THE REGULATION OF GROWTH IN THE DISTAL ELONGATION ZONE OF MAIZE ROOTS

FINAL REPORT
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PRINCIPAL INVESTIGATOR
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Goals:
The major goals of the proposed research were 1. To develop specialized software for automated whole surface root expansion analysis and to develop technology for controlled placement of surface electrodes for analysis of relationships between root growth and root pH and electrophysiological properties. 2. To measure surface pH patterns and determine the possible role of proton flux in gravitropic sensing or response, and 3. To determine the role of auxin transport in establishment of patterns of proton flux and electrical gradients during the gravitropic response of roots with special emphasis on the role of the distal elongation zone in the early phases of the gravitropic response.

Abstract from Original Proposal:
We have characterized a special zone of cells near the apex of maize roots. We refer to this group of cells as the distal elongation zone (DEZ). The DEZ is exceptionally responsive to environmental stimuli, exhibiting rapid changes in growth in response to gravistimulation. Prior work on the response of roots to gravity has ignored this special zone of cells. During the past several years we have developed computer software that allows us to use video digitizing to examine precise growth patterns both in the DEZ and the main elongation zone (MEZ). We propose to use this methodology to examine the physiological basis of growth regulation in the DEZ with special emphasis on the nature of gravi-induced growth modification in this zone. Prior research on the control of root growth by wall pH, growth hormones (e.g. auxin), and calcium has been compromised by the fact that growth measurements were made on the entire root including both the DEZ and the MEZ which we now know exhibit widely differing (in some cases opposite) responses to these potential growth regulating factors. In order to understand the nature of the gravitropic response of roots, we need to understand the motor mechanism (differential growth mechanism) mediating root reorientation in a gravitational field. The work proposed here examines the interaction of auxin, acid efflux, and calcium in the control of elongation in the DEZ as compared with the MEZ and seeks to use this information to identify the key components of the gravitropic motor response. The proposed work will lead to further advances in computerized video analysis of growth in plants, a technique that should become increasingly important to plant research in space

where there is a need for maximum extraction of data from limited numbers of experimental samples.

Accomplishments

1. Establishment of constant gravistimulation conditions -- new revelations on the nature of root gravitropism.

During our tests of the performance of surface pH marking beads it became clear that, because of the buffering capacity of the beads, it would be advantageous to develop a method for long-term gravistimulation to maintain fixed rates of proton efflux over a prolonged period. Fortunately, during the previous funding period we had developed a device (called ROTATO) capable of manipulating an actively responding root so that the root tip remains constantly gravistimulated. Using this device, we learned that roots display long-term non-damping gravitropic responses as long as the input signal (gravistimulation) remains constant. As controls for these experiments, we examined the shut-off time for gravitropism under conditions where the tip of a responding root is suddenly reoriented and automatically maintained in a vertical orientation. This led to the startling finding that roots with the tips vertical (no gravistimulation) but with portions of the elongation zone displaced from vertical continue to sense gravity for an extended period of time! The startling conclusion is that there is gravity sensing outside of the root cap. This alters the dogma of root gravitropism that has been in force for more than 150 years. A manuscript on this finding (see publication list item #5) is currently under review. We believe that this finding will have a major impact on our understanding of gravitropism and will provide an answer to some puzzling features of gravitropism that have heretofore been difficult to reconcile. It also opens a whole new sub-field of gravitropism relating to the nature of the second gravity sensor and emphasizes the importance of the findings outlined below relative to the control of gravitropic responses in the distal elongation zone (DEZ).

2. A role for potassium ions and protons in gravitropic signaling and/or response -- new data on molecular mechanisms underlying gravitropism.

After determining that there is long-term proton efflux within the distal elongation zone of graviresponding roots during long term gravistimulation, we turned our attention to the issue of counter ions that might be involved in electrically balancing this proton flux. This approach was slightly different from the original plan in that it focused on the sub-cellular basis of the flux patterns more than on the precise zone of efflux activity. However, this approach proved to be particularly fruitful. We examined the potential involvement of both anions and cations (potassium) in proton flux related to gravitropic signaling/response in the following ways. We determined that the anion channel blocker, NPPB severely retards root gravitropism without inhibiting root growth. Furthermore, it appears to interfere most strongly with the DEZ response, the region in which gravity induced proton efflux is most active. We established that this is not simply a general toxic effect because, in addition to the fact that NPPB causes no growth inhibition, it failed to interfere with root phototropism; to the contrary, roots treated with NPPB showed enhanced root phototropism. In collaboration with Edgar Spalding at the

University of Wisconsin, we learned (based on patch clamp experiments) that protoplasts from roots of Arabidopsis have an anion channel at the plasma membrane that is highly sensitive to NPPB. These results indicate that the activation of anion channels may be a crucial early event in the gravitropic response of Arabidopsis roots. We extended these observations to examine the potential role of potassium as a counterbalancing cation during gravitropic signaling. Our approach was to use mutants displaying T-DNA disruptions in the Arabidopsis K^+ channel genes AKT1 and AKT2. These mutants (e.g. akt1-1) show a reduction in gravity perception (extended presentation time) and both a delay in initiation of curvature as well as a reduction in the curvature rate. In collaboration with the Simon Gilroy laboratory at Penn State University, we also learned that the mutants lack the cytoplasmic and wall pH changes characteristic of gravity perception by the columella cells of the root cap. These results indicate that the AKT family of K^+ channels are closely associated with gravity sensing and gravitropic growth responses in roots. These experiments are leading us toward a molecular understanding of gravitropic signaling. The key items from the publication list relevant to these accomplishments are items #4 and #9.

3. <u>Relationship between ion fluxes and responses of roots to imposed electrical gradients in microgravity and under 1g.</u>

The results outlined above indicated that ion movement/electrical events play a key role in gravitropic signaling and or the gravitropic motor response. In view of this new evidence, we investigated the ability of imposed electrical fields to activate differential elongation in selected regions of the root elongation zone. Further incentive for these experiments was provided by our investigations on board STS-95 during which we measured the response of roots to applied electrical gradients under microgravity. The following key findings arose from these investigations. 1. Exposure of roots to an exogenous DC electrical field induces curvature in opposite directions within two distinct zones of the root, the central elongation zone (CEZ, curvature toward the positive electrode) and the DEZ (curvature toward the negative electrode). These oppositely-directed responses could be reproduced individually by localized electric field application to the region of response. This indicates that both are true responses to the electric field, rather than one being a secondary response to an induced gravitropic stimulation. The individual responses differed in the type of differential growth giving rise to curvature. In the CEZ, curvature was driven by inhibition of elongation, while curvature in the DEZ was primarily due to stimulation of elongation. This stimulation of elongation is consistent with the growth response of the DEZ when the root is gravistimulated. It appears that artificial imposition of an electrical gradient in the DEZ induces a curvature pattern similar to that which occurs in the DEZ of gravistimulated roots. Since we know that the DEZ develops natural electrical gradients upon gravistimulation, it appears that the electrical gradient is a key factor in either signaling or motor activation in the DEZ.

When we applied an electric field to roots growing in microgravity during STS-95 we observed immediate inhibition of root elongation, a response that did not occur under 1g. We interpret this result as an indication that, in the absence of a gravity stimulus, the sensitivity of the root to an applied electric stimulus is increased. We investigated the physiological basis for this difference in ground based and space flight electrotropic responses by measuring the dependence of growth and electrotropism on applied field strength in ground-based experiments. Application of higher field strength in ground based experiments caused growth inhibition and reduced curvature similar to that observed in flight experiments using weaker field strength. The results indicate that the microgravity conditions of space cause an increase in the sensitivity of roots to electrical stimulation. We conclude that induction of electrotropic curvature during space flight will require reduced stimulus strength. We hypothesize that the shift in electrical sensitivity under microgravity is closely linked to the strong influence of gravity on endogenous electrical patterns under 1g and the probability that the background electrical gradient against which imposed fields are measured is strongly modified under microgravity. The key items from the publication list relevant to these accomplishments are items #1, 3, and 10.

Papers published during this funding period. (Italics indicates directly related to this project and funding period. Normal text indicates publications resulting from unanticipated directions generated during this research)

1. *Wolverton C, Mullen JL, Aizawa S, Yoshizaki I, Kamigaichi S, Mukai C, Shimazu T, Fukui K, Evans ML, Ishikawa H (1999) Inhibition of root elongation in microgravity by an applied electric field. J Plant Research 112:493-496*

2. Mullen JL,Wolverton C, Ishikawa H, Evans ML 2000 Kinetics of constant gravitropic stimulus responses in Arabidopsis roots using a feedback system. Plant Physiol 123: 665-670

3. *Wolverton C, Mullen JL, Ishikawa H, Evans ML 2000 Two distinct regions of response drive differential growth in Vigna root electrotropism. Plant Cell & Envir 23: 1275-1280*

Papers submitted and currently undergoing review.

4. *Wolverton C, Lewis B, Evans ML, Ishikawa H, Spalding E. (2001) An anion channel inhibitor selectively blocks Arabidopsis root gravitropism but not phototropism. Plant Physiology*

5. *Wolverton C, Mullen JL, Ishikawa H, Evans ML (2001). Root gravitropism in response to a signal originating outside of the cap. Planta*

Papers to be submitted during the next few months (in final stages)

6. Wolverton C, Mullen JL, Ishikawa H, Evans ML (2001) Maize root gravitropism: A re-examination of response kinetics and dose-response relationships using a gravity compensation device. Plant Physiology

7. Zhao H, Hertel R, Ishikawa H, Evans ML (2001) Species differences in ligand specificity of auxin-controlled elongation and auxin transport: comparing Zea and Vigna. Physiologia Plantarum

8. Wolverton C, Mullen JL, Ishikawa H, Hangarter R, Evans ML (2001) Characterization of negative phototropism in primary roots of maize. Planta

9. *Hirsch R, Wolverton W, Fasano J, Minnich P, Ishikawa H, Evans ML, Gilroy S. (2001) The role of K+ channels in the gravitropic responsse of Arabidopsis roots. Plant Cell.*

10. *Wolverton C, Mullen JL, Ishikawa H, Evans ML (2001) Effects of applied electric fields on the growth of Vigna mungo roots under 1 g and during space flight. Physiologia Plantarum*

New Technology

The research supported by this grant has led to improvements in the computer-controlled seedling positioning device developed during the prior funding period. This device, (nicknamed ROTATO) is a powerful new tool for analysis of plant tropic responses, including phototropism and gravitropism. In addition to improvments in the ROTATO hardware and software itself, we have developed an ancillary control program that improves the flexibility of ROTATO. This program is dubbed "ROTINI." ROTINI allows the user to input any particular plant manipulation protocol. In conjunction with ROTATO, the plant is then exposed to that specific sequence of manipulations. As examples of the kinds of experiments made possible by this technology, we can expose roots (or shoots) to a given pattern of gravistimulation such as slow rotation to a fixed value, rapid rotation to a fixed value, repeated pulse stimulations, and so forth. This opens the door to a new era of quantitative analysis of gravistimulation and response. We predict that application of this new technology will lead to significant new insights into the nature of gravisensing in plants.

Unencumbered Balance

None

Figures

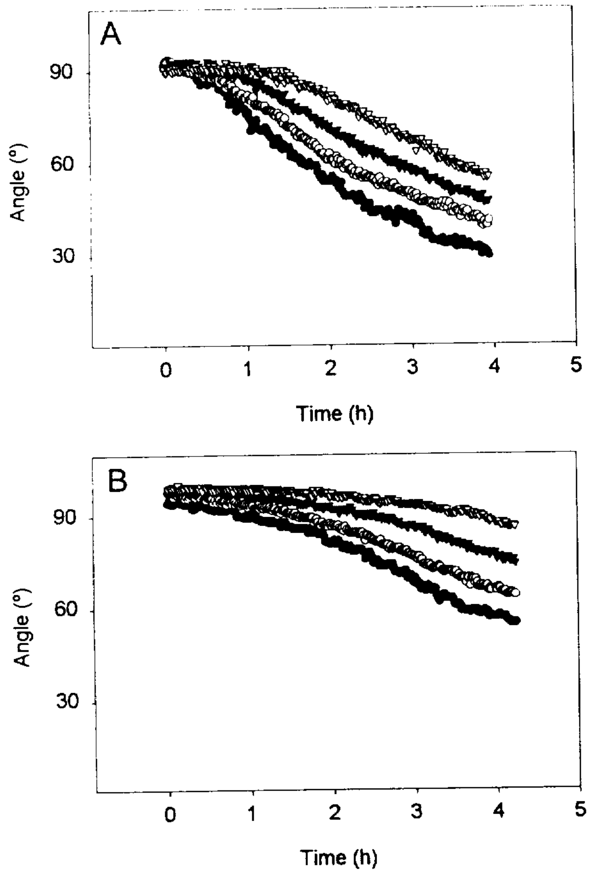

Figure 1. The akt1 mutant of Arabidopsis lacks early phase (most likely DEZ-mediated) gravitropism and has impaired late phase gravitropism. Part A shows the kinetics of gravitropism in wild type roots. The lines represent the change in angle of a series of apical root segments following gravistimulation. The pattern of line separation provides information on the location of curvature initiation. Note the early initiation of curvature and the rapid rate of curvature. Part B shows the kinetics of gravitropism in roots of the akt1 mutant. Note that the early phase of gravitropism is missing and that even the prolonged curvature is reduced in rate. The impairment of gravitropism in this mutant may relate to impaired proton/K^+ exchange or to impaired osmoregulation.

The key point of this figure is that impairment of potassium uptake severely curtails gravitropism. The response of the distal elongation zone to gravity is especially affected, indicating that proton/potassium exchange or some role of potassium in electrical signaling may be a key factor in signal transduction for this component of gravitropism.

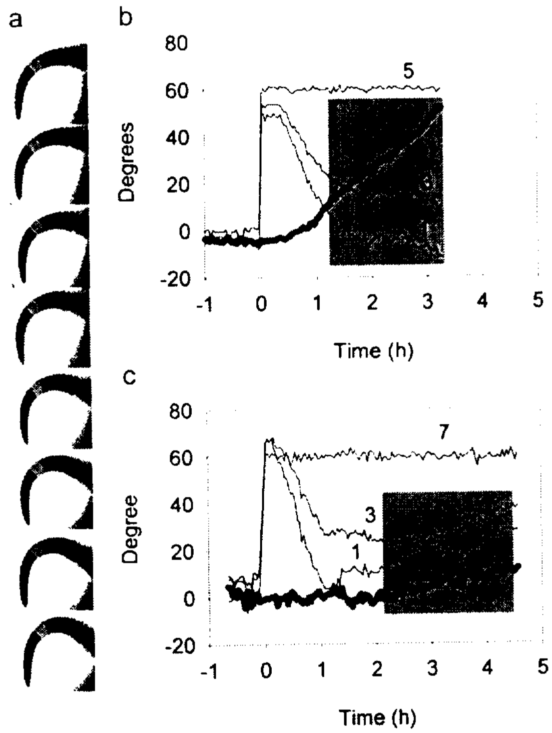

Figure 2: Signals arising from outside the root cap can contribute to gravitropic curvature. A. Images of a root responding to gravity with the cap at vertical. The root was initially stimulated at 60° with the highlighted segment (4-5 mm from the root tip) maintained at 60° throughout the response. The images show changes in root shape during the 2 h time period after the root tip reached vertical. The root generated 30° of curvature during this time as indicated by the rotation of the root base. B. Data collected from an experiment identical to that described in part A. The lines labeled 1, 3, and 5 represent change in angle of root segments 1, 3 and 5 which are located at 0-1, 2-3, and 4-5 mm respectively from the root tip. Segment 5 was maintained at 60° while segments 1 and 3 were allowed to curve toward vertical. The heavy ascending line represents the rotation required to maintain segment 5 at 60°. Continued generation of curvature after the root tip reaches vertical is evident from the fact that segment 1 remains vertical despite the rotation of the root required to maintain segment 5 at 60° (shaded region). This experiment was repeated 9 times with similar results. C. Control: the region 6-7 mm from the tip (segment 7) was maintained at 60°. The absence of continued curvature after the root tip (segment 1) reaches vertical is evident from the upward displacement of this segment at the same rate as rotation (shaded region).

The key point of this figure is that roots continue to sense gravity even when the root tip (cap) is vertical (non-gravistimulated). This suggests that there is a sensor outside of the root cap, a finding that is contrary to the dogma of gravitropism that has existed for more than 150 years. This finding should have a profound effect on current efforts to link sensing and response during gravitropism.