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Inhibition of root elongation in microgravity by an applied electric field

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Abstract Roots grown in an applied electric field demonstrate a bidirectional curvature. To further understand the nature of this response and its implications for the regulation of differential growth, we applied an electric field to roots growing in microgravity. We found that growth rates of roots in microgravity were higher than growth rates of ground controls. Immediately upon application of the electric field, root elongation was inhibited. 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. Further space experiments are required to determine the extent to which this sensitivity is shifted. The implications of this result are discussed in relation to gravitropic signaling and the regulation of differential cell elongation in the root.

Key words:Electric field, Growth, Microgravity, Root, Vigna

Introduction

Plant roots begin to respond to gravity in a region between the meristem and the central elongation zone [CEZ] that has been called the distal elongation zone [DEZ] (Ishikawa *et al.* 1991, Zieschang and Sievers 1991). The cells making up this region are in a transition phase between division and expansion. Differential polar expansion can be initiated by external stimuli such as gravity or touch, but the mechanism governing the switch to rapid differential expansion is unknown but apparently auxin-independent (Ishikawa and Evans 1993).

One possible candidate for controlling DEZ cell expansion is electrical signaling. Vertical roots generate a pattern of current consisting of influx in the meristem and DEZ and efflux in the CEZ (Iwabuchi *et al.* 1989, Weisenseel *et al.* 1992). This current pattern changes upon rotation of the root from vertical to horizontal, resulting in efflux along the upper flank of the DEZ (Collings *et al.* 1992, Iwabuchi *et al.* 1989). Electrical changes have also been observed at the single-cell level. Membrane potentials of cells along the upper side of horizontal roots were shown to hyperpolarize, while depolarization was observed along the lower side (Ishikawa and Evans 1990a).

To further address the role of electrical changes as they relate to DEZ cell expansion, we have coupled high-resolution image analysis with exogenous application of an electric field. Previous results suggest some variability in the direction of response. Ishikawa and Evans (1990b) concluded that roots curve toward the anode (+), while Stenz and Weisenseel (1993) concluded that healthy, intact roots curve toward the cathode (-). In both cases roots were occasionally observed curving in both directions. This bidirectional curvature was attributed to an electrotropic response followed by a gravitropic response. This conclusion was supported by the lack of a bidirectional curvature in decapped roots, which were presumably unable to sense the gravity stimulus.

To test more rigorously the true nature of the electrotropic response and its interplay with the gravitropic response, we utilized the microgravity environment of low Earth orbit. Plants were flown aboard the U.S. Space Shuttle Discovery STS-95 in October-November, 1998. We were specifically interested in whether roots curve toward the anode, cathode, or both in the absence of gravity.

Materials and Methods

Plant Material and Growth Conditions

Seeds of *Vigna mungo* L. and caryopses of *Zea mays* L. cv. Merit were sterilized with 5.25% sodium hypochlorite (Clorox) for 2-3 min and placed on Petri dishes between paper towels moistened with distilled water. After 48 hr in a growth chamber with continuous illumination at 25 C, germinated seedlings were transferred to flight dishes. The dishes contained 0.8% (w/v) agarose (Life Technologies, Inc., Rockville, MD, USA), 1 mM KCl, 1 mM $CaCl_2$, 1 mM MES buffer, pH 5.8. Specialized inserts for standard 60 mm Petri dishes containing parallel platinum electrodes were designed for application of an electric field in microgravity (Fig. 1) (Chiyoda Corp., Yokohama, Japan). The dish was designed with simple wire leads that connect to a separate battery pack. The battery pack contained a voltage selector and a LED current indicator to ensure proper electrical connectivity. Immediately after transfer of seedlings, flight dishes were placed at 4°C, where they

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remained throughout launch and flight until removed in flight by the crew.

Orientation of roots for electrotropism

Upon removal from 4∞ C storage on flight, dishes were placed in unilateral light for 2 h inside the Spacehab single module (Spacehab Inc., Washington, D.C., USA). Unilateral light was applied by placing the petri dish inside a black holder containing a slit on one side. This assembly was placed adjacent to a white fluorescent lamp (estimated fluence rate received by plants £ 10 mmol m^{-2} s^{-1}). The purpose of the chamber was to provide a directional cue during cold recovery so that roots would begin elongating parallel to the electrodes. The majority of roots showing a phototropic response to visible light exhibit negative phototropism (Hubert and Funke 1937). This includes roots of maize (Schneider 1964) and *Arabidopsis* (Okada and Shimura 1992).

In a related experiment, we tested the effectiveness of unilateral light for orienting seedlings in microgravity. The effectiveness of this treatment is demonstrated in Fig. 2, which shows seedlings of *Arabidopsis* before and after phototropic stimulus. We found significantly less variance ($P = 0.017$, f-test) in angle of hypocotyls of seedlings exposed to unilateral light compared with dark (data not shown). Likewise, the roots exposed to unilateral light before electric field application elongated parallel to the electrodes (Fig. 1). Therefore, we believe the unilateral light treatment was helpful for maintaining the parallel orientation of roots relative to electrodes.

Time-lapse video recording of growth

Following the recovery period, dishes were mounted to the lens hood of a NASA-modified Hi-8 video recorder (Canon Inc., Tokyo, Japan) and data collection was initiated. After approx. 1.5 h, the crew applied an electric field to the roots for approx. 30 min. A total of 10 h of time-lapse video was obtained for two separate dishes, each containing 3 seedlings.

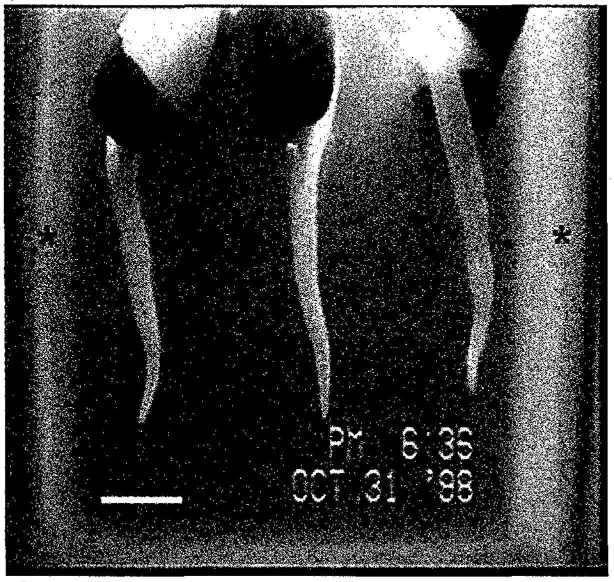

Fig. 1 Flight dish containing insert for electric field application. (*), platinum electrodes. Bar = 500 mm.

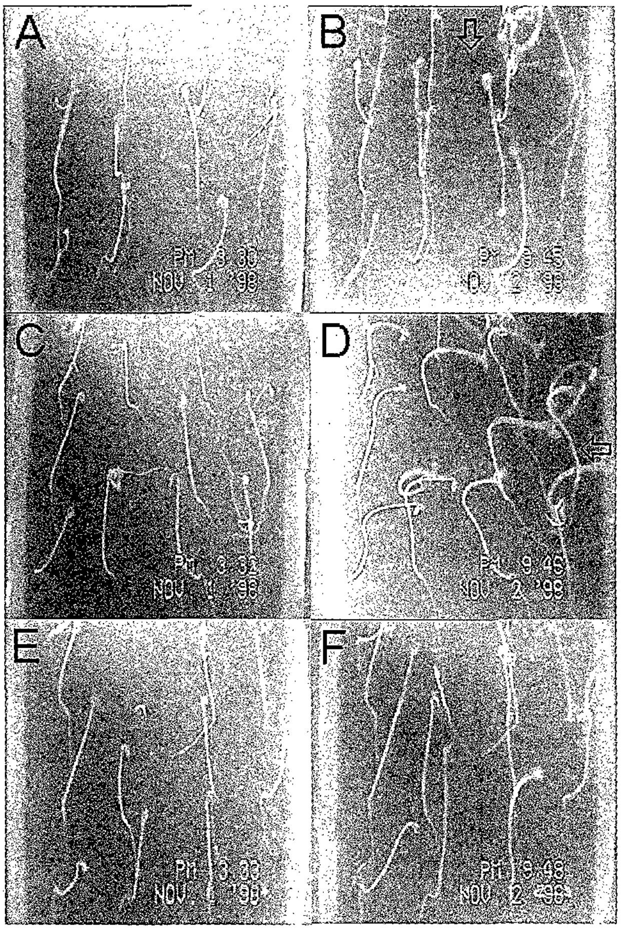

Fig. 2 Phototropism of *Arabidopsis* hypocotyls in microgravity. A, C, and E, seedlings before light treatment. B, D, and F, seedlings after 30 h light from above, light from right, and darkness, respectively. Arrows indicate direction of unilateral light.

Analysis of growth

Time-lapse video of growth and electrotropic response was analyzed in two ways. Custom software written in our laboratory and used routinely for growth analysis (Mullen *et al.* 1998) was applied to video playback. However, due to frequent vibrations and changes in light intensity in the flight cabin, this technique did not provide high accuracy. Therefore, we sampled images from the time-lapse video, imported them into an image analysis package (ImageTool 1.28, a port of NIH Image, University of Texas Health Science Center in San Antonio, San Antonio, TX, USA) and measured pixel-calibrated lengths of roots over time. Data acquired in either manner were imported into a spreadsheet application (Microsoft Excel 97, Microsoft Corp., Redmond, WA, USA, or SigmaPlot 4.0, SPSS Inc., Chicago, IL, USA) and analyzed using standard spreadsheet operations.

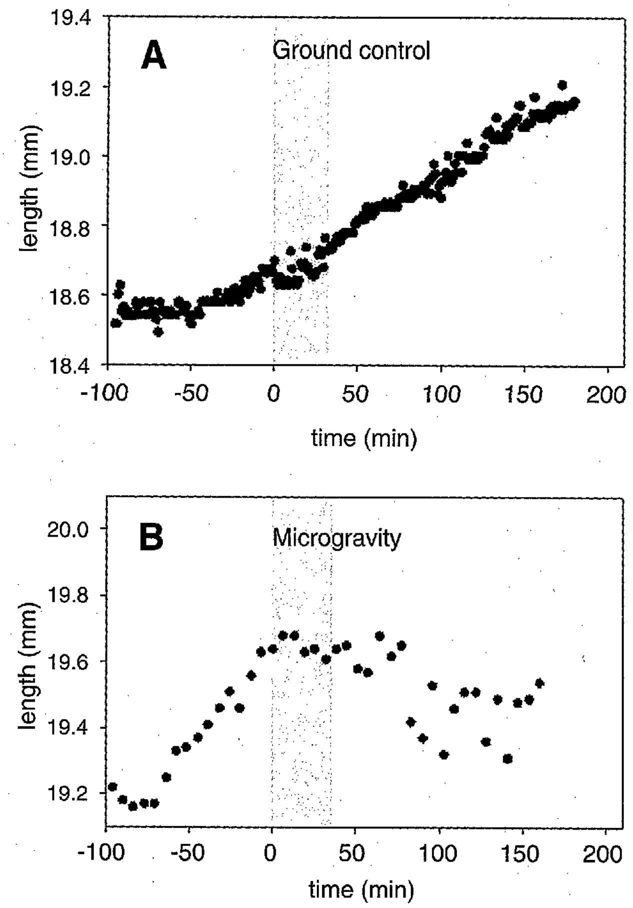

Fig. 3 Effect of electric field application on root growth. A field of 1.5 V cm^{-1} was applied at 0 min for ~30 min (shaded area). A, representative growth data for a root of *Vigna mungo* in 1 *g* (n = 12). B, representative growth data for a root of *Vigna mungo* in microgravity (n = 3).

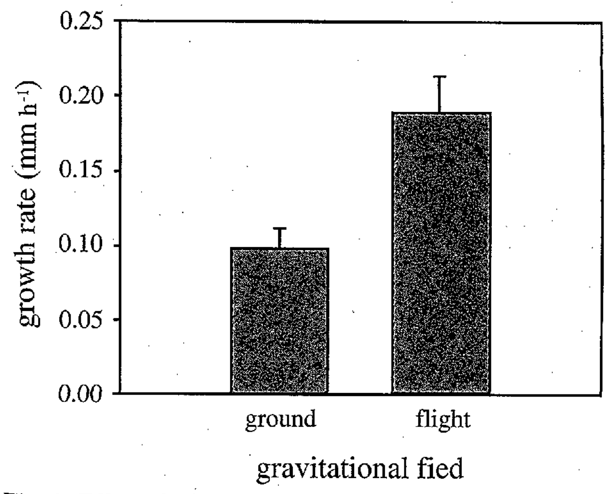

Fig. 4. Effect of microgravity on root elongation rate. Growth rate was calculated over the 2 h prior to electric field application. Error bars indicate SE; n = 12 for ground controls, 3 for flight experiments.

Results

Following 2 h of recovery in unilateral light, ground control seedling roots appeared to be healthy and elongating rapidly (Fig. 3a). This indicates that the roots were able to recover from prolonged cold treatment. When compared to the sample data in Fig. 3b from flight seedlings, it is apparent that the absolute rate of elongation before application of the electric field was higher in microgravity than in 1 *g*. This observation is further substantiated by the calculated average growth rates of ground and flight roots (Fig. 4). While the growth rate of ground control roots was 0.10 ± 0.01 mm h^{-1} (n = 12), the growth rate of roots in microgravity was 0.19 ± 0.02 mm h^{-1} (n = 3, Fig.5 and Fig.6), a significant difference (P= 0.01, t-test).

Upon application of an electric field of 1.5 V cm^{-1} in microgravity, elongation of primary seedling roots ceased and elongation remained inhibited even after removal of the field (Fig. 3b). In contrast, the same electric field applied in 1 *g* had little long-term effect on elongation rates (Fig. 3a).

Discussion

When an electric field is applied to vertical roots, the response is a bidirectional curvature (Ishikawa and Evans 1990b, Stenz and Weisenseel 1992). The two possible interpretations of this result are that curvature in both directions is in response to the electric field or that curvature in one direction is a response to the electric field and curvature in the opposite direction is a response to the introduced gravity stimulus. Decapping experiments support the latter conclusion, because removing the root cap, which renders roots non-responsive to gravity, appears to eliminate one portion of the dual electrotropic response (Ishikawa and Evans 1990b).

Because we observed strong growth inhibition upon application of an electric field to roots in microgravity, our results do not resolve the bidirectional nature of the response in ground controls. However, our findings lend insight into the interplay between the gravitropic response and electrical events. Inhibition of elongation in microgravity by an applied electric field suggests an altered sensitivity to the electrical stimulus in the absence of a gravity stimulus. If the electrotropic response occurs by altering endogenous electric signals in the root environment and if the magnitude of these endogenous signals is decreased in the absence of a gravity stimulus, then the effectiveness of the electric field applied in microgravity is expected to be greater. Several implications follow from this interpretation of our results.

If the effectiveness of the applied electric field is greater in microgravity, then similar inhibition of elongation should be observed at higher electric field strengths in 1 *g*. Indeed, preliminary evidence indicates that such inhibition of elongation occurs at higher electric field strengths in 1 *g* (C. Wolverton, J.L. Mullen, M.L. Evans, and H. Ishikawa, unpublished data). This supports the idea that the threshold electrical sensitivity is shifted in microgravity.

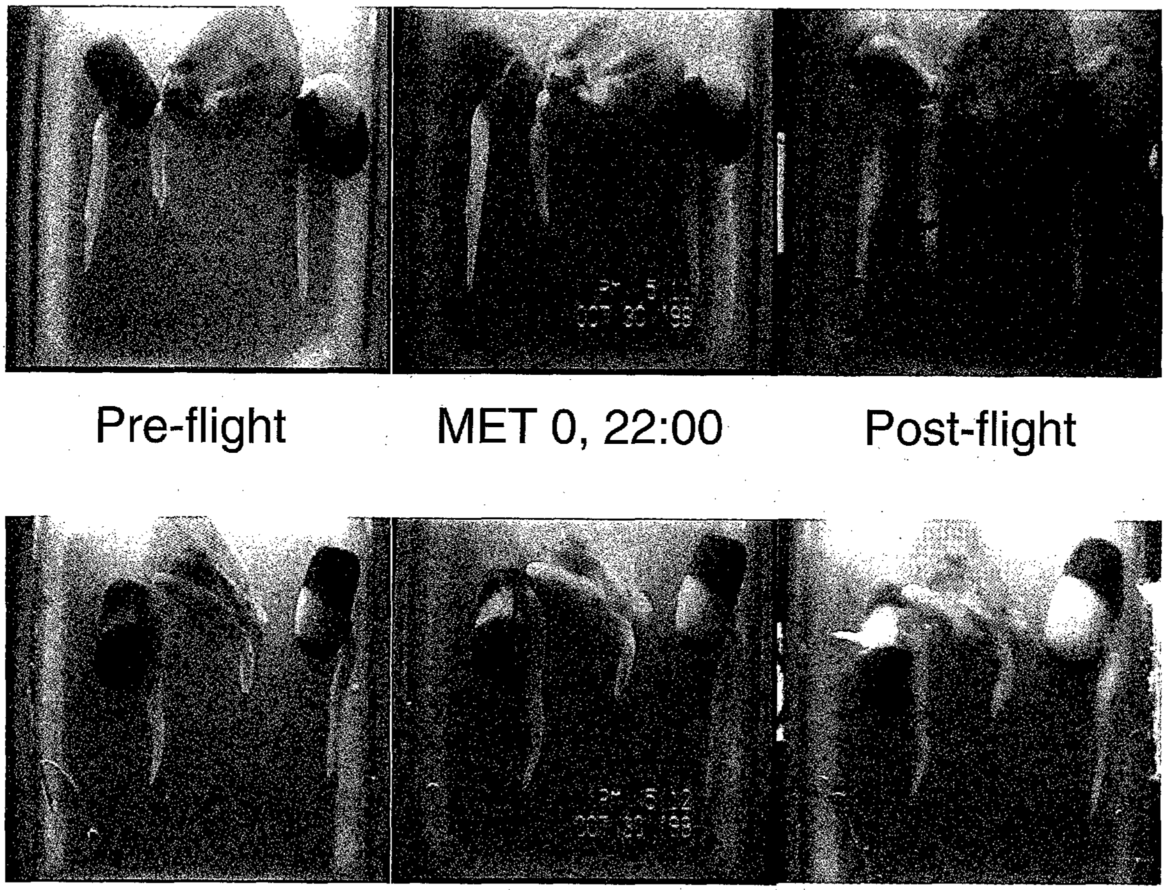

Fig.5 Electrotropism of bean(*Vigna mungo*) and corn(Zea mays) roots in microgravity. Experiment was conducted at the first day of the flight (MET 0). Top: No application of the electric field in microgravity, Bottom: Application of the electric field in microgravity.

A further implication of this conclusion is that the gravity signal introduces or modulates some endogenous electrical property of the root. This would be in agreement with previous findings detailing changes in electrical properties observed after application of a gravity stimulus (for a review, see Weisenseel and Meyer 1997). Modulation of an electrical signal by gravity may also help to explain the observation of a greater elongation rate in microgravity. In the absence of a regulating gravity signal, it is possible that control over cell elongation is altered. There is conflicting evidence in the literature for microgravity effects on root elongation, with Brown *et al.* (1995) finding increased rates, Johnsson *et al.* (1996) finding decreased rates, and Legué *et al.* (1996) finding no difference. However, none of these reports was capable of the high temporal and spatial resolution achieved in this report.

We are interested in the mechanism by which the gravity stimulus exerts this electrical effect on the root. To what extent is the effectiveness of an applied electric field shifted in the absence of gravity? Can the bidirectional response be induced in microgravity at lower field strengths? Is electrical signaling involved in regulating the transition to cell elongation in the DEZ?

In conclusion, the inhibition of elongation by an applied electric field in microgravity argues for some involvement of electrical signaling in root growth and gravitropism. Further microgravity experiments are necessary to determine the magnitude of gravity-induced electrical signals and whether differential growth can be triggered by an applied electric field in microgravity.

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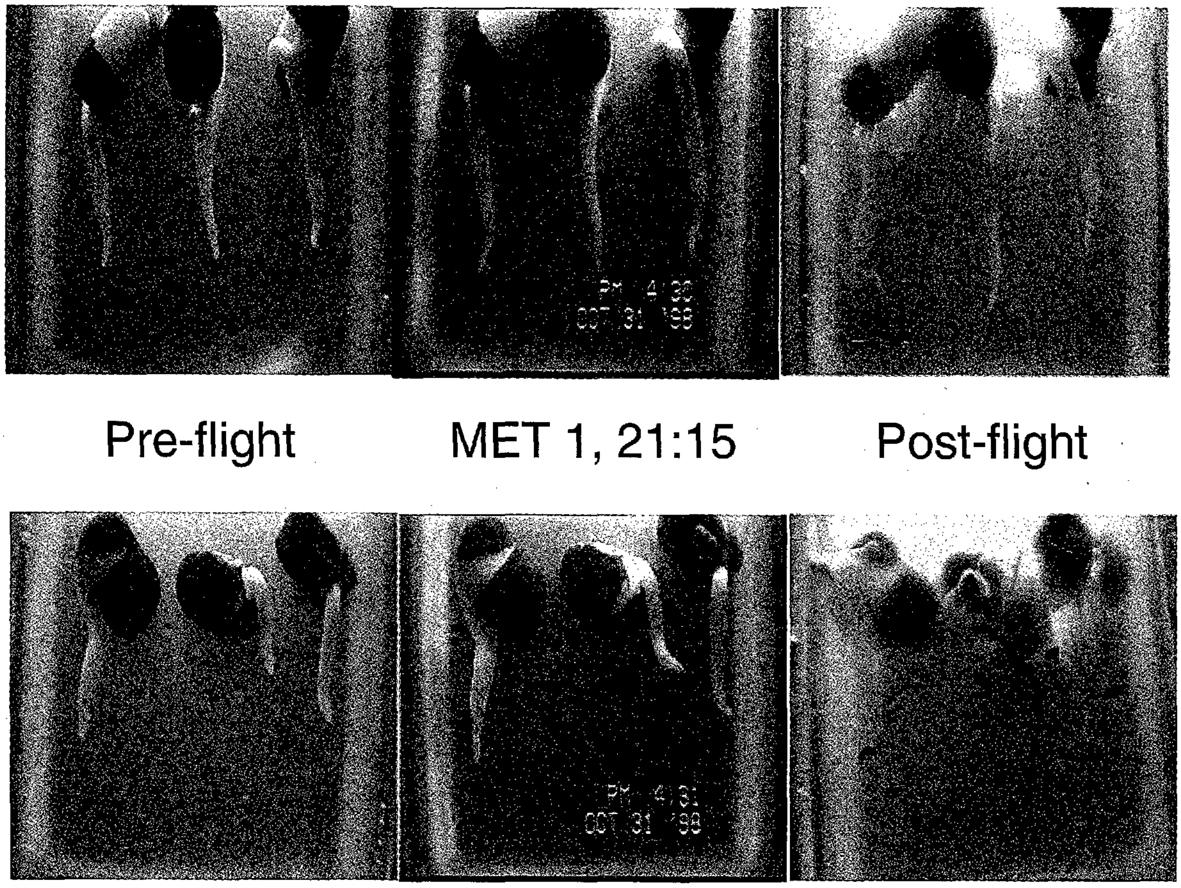

Fig.6 Electrotropism of Vigna mungo roots in microgravity. Experiment was conducted at the second day of the flight (MET 1). Top: Application of the electric field in microgravity, Bottom: No application of the electric field in microgravity.

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