Growth, Gravitropism, and Endogenous Ion Currents of Cress Roots (*Lepidium sativum* L.)

Measurements Using a Novel Three-Dimensional Recording Probe

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ABSTRACT

A novel, three-dimensional recording, vibrating probe was used for measuring the density and direction of the endogenous ionic current of cress roots (*Lepidium sativum* L.) bathed in low salt media (artificial pond water, APW). Roots submerged in regular APW and growing vertically show the following current pattern. Current of 0.7 microampere/square centimeter density enters or leaves the root cap; the current changes direction frequently. Current of 1.6 microamperes/square centimeter enters the meristem zone most of the time. Maximum current with a density of 2.2 microamperes/square centimeter enters the apical elongating zone, i.e., between 0.8 and 1.2 millimeters behind the root tip. The current density decreases to 1.4 microamperes/square centimeter at 2 millimeters, i.e., in the central elongating zone, and to 1.0 microamperes/square centimeter at 3 millimeters, i.e., in the basal elongating zone. The current direction changes from inward to predominantly outward between 1.2 and 3 millimeters behind the tip. Measurements on opposite flanks of the roots indicate that the current pattern is fairly symmetrical. After placing the roots horizontally, the density of the endogenous current remains stable, but the current direction changes at the root cap and in the meristem zone. The current leaves the root on the upper side and enters on the lower side, causing a highly asymmetrical current pattern at the very tip. The current pattern at the upper and lower side further away from the tip remains the same as in vertical roots. Roots submerged in low Ca²⁺ APW show a very different current pattern, no gravitropism, and no change of the current pattern after horizontal orientation. In these roots current enters the root cap and the basal elongating zone and leaves the apical elongating zone. Three conclusions are drawn from these results: First, plant roots elongate by two different modes of growth that are correlated with different current directions. They grow by cytoplasmic enlargement at sites of inward current and by turgor-driven elongation at sites of outward current. Second, a change in the current pattern at the root cap and in the meristem zone is a clear indicator of later gravitropism. Third, Ca²⁺ ions are involved in the gravstimulated change in the current pattern, probably affecting the activity of plasmalemma H⁺-ATPases.

Plant roots show some remarkable electrical phenomena, for instance membrane potentials that respond to gravity and ionic currents that traverse the root and change direction upon gravistimulation. Changes in membrane potential have been measured by Behrens et al. (3) in columella cells of cress roots and by Ishikawa and Evans (11) in cortical cells of the elongating zone of bean roots. Both columella and cortex cells respond to gravistimulation on the upper side with hyperpolarization and on the lower side with depolarization.

Endogenous currents of roots have been measured to date in approximately 20 different species. The results of four species are summarized briefly to show the advancement of measurement techniques and some typical features of such currents. In 1962, Scott and Martin (27), using static electrodes placed at the surface of vertically growing bean roots bathed in KCl medium, found that current leaves the root hair zone and enters along the root cap, meristem, and elongating zone. In NaCl medium the current reversed direction at the root cap and the meristem. With the aid of a one-dimensional recording, vibrating probe near the surface of horizontally growing barley roots, Weisenseel et al. (32) discovered an endogenous current that entered the root cap, meristem, and apical elongating zone, and left at the root hair zone. With the same technique, Miller and Cow (17) measured in horizontally placed maize roots an outward current at the root cap and the root hair zone and an inward current at the meristem and elongating zone. Decreasing the pH of the medium around the maize roots increased the density of the inward current, the area of current influx, and the growth rate. Increasing the pH had the opposite effect. Employing a two-dimensional recording, vibrating probe Ruhore et al. (25) found in young, lateral roots of radish tissue an outward current at the root base and an inward current along the root cap, meristem, and elongating zone. Omitting the growth hormone IAA from the medium stimulated both growth rate and current density.

These examples suffice to demonstrate three facts. First, growing plant roots drive ionic current through themselves and the surrounding medium. Second, the current varies in density and direction in different media. And third, the current is affected by the gravisensitivity and gravistimulation of the root. However, to draw firm conclusions from available data about the density and pattern of endogenous currents and the induced changes is risky because current density is a vector. All methods applied so far were incapable of measuring vectors. Thus, an observed current increase or decrease could be

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could have been affected by a change in the direction of the current, or changes in current direction might have gone unnoticed when the current density had changed simultaneously. Therefore, what is needed to obtain the proper information about endogenous currents and their role in growth and gravitropism of plant roots is a method to measure current vectors. We have developed such a technique during the last few years based on the pioneering work of Jaffe and Nuccitelli (14) and applied it to investigate the endogenous current of cress roots. We have used cress roots in order to complement previous measurements with a one-dimensional vibrating probe (4, 13) and to obtain true current densities and current patterns. We also wanted to compare endogenous currents of graviresponding and nongravi-
responding roots. In this respect we follow up the stimulating thoughts of Lund (16).

MATERIALS AND METHODS

Plant Material and Culture Conditions

All experiments were carried out with seedlings of garden cress (Lepidium sativum L., Chrysant, Bonn, FRG). Dry seeds were presoaked in artificial medium for 5 h and then grown in the dark for 20 h at 25 ± 1°C in a container with high humidity. In the container, 30 seeds were attached to the bottom edge of a horizontal strip of wet filter paper, dipping into the experimental medium. The seeds were oriented with their germ pore facing down, so that the root was free to grow vertically. After about 20 h, most of the seeds had developed a straight main root with a length of 10 to 15 mm. Five of the seedlings from each container were selected for further experimentation.

Most of the experiments were carried out in regular APW-1, which contained the following salts: 1.0 mM NaCl, 0.1 mM KCl, 0.1 mM CaCl₂, 1.0 mM Mes, adjusted to pH 6 with Tris. This low salt medium is frequently used to grow freshwater organisms and it contains the main salts of a natural soil solution. Several experiments were carried out in low Ca²⁺ APW (APW-2). APW-2 contained the following salts: 1.0 mM NaCl, 0.1 mM KCl, 0.1 mM MgCl₂, 0.1 mM EGTA, 1.0 mM Mes, adjusted to pH 6 with Tris. APW-1 was made up with demineralized water, APW-2 with demineralized water poured before use through a column of Chelex beads to further reduce Ca²⁺. All media were aerated overnight before adjustment of the pH. APW-2 was used to inhibit gravitropic bending of horizontally oriented cress roots. Addition of EGTA turned out to be necessary to reduce the free Ca²⁺ concentration to a level that stops gravitropism, i.e. to 1 to 2 μM, as measured with an ion selective electrode (Orion, Colora, Freiburg, FRG). Without EGTA, the free Ca²⁺ concentration, due to contamination, was sufficient to support gravitropic curvature of most cress roots.

Current Measurements

Five seedlings with a well-developed root were transferred into the bore of Lucite holders (cf. ref. 4) and fastened with a drop of 35°C hot APW containing 1% agar. The seedlings were then kept for 1 h in a container filled with experimental medium up to approximately 3 mm behind the tip of the roots. After adaptation to the experimental medium, one seedling was selected for current measurements, mounted onto a micromanipulator and dipped in a plastic Petri dish filled with experimental medium. The dish had a bottom and a front window made of cover glass. An inverted microscope (IM 35, Zeiss, Oberkochen, FRG) equipped with a videocamera and a horizontal stereomicroscope (Leitz, Wetzlar, FRG) served for viewing the root from below and in front. The light of the microscope, filtered through a 5-mm thick heat-absorbing glass (KG1, Schott a. Gen., Mainz, FRG) was used to illuminate the seedling on the stage. The other four seedlings remained in the container and served as controls for growth and gravitropism.

The current produced by the root was measured with a novel type of vibrating probe. This probe measures the total current density as well as the x, y, and z component of the current, yielding a three-dimensional vector of current density (31). In this paper the axes x and y define the horizontal plane, and axes x or y and z define the vertical plane. Briefly, the probes were fabricated from parylene C insulated stainless steel electrodes (SS300305 A, Micro Probe Inc., Clarksburg, MD) shortened to a length of 15 mm. The uninsulated tip was plated with layers of gold and platinum, and finally a ball of 30 μm platinum black was deposited. The probe was mounted on the head of a tube that was supported by two triangular-shaped plates of spring metal. The tube and the probe formed an angle of 50° with the horizontal plane. A similar electrode with a tip of 60 to 70 μm diameter served as a reference electrode. Both electrodes were carried by a rotating stage. The tip of the vibrating probe remained centered with an accuracy of 1 to 2 μm during rotation. The whole rotating stage could be moved in three directions, i.e. horizontally (x and y direction) and vertically (z direction).

The probe was vibrated lengthwise with 317 Hz over a distance of usually 35 μm. The vibrations were generated by an oscillator, transformed by an electromagnet into movement (Walz, Effeltrich, FRG), and transmitted to the probe via a liquid-filled Teflon tubing and metal bellows. The AC signals generated by the probe at sites of current flow were amplified 1000-fold (preamplifier 5006, EG a. G, München, FRG) and fed into a Lock-in amplifier (PAR, EG a. G). The DC-signals from the Lock-in amplifier were recorded with an IBM PC/AT computer. The probe was calibrated to read current densities, i.e. μA/cm², by vibrating it in an electric field of known strength. This field was established in a rectangular Lucite chamber with two opposite plates of Ag/AgCl. The chamber was filled with the same medium as used for the subsequent measurements, and current of 4.0 or 6.0 μA, provided by a constant current source (Picoammper source 261, Keithley, München, FRG), was passed through the medium. Repeated calibration with current densities from 1.0 to 5.0 μA/cm² always yielded a linear response of the probes. A one-point calibration just before each experiment was therefore sufficient.

With the roots, current was measured at a distance of approximately 100 μm from the surface and at seven sites along the first 3 mm of the tip (cf. Fig. 2), i.e. at the root cap

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2 Abbreviation: APW, artificial pond water.
(positions 1 and 2), the meristem zone (position 3), the apical elongating zone (positions 4 and 5), the central elongating zone (position 6), and the basal elongating zone (position 7). At each site the current density was measured at three different angles. The three measurements, each lasting 20 s, were then used to calculate the current vector at the measurement site. The algorithm for the calculation and a graphics program to plot the vectors (cf. Fig. 1) were stored in the computer. All measurements were carried out at 21 ± 2°C and under white light conditions of a few mW/cm². Preliminary experiments, comparing the effects of green safety light and white light, showed no effect of light on the current pattern and density of cress roots.

RESULTS
Current Pattern and Current Density of Vertically Growing Roots

Over the last 2 years we measured and evaluated the endogenous current of about 90 different Lepidium roots. Figure 1 shows a typical example of the current measured in a vertically oriented root after transfer to APW-1. As the current vectors of the first measurement indicate, current enters the root cap at position 1, leaves at position 2, enters the meristem zone (position 3) and the apical elongating zone (positions 4 and 5). Current leaves the root at the central elongating zone (position 6) and the beginning of the root hair zone (position 7). In this and all subsequent figures, current vectors (arrows or dots) pointing toward the drawing of the root indicate inward current and vectors pointing away from the root indicate outward current. During the next two measurements the direction of current was quite stable at sites 3, 4, 5, 6, and 7, but changed direction at sites 1 and 2. Changes in current direction at these sites were common and are apparent in the next figure, which summarizes the data from 32 vertically oriented roots growing in APW-1 (Fig. 2). Each root was measured two to five times during 1 to 3 h.

At the start of current measurements the roots measured 10 to 12 mm in length. At the end of the experiment they had grown to 12 to 15 mm. The average growth rate (±se) at the beginning was 10.1 ± 0.7 μm/min, then it declined to 9.4 ± 0.8 μm/min after 1 h, to 8.0 ± 0.5 μm/min after 2 h, and to 7.2 ± 0.6 μm/min after 3 h. However, large differences in growth rate between different roots were common and considerable variability occurred within the same root during the time of current measurement. Moreover, all roots showed irregular and sometimes wide movements of their tip when viewed and traced from below.

In Figure 2a all vectors measured in the 32 roots are presented according to their x and z coordinates, the coordinates running normal and parallel to the longitudinal axis of the root, and irrespective of their density. The y coordinate, i.e. the tangential direction, is omitted for clarity. Most current vectors possess a tangential, or y component, however, indicating that the current spirals around the root (cf. Fig. 1). The mean current vectors in the x-z plane and their changes in direction and density with time are illustrated in Figure 2b. The average current densities during 3 h of measurement are presented in Table I.

Figure 1. Typical example of the pattern and density of endogenous ionic current traversing a vertically growing root of L. sativum bathed in low salt medium (APW-1). The left side illustrates schematically the root tip and the seven sites of measurement which are located along the root median at a distance of approximately 100 μm from the root surface. The right side shows the current density vectors measured in sites 1 to 7 at time 0, i.e. immediately after transfer of the seedling to the microscope stage (top), at 1 h (center), and at 2 h (bottom). All vectors are drawn to the same origin, and dotted lines are added to visualize the spatial orientation of the vectors.

Figure 2 clearly shows that the direction of the endogenous current of cress roots fluctuates between inward and outward current at the root cap. In the meristem zone the direction of current becomes more stable, and 72% of all vectors point toward the root and report inward current. In the apical elongating zone practically all vectors show inward current. Further along behind the root tip the number of outward pointing current vectors increases again, rising to 74% at the beginning of the root hair zone.

The mean current density increases from the root cap to a maximum at the apical elongating zone and then decreases again (Table I). This pattern of current density confirms earlier results, but the increase in current density from the root cap to the apical elongating zone is in fact only about threefold, i.e. much less than inferred from measurements with one-dimensional recording probes (4, 17). The rather steep incli-
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Figure 2. Synopsis of all current density vectors measured in 32 different, vertically growing roots of *L. sativum* bathed in APW-1. Each root was measured two or five times during 1 and 3 h, respectively. a (left side), Schematic drawing of a root tip and location of measurement sites; (right side) current density vectors positioned according to their *x* and *z* direction irrespective of current density. Each vector is represented by a dot or asterisk. (The average current densities are presented in Table I. The *y* component of the vectors is omitted for clarity.) b (right side), Mean current density vectors in the *x* and *z* direction at various hours after the start of the measurements.

Table I. Mean Current Density Near the Surface of Vertically Growing Roots of *L. sativum*, Bathed in a Low Salt Medium (APW-1)

<table>
<thead>
<tr>
<th>Position*</th>
<th>Current Density ± SE μA/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 and 2 (root cap)</td>
<td>0.73 ± 0.04b</td>
</tr>
<tr>
<td>3 (meristem zone)</td>
<td>1.62 ± 0.11</td>
</tr>
<tr>
<td>4 and 5 (apical elongating zone)</td>
<td>2.15 ± 0.11</td>
</tr>
<tr>
<td>6 (central elongating zone)</td>
<td>1.35 ± 0.15</td>
</tr>
<tr>
<td>7 (basal elongating zone)</td>
<td>0.97 ± 0.12</td>
</tr>
</tbody>
</table>

* For exact location of measurement sites, see Figure 2. b Mean current density of 32 different roots. Each root was measured two or five times during 1 or 3 h in APW-1.

Table II. Symmetry of the Endogenous Current Measured Near the Surface of Vertically or Horizontally Oriented Roots of *L. sativum* Bathed in APW-1

<table>
<thead>
<tr>
<th>Position*</th>
<th>Symmetryb %</th>
<th>Vertical roots</th>
<th>Horizontal roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>64</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>86</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>93</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>93</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>71</td>
<td>67</td>
<td></td>
</tr>
</tbody>
</table>

* For sites of measurement, see Figures 2 and 4. b Symmetry is defined as the percentage of current vectors on opposite sides of the root in the same site and at the same time that show a common current direction, i.e. either toward or away from the root. The results from seven vertically and seven horizontally oriented roots have been pooled.
nation of the current vectors at the root cap is most likely the reason of the previous underestimation of the current density at the root cap.

With another seven roots the symmetry of the current pattern and density of vertically growing roots was investigated more closely. With these roots the current was always measured twice at each site by moving the probe back and forth between left and right side of the root. The results of the measurements are summarized on the left side of Table II, which shows that the current direction and density are quite symmetrical around vertically oriented roots. The smaller symmetry at the root cap and at the beginning of the root hair zone reflects the fluctuations of current direction at these sites. It also indicates some independence of the two flanks of a root in their capability to either emit or take up current.

Current Pattern and Current Density of Graviresponding Roots

The purpose of the following measurements was twofold. First, to reinvestigate gravity-induced changes of the current pattern of Lepidium roots more thoroughly than has been possible with a one-dimensional probe (4, 13), and second, to provide the data for a comparison of graviresponding with nongravireacting roots. All measurements were carried out in APW-1, and they always began with two measurements along the root in vertical orientation. (The data from vertically oriented roots are included in Fig. 2.) After about 1 h the roots were oriented horizontally and kept in this position for 2 h. During this time each root was measured five times either along its upper surface or along its lower surface from sites 1 to 5, and finally one measurement each was made at sites 6 and 7. The first measurement at the horizontal root was conducted within 5 to 20 min after rotation.

Figure 3 shows two typical examples of the current pattern and density of two roots approximately 20 min after horizontal orientation, one measured at the upper surface (Fig. 3, top) and one at the lower surface (Fig. 3, bottom). Most striking and distinct from vertical roots is the outward current on the upper side at the root cap and the meristem zone. At the same sites on the lower side, current enters the root. At sites 4 and 5, i.e. the apical elongating zone, the current vectors of both sides point toward the root as in vertical roots.

Figure 4 summarizes the results obtained with 18 different roots, nine measured along the upper surface and nine along the lower surface. The figure shows the mean current density vectors at different times after horizontal orientation, and their x and z directions. (Again, the y component has been omitted for clarity.) During measurements the roots elongated with an average rate (±s.e.) of 7.5 ± 1.0 μm/min and curved downward with 34 ± 5° at 2 h. The average site of maximum curvature was at 1.6 mm behind the tip. (Note that gravitropic curvature of roots in aqueous medium is always less than that of roots growing in humid air.)

Figure 4 clearly shows that the direction of endogenous current on the upper and lower side of horizontal roots further away from the tip, i.e. at sites 4 to 7, is quite similar to vertical roots. Current enters in the apical elongating zone and leaves at the beginning of the root hair zone. However, the current pattern is very different from vertical roots at the very tip, i.e. in sites 1 to 3. There, current leaves the root on the upper side for a prolonged time after horizontal placement and enters it on the lower side. It takes about 0.5 h at the root cap, and about 2 h in the meristem zone before the current vectors begin to point toward the root again and signal the end of the gravistimulated outward current phase. The mean current densities are more or less the same in horizontal and vertical roots (Table III). But in a few roots, a significantly higher current density was measured at the lower surface compared with the upper surface, in particular at the apical elongating zone.

As with vertical roots, seven horizontally placed roots were investigated for symmetry of the current direction and density
The following experiments were carried out to measure the pattern and density of endogenous currents of roots submerged in low Ca\(^{2+}\) APW, in particular to elucidate the current pattern of horizontally oriented roots that do not show a gravitropic growth response. At first, several media with low Ca\(^{2+}\) concentration were tested to find one that prevented roots from responding gravitropically, but not from growing normally, at least for several hours. Replacement of CaCl\(_2\) of APW-1 with MgCl\(_2\) was not enough to stop gravitropism in most roots. Only addition of an extra 0.1 mM EGTA prevented practically all roots from responding to horizontal orientation with differential flank growth. In this medium (APW-2) *Lepidium* roots elongated with an average rate (±se) of 9.9 ± 1.0 \(\mu\)m/min when growing vertically, and with 7.0 ± 1.1 \(\mu\)m/min in horizontal position during the current measurements.

The current pattern of the roots growing in APW-2 was a real surprise. In roots oriented vertically (Fig. 5a, Table IV), mainly inward current was found at the root cap and in the basal elongating zone. Outward current dominated in the apical and central elongating zones. Thus, in low Ca\(^{2+}\) APW, *Lepidium* roots grow with a drastically altered current pattern, i.e. with a pattern that has one central and extended current source, or area with outward current, flanked by two current sinks, or areas with inward current. This current pattern is just the opposite of the one found in roots growing in medium with a higher concentration of Ca\(^{2+}\) (APW-1). There, the main current source lies in the basal elongating zone and root hair zone, and the main current sink is located in the meristem and apical elongating zone.

When the roots were oriented horizontally (Fig. 5b), the current pattern remained practically unchanged. Current again entered the root cap and the basal elongating zone on the upper and lower side and left the root at the apical elongating zone on both sides. As in vertical roots, current at 18 different, horizontally placed *L. sativum* roots bathed in APW-1. In nine roots, the current pattern and density was measured at the upper surface, and in nine other roots current was measured at the lower surface. The average time after horizontal orientation is indicated in minutes next to each vector. The schematic drawing in the middle shows the root at the beginning of the measurements and illustrates the measurement sites.

by measuring the current alternatingly at the upper and lower surface of the same root. Each root was measured three times during 2 h in the horizontal position, and the results are summarized in Table II (right side). The results support the measurements presented in Figures 3 and 4, i.e. they also show the highly asymmetric current pattern at the meristem region. There, only 14% of the vectors show current flowing in a common direction on opposite sides of the root. The measurements also indicate that at most sites there is less symmetry of current direction in horizontal roots than in vertical roots. The early asymmetry of the current pattern at the root cap is lost in Table II due to summarizing the data from 2 h of measurements.

**Current Pattern and Current Density of Nongraviresponding Roots (Roots in Low Ca\(^{2+}\) Medium)**

It is well known that Ca\(^{2+}\) ions play a major role in root gravitropism (7, 20), and that a low external Ca\(^{2+}\) concentration stops gravitropic curvature (19).

<table>
<thead>
<tr>
<th>Position*</th>
<th>Current Density ± se</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vertical roots</td>
<td>Horizontal roots, upper surface</td>
</tr>
<tr>
<td>1 and 2</td>
<td>0.62 ± 0.06(^b)</td>
<td>0.85 ± 0.05</td>
</tr>
<tr>
<td>3</td>
<td>1.72 ± 0.17</td>
<td>2.08 ± 0.13</td>
</tr>
<tr>
<td>4 and 5</td>
<td>2.18 ± 0.19</td>
<td>1.94 ± 0.13</td>
</tr>
<tr>
<td>6</td>
<td>1.44 ± 0.25</td>
<td>1.43 ± 0.25</td>
</tr>
<tr>
<td>7</td>
<td>1.10 ± 0.19</td>
<td>1.13 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>Lower surface</td>
<td></td>
</tr>
<tr>
<td>1 and 2</td>
<td>0.52 ± 0.05(^b)</td>
<td>0.90 ± 0.08</td>
</tr>
<tr>
<td>3</td>
<td>1.45 ± 0.19</td>
<td>1.76 ± 0.34</td>
</tr>
<tr>
<td>4 and 5</td>
<td>2.28 ± 0.16</td>
<td>2.33 ± 0.16</td>
</tr>
<tr>
<td>6</td>
<td>1.19 ± 0.22</td>
<td>1.67 ± 0.21</td>
</tr>
<tr>
<td>7</td>
<td>0.98 ± 0.21</td>
<td>0.87 ± 0.13</td>
</tr>
</tbody>
</table>

* For location of measurement sites, see Figures 2 and 4.

\(^b\) Mean current density of nine different roots, each measured twice in vertical orientation and five times in horizontal orientation.
Figure 5. Distribution of the current density vectors of 10 L. sativum roots bathed in low Ca²⁺ medium (APW-2). Each dot or asterisk represents one vector with its x and z coordinates (otherwise as Fig. 2). a, Direction of vectors of roots growing vertically. Each root was measured twice in 1 h. b, Direction of vectors of roots after horizontal orientation. Each root was measured five times in 2 h. Five roots were used for measurements on the upper side, and five roots for measurements on the lower side. (The current densities of these roots are summarized in Table V.)
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Table IV. Comparison of the Current Patterns of Endogenous Current Near the Surface of L. sativum Roots Growing Vertically in Regular Low Salt Medium (APW-1) or Low Ca⁺⁺ Medium (APW-2)

<table>
<thead>
<tr>
<th>Position*</th>
<th>Percent Current Density Vectors Indicating Inward current</th>
<th>Outward current</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>APW-1</td>
<td>APW-2</td>
</tr>
<tr>
<td>1 and 2</td>
<td>50b, 85c</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>40</td>
</tr>
<tr>
<td>4 and 5</td>
<td>96</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>57</td>
<td>35</td>
</tr>
<tr>
<td>7</td>
<td>26</td>
<td>70</td>
</tr>
</tbody>
</table>

* For sites of measurement, see Figure 2. b Data from the 32 roots presented in Figure 2. c Data from the 10 roots presented in Figure 5.

The meristem and the central elongating zone fluctuated between the inward and outward direction, but inward current predominated on the lower flank of the central elongating zone.

In roots submerged in APW-2, the current density was generally larger than in roots growing in APW-1 (Table V). In vertically oriented roots of the first investigated batch of five roots (Table V, upper half) the current density was enhanced by a factor of 1.1 to 1.9. In the second batch of five roots (Table V, lower half) the current density was raised by a factor of 2 to 5. The difference in current density between the two batches of *Lepidium* roots was real, but the reason for the difference is unknown. We also noted that the growth rate of the batch with the enhanced current density was approximately 20% larger. Nevertheless, the distribution of current density along the root was similar in both batches and the same as in roots growing in APW-1, i.e. maximum current density occurred at the apical elongating zone (positions 4 and 5). After rotation of the root into a horizontal position, the current density decreased to some extent at most sites, except at the very tip, where an increase was measured (Table V). The apparent difference in current density between the upper and lower surface shown in Table V is probably not real. Rather, it reflects the fact that the two batches of roots differed so grossly in current density.

**DISCUSSION**

We will focus the following discussion on the patterns of current produced by vertically and horizontally oriented roots of *L. sativum*. We believe that some interesting information concerning root growth and gravitropism can be obtained from the various current patterns observed during this investigation.

Current Traversing Vertically Growing Roots

Cress roots immersed in regular APW-1 grow with one main and persistent current sink, i.e. a site of inward current at the meristem and the apical elongating zone. The main current source, i.e. the site of outward current, is localized in the basal elongating zone and root hair zone. At the root cap and in the central elongating zone, current direction may change with time from inward to outward current, and vice versa. This result confirms previous measurements with cress roots by Behrens et al. (4) and Iwabuchi et al. (13). Moreover, the pattern of endogenous current now firmly documented in cress roots seems to be the most common pattern found in plant roots. A similar pattern is reported for bean roots (6, 10, 27), barley roots (32), corn roots (17), wheat roots, oat roots, and several others (18), pea roots (9), and radish lateral roots (25).

Does this current pattern provide information about the physiology of root growth? Most likely the current pattern indicates two different modes and sites of growth, namely growth dominated by increase of cytoplasm, and growth dominated by turgor-driven cell elongation. We believe that the first mode of growth (cytoplasmic growth) is connected with inward current and that it necessitates the uptake of organic nutrients such as sugars and amino acids. The second mode of growth (turgor growth), we believe, is related to outward current and requires the uptake of ions, for instance K⁺ and Na⁺. We assume that the organic nutrients needed for cytoplasmic growth are taken up from the apoplast by symport with H⁺ ions, and that the ions required for cell elongation are taken up either via antipor or symport with H⁺ from the medium. We further assume that the antipor system provides all the H⁺ ions that are used by the symport mechanism, i.e. H⁺ ions may be used very economically by the growing root. Most likely, the second mode of growth contributes more to the overall elongation of a root than the first mode.

Our hypothesis of two modes of root growth is supported by the following observations: There is abundant evidence indicating that a major component of the endogenous current of roots is H⁺, and that these H⁺ are taken up by the root tip and extruded by proximal, more mature root zones (10, 17, 22, 32). Growth measurements in wheat roots (5), cress roots

Table V. Current Density Near the Surface of L. sativum Roots Growing at First Vertically for 1 h and Then Horizontally for 2 h in Low Ca⁺⁺ Medium (APW-2)

<table>
<thead>
<tr>
<th>Position*</th>
<th>Current Density ± se</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vertical roots</td>
</tr>
<tr>
<td></td>
<td>µA/cm²</td>
</tr>
<tr>
<td>1 and 2</td>
<td>0.79 ± 0.11b</td>
</tr>
<tr>
<td>3</td>
<td>3.03 ± 0.68</td>
</tr>
<tr>
<td>4 and 5</td>
<td>3.57 ± 0.40</td>
</tr>
<tr>
<td>6</td>
<td>1.79 ± 0.28</td>
</tr>
<tr>
<td>7</td>
<td>1.20 ± 0.25</td>
</tr>
</tbody>
</table>

* For location of measurement sites, see Figure 5. b Mean value of five different roots. Each root was measured twice in vertical orientation and five times in horizontal orientation.

...
(28), and maize roots (1) indicate different regions within the elongating zone. In wheat roots, cells of the elongating zone enlarge in two phases, a slow phase and a subsequent rapid phase. In cress roots, cells do not begin to elongate until about 1.4 mm behind the tip. In maize roots, cells increase in cytoplasm in the meristem zone and in a distinct zone behind the meristem and they elongate further away from the tip. Organic nutrients are present in the apoplast of the root tip, delivered there by the phloem (26).

If outward current is an indicator of turgor-driven cell elongation and inward current of cytoplasmic growth, then roots bathed in low Ca\textsuperscript{2+} medium (APW-2) grow with a different strategy. The apical elongating zone now is the main current source, and the basal elongating zone is the main current sink. This suggests that the phase of cytoplasmic growth following cell division is more or less abandoned in favor of immediate cell enlargement. Such a growth pattern can probably not be sustained for very long, as the time a root cell has to accumulate cytoplasm is very much reduced. This may explain that roots in low Ca\textsuperscript{2+} medium slow down in growth and stop growing within 1 d or less (5) (our observations).

We are positive that the change in current pattern observed in APW-2 is correlated with the low Ca\textsuperscript{2+} activity, but we can only hypothesize how the low external Ca\textsuperscript{2+} concentration effects a change in current pattern. Recently, plasma membrane H\textsuperscript+-ATPases have been detected by immunological techniques in the epidermis and stele of roots (23). Assuming first that these H\textsuperscript{+}-ATPases are the source and driving force for the endogenous current, and second, that they have an optimum requirement for Ca\textsuperscript{2+} (cf. ref. 29), the change of current pattern in low Ca\textsuperscript{2+} medium becomes comprehensible. Normally, there is a higher calcium concentration in the apex and less calcium in the elongating zone of roots (5, 19, 22). This gradient of calcium along the root may be responsible for a difference of H\textsuperscript{+}-ATPase activity, namely low activity in the very tip and high activity further away from the tip. In low Ca\textsuperscript{2+} medium the calcium concentration in the root tip may fall to stimulating levels for the H\textsuperscript{+}-ATPase, and to inhibitory levels in the basal elongating zone. This would reverse the direction of current flow as observed in APW-2.

**Current Traversing Horizontally Oriented Roots**

After strong gravistimulation, a drastic and long-lasting change in current pattern occurred at the cap and the meristem zone of *Lepidium* roots bathed in APW-1. At the upper side, current left the root cap and the meristem zone, whereas at the lower side current entered at the same sites, generating an asymmetric current pattern at the very tip. This result confirms earlier reports about gravistimulated changes of endogenous current at the root cap (4) and in the meristem zone (13).

No change of current pattern was observed in horizontally placed roots submerged in low Ca\textsuperscript{2+} APW, i.e. in a medium in which the roots did not respond to gravity with curvature. This observation clearly suggests an involvement of Ca\textsuperscript{2+} in the change of current direction at the tip of graviresponding roots. We believe that the origin of the change might be a reduction in Ca\textsuperscript{2+} concentration on the upper side of the horizontally oriented root and an increase on the lower side. This assumption is supported by several reports about a downward movement of Ca\textsuperscript{2+} in the root tip after gravistimulation (15, 21), by measurements of Ca\textsuperscript{2+} in horizontal coleoptiles (8), and by the current pattern of cress roots measured in low Ca\textsuperscript{2+} APW (this investigation). Our results also suggest that an asymmetric current pattern at the root cap and in the meristem zone is an early and clear indicator of a subsequent gravicurvature, and vice versa. If we again associate outward current with cell elongation, then gravitropic curvature in horizontally oriented cress roots begins already in the meristem zone. Some measurements at the tip of gravistimulated roots support this conclusion (2, 12).

With the present results about the change in current pattern of graviresponding roots in mind, we will return to the unstable current pattern at the apex of roots oriented vertically. In these roots, the current at the cap frequently changed direction between inward and outward. These fluctuations most likely reflect the fact that vertically growing roots are gravistimulated roots during all of the time. One flank of the root cap functions part of the time as upper side and part of the time as lower side.

This interpretation of the current pattern at the root cap is supported by three observations. First, gravistimulation causes outward current at the upper side and inward current at the lower side of the root apex. Second, nongraviresponding roots, for instance roots in low Ca\textsuperscript{2+} APW or young lateral roots (24), show mainly inward current at the root cap (25). Third, vertically growing cress roots frequently deviate from the direction of the g-vector due to growth movements, and even slight deviations are sufficient for gravistimulation (30).

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**LITERATURE CITED**