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Communication

A Two-Dimensional Vibrating Probe Study of Currents around Lateral Roots of *Raphanus sativus* Developing in Culture[1]

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ABSTRACT

A computer-assisted, two-dimensional vibrating probe was used to study the ionic currents around developing lateral roots of *Raphanus sativus in vitro*. This system allowed us to superimpose current vectors on the video image of the roots. In a young lateral root, current entered the cap, meristematic, and elongation zones and exited the primary root surface close to the base of the lateral root. As the lateral root grew, current began to exit from its basal (cell maturation zone) end. The densities of currents entering the apical portion of the faster-growing lateral roots in a medium lacking indole 3-acetic acid were about twice as large as those entering the apical region of the slower-growing lateral roots in indole 3-acetic acid-supplemented medium.

Roots (2, 9–11, 18) are known to generate steady ionic currents that are associated with their morphological polarity. The extracellular component of these currents produces an electrical field in an aqueous medium that can be detected by a vibrating probe (7). The currents are believed to be part of a feedback mechanism that acts to maintain developmental polarity. Their role in development is supported by the fact that a change in the magnitude and/or pattern of ion flow is one of the primary events in the developmental response to an external stimulus (2, 3). In addition, artificially applied electric fields are also known to affect development (15). Various ways in which endogenous currents might interact with developing organisms have been proposed. The electrical fields set up by the currents could affect development by causing an electrophoretic (or electro-osmotic) redistribution of the charged entities in the cytoplasm (8, 20) and/or the plasma membrane (17). Also a localized influx of a specific ion, such as Ca^{2+} or H^+ might result in a cytoplasmic gradient which could provide positional information via its effect on the cytoskeleton or other cytoplasmic components (5).

A number of studies, using the one-dimensional vibrating probe, have demonstrated steady ionic currents around the apical portions of growing primary roots of intact seedlings (2, 3, 6, 9–11, 18). One recent paper (11) reports a set of current measurements near the primary root surface of a tobacco seedling prior to lateral root emergence, and another set of measurements around a 1.5 mm long lateral root. Lateral roots can also be induced on root segments growing in culture (4). The media that are used to grow such organ cultures (4) have relatively high ionic concentration, *e.g.* K^+ is about 20 mM in the Murashige and Skoog (13) medium compared to about 0.1 mM found in soil solution. We were interested to see if the lateral roots developing under such artificial culture conditions would have endogenous currents similar to those detected around primary and lateral roots growing under more physiological ionic conditions. Culturing root segments in a defined medium offers two other advantages; first, growth conditions can be easily manipulated, and second, effects of exogenous hormones can be tested without interference from the endogenous hormones supplied by the shoot or the tip of the main root in an intact seedling.

The use of a circularly oscillating probe, based on the design of Nuccitelli (14), permitted us to obtain a two-dimensional picture of the current pattern in the horizontal plane. With a conventional one-dimensional probe, two measurements at each point are required to detect the two orthogonal components of the current vector. This necessitates rotating the probe or the root by 90° and taking a second reading in exactly the same location as the first. Such precise positioning of the probe is difficult, and such measurements would be impossible in cases where rapid changes in current magnitude or direction occur.

MATERIALS AND METHODS

Radish (*Raphanus sativus* L. cv 'Scarlet Globe') seedlings were grown under sterile conditions in the dark at 23°C for 3 to 4 d in 0.1 mM $CaCl_2$. Root segments measuring 2.5 cm were excised, starting 0.5 to 1.0 cm behind the root tip. These segments were cultured in medium composed of Murashige and Skoog (13) salts, 3% sucrose, 10 mM Mes, and 10 mM succinic acid, with or without 30 μM IAA (pH 6.0). Each root segment was cultured in 25 mL of the medium in a 100 mL flask and incubated on an orbital shaker (80 rpm) in the dark for 4 to 6 d. Root segments that had lateral roots of the desired size were selected and mounted at the bottom of a Petri dish (35 × 10 mm) with a small amount of silicone cone grease applied to either end of the segment. The root segment was

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covered with 5 mL to aerated medium (resistivity = 190 Ω·cm). The measurements were carried out on a Nikon Diaphot inverted microscope.

A 20 μm diameter platinum-black ball, electroplated at the tip of an otherwise insulated stainless steel wire, was used as the electrode (16). The electrode was moved in a 35 μm diameter circle in the horizontal plane by an arrangement that uses two piezoelectric elements mounted perpendicularly to each other. The ends of a piece of titanium foil, bent in the middle, were connected to the piezoelectric elements to form a parallelogram. The electrode was mounted on the middle of the titanium front piece with a lucite block (see ref. 14 for details of construction). Two sine waves of the same frequency, but differing in phase by 90°C, were applied to the two piezoelectric elements; a circular motion was achieved at or near the resonance frequency by fine adjustment of the phase and amplitude of the two signals. The sensing electrode and a stationary platinum black reference electrode were connected to a low noise differential preamplifier (Applicable Electrotechnics, W. Yarmouth, MA). The output of the preamplifier was fed to a two-phase lock-in amplifier (model 5204, Princeton Applied Research, Princeton, NJ) that detected both the in-phase and quadrature components of the signal. The two outputs of the lock-in amplifier were digitized by an A-D converter and analyzed by an IBM PCXT computer using software supplied by the Vibrating Probe Co (Davis, CA). The computer calculated the average current vector from the two orthogonal components and displayed it on the screen, superimposed on the video image of the root.

RESULTS

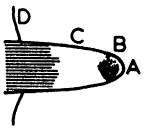
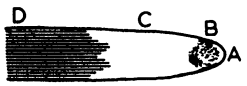
The primary root segments cultured in IAA-supplemented medium produced a large number of lateral roots (10.3 lateral roots/cm of primary root) and their growth rate was about 100 $\mu m \cdot d^{-1}$ (see Table I). A representative root segment cultured in IAA-supplemented medium for 5 d is shown in the inset in Figure 1A. This effect of IAA on root initiation and growth is well known (4). On the other hand, the root segments cultured in IAA-free medium produced a smaller number of lateral roots (3.7 lateral roots/cm of primary root) that elongated faster at an average rate of 1250 $\mu m \cdot d^{-1}$. The inset in Figure 2A shows an example of one such root segment cultured in IAA-free medium for 5 d. Current measurements were carried out on lateral roots positioned horizontally, with the probe oscillating in the horizontal plane. Unlike primary roots, young lateral roots do not exhibit positive gravitropism (12). Our own tests on the lateral roots produced in culture confirmed this (data not shown). Therefore, gravity-induced changes in the current pattern on the upper and lower side of the horizontally placed primary roots reported in some earlier studies (2, 3) should not occur in our system. In any case, past studies on the main root have suggested that the current pattern mapped on either side of a horizontally placed root is similar to that found around a vertically growing root (10, 11, 18).

It was possible to see the root primordia under the microscope even before they emerged from the primary root surface. Despite many attempts, we did not observe any consistent current pattern at the surface of the primary root before the lateral root primordium emerged. In some cases the current was either all inward or all outward and in other cases regions of both inward and outward current were seen at the site of lateral root emergence. Endogenous currents were observed at all stages after the emergence of the lateral root from the primary root surface, in roots developing in media both with and without IAA. Figures 1 and 2 show representative recordings of current measurements around lateral roots. Figure 1, A to C, shows lateral roots at various stages of development in IAA-supplemented medium. Figure 1, D to F, shows the current pattern around these lateral roots. During the early stages, inward current was detected at the root cap (this structure is very small in the lateral roots growing in culture), meristematic, and cell elongation zones. Usually, the largest inward current was detected at the cell elongation zone. An outward current was observed at the primary root surface area surrounding the emerged lateral root (Fig. 1, D and E). As the lateral root developed further, the outward currents were found to exit from that root's own basal region (zone of cellular maturation) as shown in Figure 1F.

Lateral roots developing in IAA-free medium have similar current patterns at comparable stages. Figure 2A is a photomicrograph of the apical portion of a 2.57 mm long lateral root developing in IAA-free medium. The current pattern recorded around this root is shown in Figure 2B. It shows clearly that current entered the root cap, the meristematic

Table I. *Growth Rate and Current Density Measurements on Lateral Roots Developing in Media with and without IAA*

The current density values indicate readings at 30 to 40 μm from the surface at various points around the lateral roots. (A) Tip of lateral root; (B) meristematic region; (C) middle of elongation zone; (D) primary root surface in the case of roots growing in IAA-supplemented medium and the zone of cell maturation and cytodifferentiation in the case of lateral roots developing in IAA-free medium. Each value represents mean ± SEM. The average age of the lateral roots was approximately 4 d.

	+IAA	−IAA
		
Root		
	Growth rate ($\mu m \cdot d^{-1}$)	
	103 ± 10 (n = 39)	1259 ± 214 (n = 24)
	Current density ($\mu A \cdot cm^{-1}$)	
A	In 0.9 ± 0.2 (n = 8)	In 2.0 ± 0.4 (n = 7)
B	In 1.1 ± 0.2 (n = 12)	In 2.7 ± 0.4 (n = 14)
C	In 2.7 ± 0.5 (n = 12)	In 4.6 ± 0.6 (n = 15)
D	Out 2.9 ± 0.6 (n = 5)	Out 4.0 ± 0.7 (n = 4)

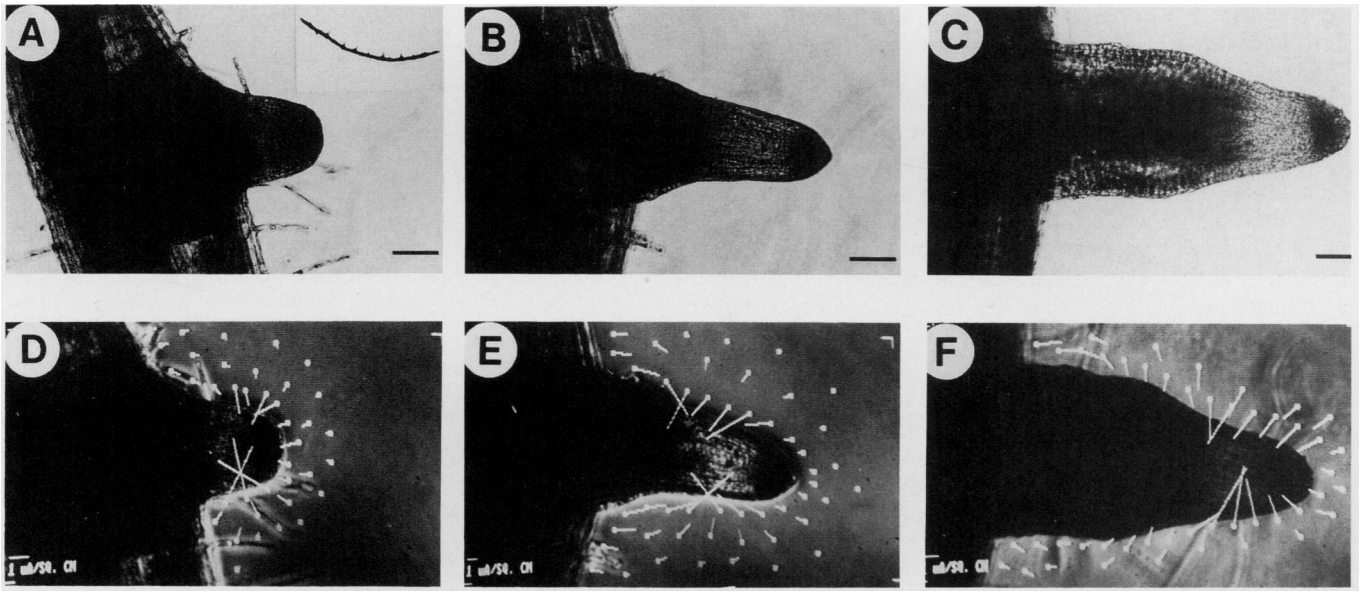


Figure 1. (A–C) Photomicrographs of lateral roots at various stages of development in IAA-supplemented medium. Scale bar, 100 μm. Approximate age of the lateral root in A, 2 d; B, 4 d; and C, 6 d. The inset in (A) shows a root segment cultured in IAA-supplemented medium for 5 d. (D–F) photographs of the video display showing the current patterns around the lateral roots. For each measurement, a reference reading was taken more than 500 μm away from the root and then the probe was brought to the measuring position close to the root. The command to take a reading and display the current vector was given by touching the screen at the center of the probe vibration with a light pen. The procedure of taking a reference reading followed by a current measurement reading was repeated at various points around the root. Each vector begins with a dot that indicates the center of the vibrational circle and extends away from the dot in the direction of the current (flow of positive charge). After each current vector was displayed individually, all of the previous vectors for that round could be displayed together in order to visualize the entire current pattern. Vector size represents the current density at the center of the oscillation. Scale bar, 1 $\mu A \cdot cm^{-2}$.

zone, and the cell elongation zone with the largest inward currents again being detected around the cell elongation zone. Current left from the more basal region of cell maturation and cytodifferentiation. The magnitude of this outward current decreased progressively toward the base. Between the zones of the inward current and the outward current there is a small region with no detectable net current either entering or leaving the root surface. This area is represented by the vectors running almost parallel to the long axis of the lateral root. Table I shows that the densities of currents entering the root-tip, meristematic zone, and elongation zone of the faster growing lateral roots in IAA-free medium were nearly twice as large as those entering the corresponding regions of the slower growing lateral roots in IAA-supplemented medium.

DISCUSSION

Our results clearly show the presence of endogenous currents around lateral roots developing in culture. They also demonstrate the power of the two-dimensional vibrating probe as an instrument for measuring extracellular currents. The current pattern that we observed around the older lateral roots is in general agreement with that seen in the primary roots of other species. The current pattern around the primary roots of about 15 other species has been inferred from one-dimensional vibrating probe measurements (2, 9–11, 18) and, with few exceptions, current is always found to enter the growing regions. In *Zea mays* seedlings the inward current was detected only at the apical part of the root cap (3). Outward current was found at the more proximal part of the root cap, meristematic, and elongation zone. A recent report (6) on primary roots of *Lepidium* seedlings showed four distinct current patterns and only one of them matches the general pattern described before. It should be noted, however, that for both *Z. mays* as well as *Lepidium sativum*, other groups have found current patterns similar to ours (2, 9). The current density values shown in Table I for lateral roots growing in IAA-free medium are of the same order of magnitude as those detected around the primary roots of the intact seedlings (2, 9–11, 18). In agreement with most of the earlier observations on primary roots (9–11, 18), our results show the largest current entering the elongation zone. This is true for lateral roots growing in media both with and without IAA. It is interesting that the inward current around the elongation zone of the faster growing lateral roots in IAA-free medium is nearly twice as large as that entering the cell elongation zone of the slower growing roots in IAA-supplemented medium, since this indicates a correlation between current entry and growth. Such a correlation between growth rate and current density has also been shown in the primary roots of *Z. mays* (9).

In addition to the vibrating probe-measured extracellular loop of the current in the aqueous medium, current loops in the apoplast of the tissue should also exist. Behrens and Gradmann (1) estimated that in their earlier vibrating probe study on *Lepidium* root (2), 75% of the current circulated within the root itself. The flow of this current in the apoplast will create an electrical field whose magnitude depends upon the apoplastic resistance. This electrical field may have an

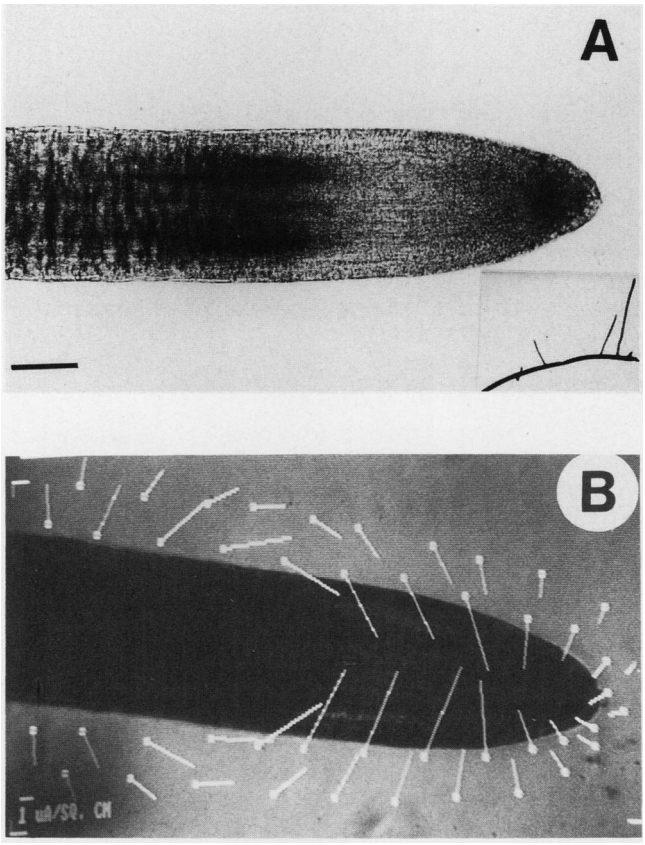


Figure 2. Current pattern around a lateral root developing in IAA-free medium. (A) Photomicrograph of the apical portion of a 2.57 mm long lateral root. Scale bar, 100 μm. The inset shows lateral roots developing on a root segment that was grown on IAA-free medium for 5 d. (B) Photograph of the video screen showing the current pattern around the lateral root. Scale bar, 1 $\mu A \cdot cm^{-2}$.

effect on the axis of cell elongation. Support for this notion comes from the investigation on the regenerating *Mougeotia* protoplasts which tended to elongate with their growth axis parallel to an applied electrical field (19). It is also interesting that in the somatic embryos of carrot, the electrical polarity can be detected as early as globular stage and the future axis of growth corresponds to this polarity (16). This again suggests the possibility that the electrical field produced by the currents might be involved in directing the axis of cell elongation.

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