of Arabidopsis and maize, indicating that uptake time is not a major contributor to the observed 15-20 min lag, 2) the kinetics of the response in the auxin-resistant mutants is similar to that of wild type indicating that the low auxin sensitivity is not caused by poor uptake of auxin or by accelerated adaptation to the hormone, and 3) very low concentrations (e.g 0.1 nM) of auxin stimulate the elongation of intact roots of Arabidopsis. This indicates that the endogenous auxin level is suboptimal in these roots, an observation with important implications for the

assessment of the role of auxin in gravitropism and for the general validity of the Cholodny-Went theory.

Analysis of the effects of gravity and thigmostimulation on surface electrical currents in maize roots. Our work on this aspect of the proposal during 1992 was largely preliminary. However, we determined that there are large surface potentials along maize roots and that tactile stimulation induces large depolarization of these potentials with a very repeatable biphasic pattern. We designed a specialized multi-chamber device for electrical isolation of restricted zones of the root and we propose to use this chamber for analysis of localized electrical changes associated with maize root responses to gravity and touch.

Papers published or in press during this funding period.

- 1991 Jones AM, Cochran DS, Lamerson PM, Evans ML, Cohen JD Red light-regulated growth. I. Changes in the abundance of indoleacetic acid and a 22-kilodalton auxin-binding protein in the maize mesocotyl. *Plant Physiol.* 97: 352-358
- 1991 Cleland RE, Buckley G, Nowbar S, Lew NM, Stinemetz C, Evans ML, Rayle DL The pH profile for acid-induced elongation of coleoptile and epicotyl sections is consistent with the acid-growth theory. *Planta* 186: 70-74
- 1991 Kiss HG, Evans ML, Johnson JD Cytoplasmic calcium levels in protoplasts from the cap and elongation zone of maize roots. *Protoplasma* 163: 181-188
- 1992 Evans ML, Young LM, Hasenstein KH. The role of calcium in the regulation of hormone transport in gravistimulated roots. *Advances in Space Research* 12: 211-218
- 1992 Evans ML What remains of the Cholodny-Went theory? (contribution to multi-author forum). *Plant, Cell and Environment* 15: 767-768 (entire forum = pp 759-794)
- 1992 Ishikawa, H, Evans ML Induction of curvature in maize roots by calcium or by thigmostimulation: Role of the postmitotic isodiametric growth zone. *Plant Physiol* 100: 762-768
- 1992 Stinemetz CL, Hasenstein KH, Young LM, Evans ML. Effect of calmodulin antagonists on the growth and graviresponsiveness of primary roots of maize. *J Plant Growth Regul* 11: 419-427
- 1992 Cha MR, Evans ML, Hangarter RP Localization of IAA-transporting tissue in *Coleus* stems and corn coleoptiles. *Plant Physiol and Biochem* 31: 263-269
- 1992 Ishikawa H, Evans ML Correlations between changes in electrical parameters and changes in cell elongation rates in gravistimulated plants. *Advances in Space Research* (in press)
- 1993 Ishikawa H, Evans ML The role of the distal elongation zone in the response of maize roots to auxin and gravity. *Plant Physiol* (in press)

New Technology

None

Property Acquired with Federal Funds

None

Unencumbered Balance

There is currently an unencumbered balance of approximately \$6000 in the budget. This money is earmarked for undergraduate assistant wages and for supplies.