Correlation between Root-Generated Ionic Currents, pH, Fusicoccin, Indoleacetic Acid, and Growth of the Primary Root of Zea mays[1]

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ABSTRACT

Correlations between root-generated ionic currents, extracellular pH, indoleacetic acid, fusicoccin, and growth were investigated. Current consistently entered the meristematic and elongating tissues of intact growing roots of Zea mays cv Golden Bantam. Mature root regions generated the outward limb of the current loop. Ion-substitution and pH-profile experiments suggested that the bulk of the ionic current was carried by H+. Calcium ions did not carry current, but calcium may regulate the proton circulation since the proton current density was slightly larger in calcium-depleted media. Increased root elongation at low pH was associated with increased current density and an extended zone of inward current. Conversely decreased elongation at high pH was associated with a reduced current density and a more restricted zone of inward current. The effect of the fungal toxin fusicoccin was to increase the current density of the inward limb of the ion current and to increase root extension. Concentrations of indoleacetic acid that reduced root growth, also reduced the density of the inward current and shortened the inward current zone. The results emphasize the point that roots are electrically contiguous over many millimeters and that the electrophysiology of root growth is best studied in intact root systems.

A significant period in the study of root-generated electrical fields was reflected in the publication in 1947 of Lund's opus "Bioelectrical Fields and Growth" (18). Recently, the topic has received considerable impetus as a result of the development of the vibrating probe, an ultrasensitive electrode capable of measuring extracellular voltage gradients (15). With the use of the probe, the actively growing root tips of several plant species have been shown to take up positive electrical current while mature root tissues generate outward current (1, 21, 22, 36). The current circuit is completed by ion flow through the external medium. Similar currents have been measured in a phylogenetically diverse range of tip growing cells and organs and it has been proposed that the circulating current may play a part in developmental control, perhaps serving to polarize cells and tissues (14). It has also been suggested that there may be a relationship between electrical polarity and the distribution of root growth regulators (33).

Considerable effort has been invested in elucidating aspects of the chemical control of root growth mediated by growth regulators. Part of this research has resulted in the development of the "acid growth hypothesis of auxin action" (6). According to this hypothesis, IAA initiates cell enlargement by causing cells to excrete hydrogen ions. The consequent acidification of the cell wall is proposed to result in the loosening of the wall matrix to an extent which allows turgor-driven cell expansion to occur. The hypothesis is still controversial and it remains to be established whether it can account for the diversity of reactions observed when intact roots are subjected to a variety of natural and synthetic growth regulators (13, 16). The observation that root tip segments acidify the medium in which they bathe has been an important foundation of the acid-growth hypothesis (6). There have, however, been several reports showing that intact roots generate areas of high pH around their growing tips (24, 36). It has also been suggested that there may be incompatibilities between transplasmalemma H+ fluxes associated with the acid-growth hypothesis, and those involved in the regulation of cytoplasmic pH, especially when the predominant form of combined-N in the soil is NO_3^- (30).

The fungal toxin fusicoccin (FC[3]) was thought initially to provide a key to understanding the mechanism of auxin action since it mimics superficially some of the effects of auxin (19, 37). While recent experimentation questions the acid-growth theory in relation to auxin action, evidence is accumulating supporting an acid growth theory of fusicoccin action (17). Different mechanisms may, therefore, be responsible for the similar reactions observed when auxin or FC are applied exogenously to tissues.

In this study we take advantage of the non-invasive nature, high resolving power and spatial resolution of a vibrating probe to investigate the magnitude, direction and ionic composition of the electrical current generated by the primary root tips of intact Zea mays seedlings. The effects of changes in external pH and exogenously applied FC and IAA on this current are also determined. The interrelationships between endogenous current, external pH, growth regulatory substances, and root development are discussed.

[3] Abbreviations: FC, fusicoccin; APW, artificial pond water.

MATERIALS AND METHODS

Experimental Media

Roots of *Zea mays* were examined with the vibrating probe when immersed in experimental media based on APW (1.0 mol m^{-3} NaCl, 0.1 mol m^{-3} KCl, and 0.1 mol m^{-3} $CaCl_2$). Solutions utilized during ion-substitution experiments were isoosmotic; K^+ and Ca^{2+} were replaced with Na^+, Na^+ with K^+, and Cl^- with SO_4^{2-}. EGTA (1.0 mol m^{-3}) was added to Ca^{2+}-free media to reduce further the extracellular calcium concentration. Media pH values were adjusted to 3.0, 5.0, 6.0, 6.5, or 7.0 using 0.1 mol m^{-3} HCl or NaOH (KOH in the case of the Na^+-free medium and H_2SO_4 for Cl^--free medium). The pH of the media were determined before and after experimentation. The media used in the IAA experiments were similar to those described above but for the addition of 10^{-4} M IAA (Sigma Chemical Co.). At this concentration IAA has been reported to inhibit primary root growth in *Z. mays* and cause an increase in the pH of the bathing medium (10). FC (Sigma Chemical Co.) was added to the various APW-based media at 10^{-6} M final concentration. This concentration has been reported to promote root elongation of *Z. mays* (25). All media were air-saturated and equilibrated at 20°C prior to experimentation.

Growth Conditions for Experimental Seedlings

Caryopses of *Z. mays* cv Golden Bantam were surface-sterilized in 15% H_2O_2 for 15 min, then washed several times with sterile deionized water. They were then imbibed in aerated APW for 24 h. Caryopses were transferred to seed trays and sown between double layers of paper tissue soaked in APW. The seed trays were kept in darkness at 18 to 20°C for 4 d. Selected seedlings were removed from the seed trays and placed in 1 L pots containing aerated medium for 24 h prior to experimentation. Great care was taken to ensure a uniformity of preexperimental growth and in preparation of material. Tissues exhibited a predictable pattern of electro-physiological activity as long as environmental influences such as light, temperature, nutrient status and osmotic pressure were carefully controlled and physical damage was prevented.

Mounting Seedlings for Vibrating Probe Examination

Intact seedlings were used exclusively. A mounting plate was cemented onto the base of a 9-cm Petri dish to support the caryopsis body and the primary root was led into the Petri dish via a slit cut in the dish wall. The caryopsis was held firmly in place and the root sealed within the slit using rapidly cooling 2% agar made up in APW. This provided a nontoxic, water-tight, seal which isolated the experimental root from the caryopsis. This was necessary since the caryopsis was found to extrude large amounts of nutrients which altered the pH and resistivity of the bathing medium. Seedlings were left to recover from the mounting process for at least 30 min and their elongation rates recorded before vibrating probe examination took place. This was essential to ensure that valid comparisons could be made between different seedlings.

Root Respiration during Experimental Examination

Root respiration rates of between 3 to 6 μmol CO_2 (kg fresh weight)$^{-1}s^{-1}$ have been reported for *Hordeum vulgare* roots (28). This is equivalent to some 350 to 700 nmol $CO_2(m^2$ surface area)$^{-1}s^{-1}$ for a root 500 μm in diameter. Our experiments were carried out in a temperature controlled room at 20°C (+2°C). From this one can assume an O_2 solubility of around 0.3 mol m^{-3} (31). In a measuring chamber containing 6.0×10^{-5} m^3 of air-saturated medium, this makes available to a single *Z. mays* primary root 18000 nmol of O_2 at the beginning of the experiment. Assuming a primary root diameter of 1,000 μm for *Z. mays* (at the upper end of the experimental root range) and a maximum length of root isolated in the chamber of 4 cm, the total respiratory area approximates to 1.2×10^{-4} m^2. Taking the upper respiration rate reported for *H. vulgare* as a guideline for *Z. mays*, one can estimate over an experimental period of 90 min (an average for the experiments undertaken) that a total of approximately 460 nmol O_2 would be consumed. Although all the O_2 in the experimental chamber is obviously not available to the respiring root (in spite of the stirring action of the probe) a consumption of 460 nmol O_2 represents only 2.6% of the total O_2 available. Fusicoccin and IAA have been reported as promoting respiratory rates in plant tissues (37) but the potential O_2 supply available over the experimental period is likely to be sufficient to accommodate any promotion of the respiration rate due to the application of either of these compounds.

The stirring action of the vibrating probe coupled with the relatively large volume of measuring medium was regarded as adequate to compensate for any ion exchange that may occur between the root and the measuring medium throughout the experimental period. The major source of error in calculating the current density was due to evaporation from the measuring chamber. This resulted in an increase in the conductivity of the measuring medium leading to an underestimation of the current density. To compensate for this a correction factor was introduced into the calculation (22).

Measurement of the Ion Current

The magnitudes and patterns of the ion currents surrounding growing *Z. mays* roots were measured with a vibrating probe as described by Miller *et al.* (22). Roots were either scanned at 250 μm intervals from the root tip back some 4 to 5 mm to mature root regions (Figs. 1, 3, and 5), at similar intervals across the zone at which current changed from inward to outward (Figs. 2 and 7) or, again at similar intervals, across a 2 mm region which represented the peak of the inward current zone (Figs. 4 and 6). The latter two procedures were adopted in order to reduce the time taken to complete the experiment in question.

It has been shown that there are rapid changes in the patterns of electrical current around the root rips of both *Lepidium sativum* (1) and *Z. mays* (3) following gravistimulation. The primary roots of *Z. mays* have been shown to be diageotropic in the dark although they become positively geotropic after prolonged growth in the dark or after exposure to light. The action spectrum of this light effect has been

shown to peak at 650 nm (34). In order to minimize the geotropic response material was prepared and experiments performed under green light (490–580 nm).

Ion-Substitution Experiments

In order to identify which ion(s) were carrying the external limb of the ion current two approaches were adopted. First, the component ions of APW were selectively removed from the bathing medium. The procedure employed during all ion-substitution experiments involved mapping the current with the root bathed in a particular medium, then flushing a new medium (minus a particular ion) through the measuring chamber. The media used in these substitution experiments are described in an earlier section. The purpose of this was to record the instantaneous effect the absence of a particular ion might have on the current density (indicating whether or not it actually participated in carrying the current). Possible longer term effects that the absence of particular ions might have on the growth of the root were avoided by minimizing the time of each ion-substitution experiment. It has been shown for several developing systems (14, 35) that different ions may account for the inward and outward currents. The usual procedure when undertaking ion-substitution experiments has been to record the effects of the substitution only at peaks of current density (both inward and outward). For small objects such as fungal hyphae or pollen grains this represents no problem, but when it comes to large objects such as Z. mays roots this procedure is undesirably time consuming because the peaks of inward and outward current may be several mm apart. The limited travel of the micro-manipulator mounting the probe combined with the restricted field of vision of the inverted microscope setup required to view the probe at the root surface, results in a good deal of careful repositioning of both the specimen under observation and the probe relative to it. The time taken to do this can lead to problems due to the long term deprivation of ions. As a compromise, therefore, we adopted a procedure where we mapped ion-substitution across the current crossover point. This could be carried out rapidly and gave information on the composition of both the inward and outward currents. The current densities associated with three separate ion-substitution experiments were mapped in the manner described and mean values calculated. This resulted in data being acquired relative to both the inward and outward current limbs over several measuring positions.

The second approach was to map the current density and pattern in media of different pH values (3, 5–7). This was carried out rapidly in order to establish how sudden increases or decreases in H^+ concentration affected the current density. Again, the time-scale of experiments was minimized to avoid longer term effects of changes in the external pH on endogenous current generation.

Root Extension Rate Measurements

Since root extension rates could not be measured readily during vibrating probe experiments, they were determined before and after probe examination took place and the averages calculated. Measurements were made on an inverted microscope with a micrometer eyepiece.

RESULTS

Self-Generated Ion Currents Around Growing Roots

Growing primary root tips of intact Z. mays seedlings were found to generate ion currents which could be detected within the aqueous medium in which they were bathed. In general, three zones could be distinguished with respect to the direction of current flow relative to the surface of the root (Fig. 1). (a) A zone of outward current associated with the root cap. (b) A zone of strong inward current beginning with the meristematic tissue and peaking approximately with the expected region of maximum cell elongation. (c) A second major region of outward current which was associated with the region of the root where cells were elongating much less vigorously and entering into a post-elongation phase. This indicates the beginning of mature root tissue.

Ion-Substitution Experiments

In order to determine which ion(s) were carrying the root-generated current, the constituent ions of APW were removed sequentially from the measuring medium and the effects on current density and pattern recorded (Fig. 2). The elimination of Na^+, K^+ and Cl^- from the measuring medium had little effect on the inward current, reducing mean peak inward current densities by 7%, 17%, and 15%, respectively. The removal of Ca^{2+} ion resulted in a small increase in mean peak inward current densities in the order of some 15%. Changes in current density of this size are of little significance since consecutive measurements under steady state growth conditions may vary by as much as 10% and additional variation can be introduced due to physical perturbations due to ion-substitution experiments. The current crossover point (except after K^+-substitution) was maintained at approximately the
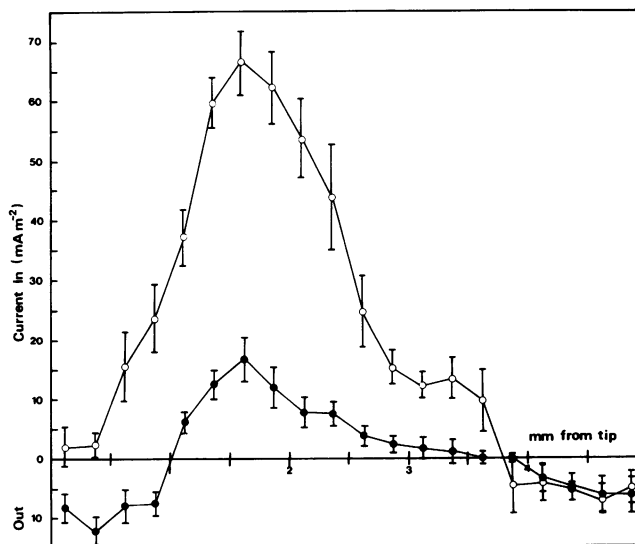

Figure 1. Pattern and density of the self-generated ion current traversing 6 d old Z. mays roots growing horizontally in APW at pH 6.0 (●). Stimulation of inward current resulting from replacement of APW with APW + 10^{-6} M fusicoccin (○) at pH 6.0. Averages from six experiments of current densities (±SEM) entering or leaving normal to the surface are illustrated at different sites along the root.
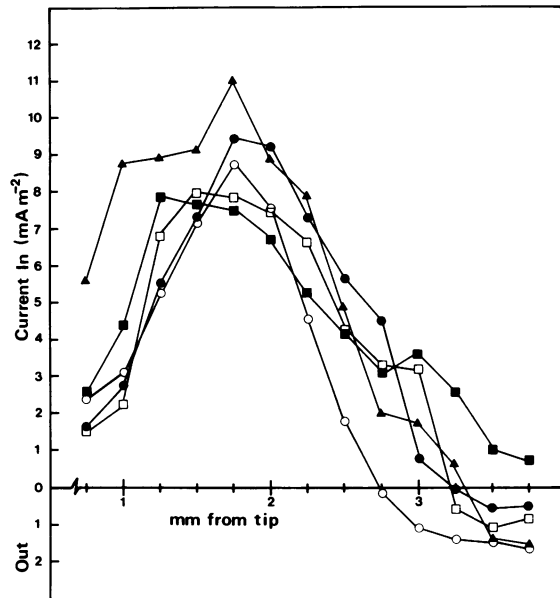
Figure 2. Ion-substitution experiments on *Z. mays* roots in APW. Six day old seedlings were used with the media adjusted to pH 6.0. The effect on the current density of removing Na$^+$ (O), K$^+$ (■), Cl$^-$ (□), and Ca^{2+} (▲) compared to an APW control (●) is shown. Each point represents the mean value from three separate ion-substitution experiments. SEM values are not shown but are of a comparative size to those given in Figure 1.

Figure 3. Relationship between the pH of the APW bathing medium and the pattern and density of current traversing 6 d old *Z. mays* roots. Media at pH 3.0 (△), 5.0 (▲), 6.0 (O), and 7.0 (●) were flushed sequentially through the measuring chamber.

same distance from the root apex (2.75–3.25 mm). The removal of Na$^+$, Ca^{2+}, and Cl$^-$ all caused a near doubling of mean peak outward current densities over the short region of root where outward current was mapped (from approximately 1 to 2 mA m^{-2}). The removal of K$^+$ ions appeared to extend the region of inward current at a low 1 mA m^{-2} level. These results suggest that these inorganic ions carry little of the inward current associated with growing *Z. mays* root tips. Since Na$^+$, K$^+$, Cl$^-$, and Ca^{2+} do not carry much current we inferred that the electrical current may be due mainly to a circulating flux of protons.

Relationship between Current Density, pH, and Root Growth

To test the suggestion that protons were carrying most of the inward current, the magnitude of the circulating current was monitored rapidly while the extracellular pH was adjusted between 3.0 and 7.0 (Fig. 3). Increasing the external pH resulted in an immediate reduction of the current density and a decrease in the elongation rate of the root (see Table I). Roots exposed to more acid pH values generated larger current densities and extended faster. These observations supported the suggestion that hydrogen ions carried the major component of the inward current and indicated a correlation between growth rate, pH, and current density. There also appeared to be a basipetal extension of the zone of inward current as the external pH fell and an acropetal compression of it as the pH increased. The effect of altering pH on the current could be detected by the time it took to make the first measurement (*i.e.* approximately 3 min).
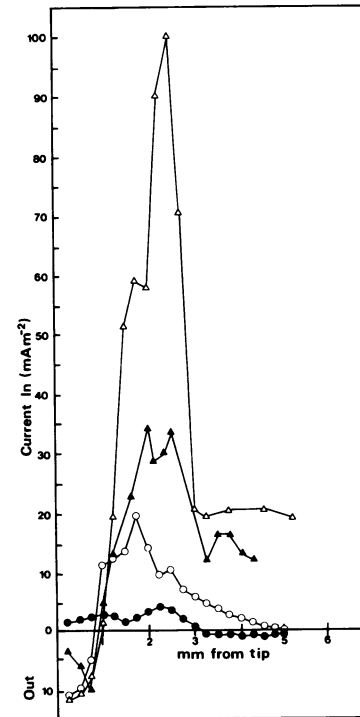
Effects of FC on the Root-Generated Ion Current and Root Growth

The effect of 10^{-6} M FC on the ionic current is illustrated in Figure 1. At pH 6.0, FC induced an approximate threefold increase in the inward limb of the current with little or no apparent stimulation of the outward limb over the length of root examined. FC treatment also reversed the small outward current associated with the root cap to an inward one. The current crossover point between the main inward and outward currents was, however, unaltered following FC treatment, being located approximately 3.75 to 4.00 mm from the root apex. The current profiles displayed in Figure 4 illustrate the relationship between FC, pH, and current density. At pH 7.0, FC induces an approximate 8-fold increase in current density, a 3-fold increase at pH 6.0 and at pH 5.0 an approximate doubling. FC stimulated the growth of intact *Z. mays* roots to varying degrees, being most effective at pH 6.0, where it induced more than a doubling of the elongation rate. At pH 3.0, 5.0, and 7.0 the promotive effects of FC on root growth were not so apparent in comparison with APW controls (see Table I).

The contribution of FC-stimulated K$^+$ influx (19) made to the enhanced inward current was determined by replacing the medium containing APW + 10^{-6} M FC with a similar K$^+$-free medium (Fig. 5). It was clear that the absence of K$^+$ had little effect on the enhanced inward current density over the length of root examined.
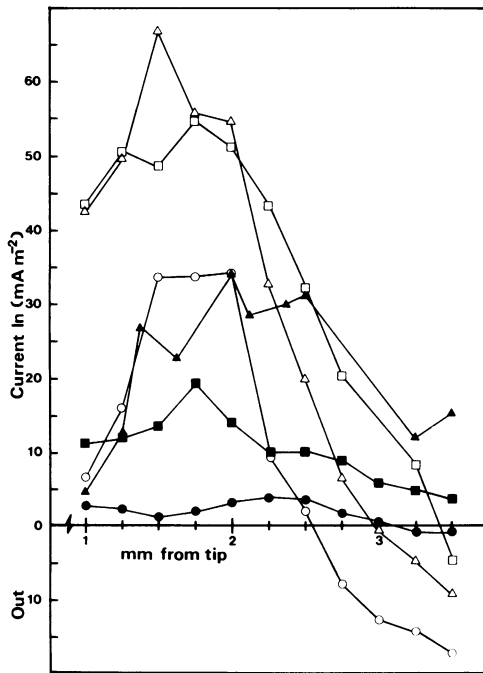
Figure 4. Enhanced root-generated current induced by 10^{-6} M fusicoccin at pH 5 ($\triangle$), 6 ($\square$), and 7 ($\bigcirc$) are compared with their respective APW controls ($\blacktriangle$), ($\blacksquare$), and ($\bullet$). Six day old seedlings were utilized.

Effects of IAA on the Root-Generated Ion Current and Root Growth

When the ionic current pattern resulting from the presence of 10^{-4} M IAA in the measuring medium was examined at pH 5.0, 6.5, and 7.0 (Fig. 6), it was clear that IAA reduced the density of the inward current. IAA also induced a shift toward the root apex of the current crossover point. These acropetal shifts were in addition to those which resulted from increasing the pH of the bathing medium (Fig. 3). This acropetal compression of the inward current zone is illustrated in Figures 5 and 6.

The current densities in the presence of IAA were again influenced by the pH of the experimental media, with higher

densities recorded at lower values. With the exception of growth rates measured at pH 3.0, the elongation of intact Z. *mays* roots was inhibited by the presence of 10^{-4} M exogenous IAA (Table I). This inhibition appeared to be pH dependent with greater inhibition at high pH. At low pH, the short-term promotion of growth resulting from the acid environment may be sufficient to swamp any growth inhibition induced by IAA. As shown previously (Fig. 3), the reduction in growth rate resulting from raising the pH of the bathing medium (in the absence of IAA) was also associated with a reduction in inward current density and a compression of the region of the root associated with inward current.

Individual ions were removed selectively from the media of roots that has been exposed to IAA at pH 7.0 (Fig. 7). Roots were scanned across the region where IAA induced a reversal from inward to outward current. The absence of Na^+ had little effect on the current pattern, indicating that Na^+ played no significant part in carrying the IAA-induced reversed current. The removal of K^+ or Cl^- increased the magnitude of the reversed current approximately twofold. When Ca^+ was removed the magnitude of the reversed current was again stimulated, but to a much greater extent, around fourfold. When Ca^{2+} was replaced the current returned to previous levels.

DISCUSSION

Ionic Current Patterns

The pattern of endogenous ion currents measured in the medium surrounding horizontally growing root tips of Z. *mays* showed that current consistently flowed into meristematic and most actively elongating tissues of the root (26). Current flowed out from the root cap and mature tissues of the primary root. Outward current was also detected associated with cells entering into a postelongating phase. The latter may also contribute a small amount to the overall elongation of the root. The lack of a nondestructive experimental method to determine the boundaries of elongating and nonelongating cells, permits only general correlations to be made concerning the developmental status of root tissues and the precise pattern
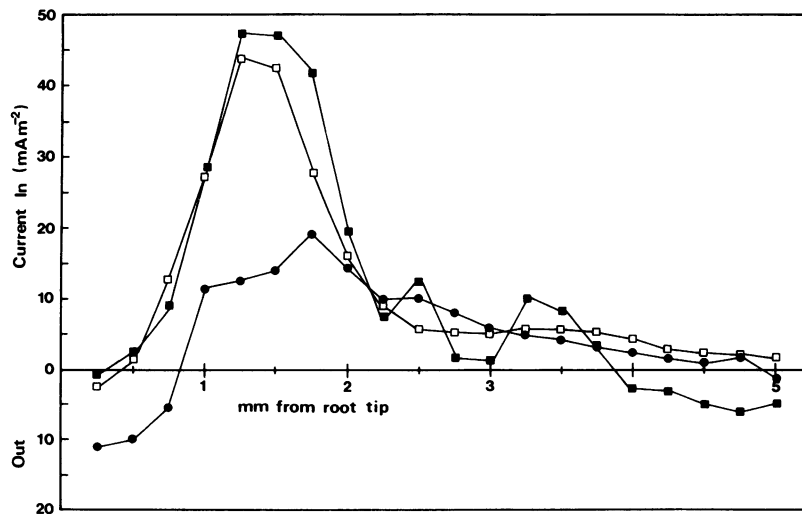

Figure 5. Effect of potassium on fusicoccin-stimulated inward current. Current patterns surrounding a 6 d old Z. *mays* root were mapped at pH 6.0 in the following sequence: an APW control ($\bullet$), APW + 10^{-6} M fusicoccin ($\blacksquare$) followed by APW − K^+ + 10^{-6} M fusicoccin ($\square$).
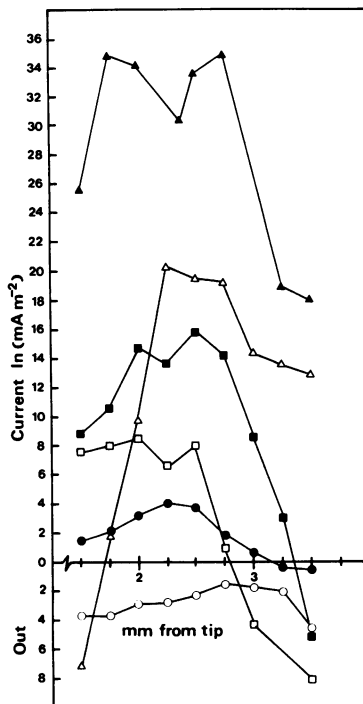
Figure 6. Effect of exogenously applied IAA on the root-generated current pattern of 6 d old *Z. mays* seedlings at different pH values. The current inhibition resulting from 10^{-4} M IAA at pH 5.0 ($\triangle$), pH 6.5 ($\square$), and pH 7.0 ($\bigcirc$) are compared with their respective APW controls: ($\blacktriangle$), ($\blacksquare$), and ($\bullet$).

Table I. Growth Rates of Primary Roots of 6 d Old Z. mays Seedlings

	Growth Rate		
pH	APW	APW + 10^{-4} M IAA	APW + 10^{-6} M FC
	$\mu m\ min^{-1} \pm$ SE		
3.0	26.67 ± 3.45	26.72 ± 3.64	31.30 ± 4.24
5.0	16.69 ± 3.38	7.35 ± 2.27	16.59 ± 0.57
6.0	8.06 ± 0.38	2.53 ± 0.56	17.82 ± 1.64
6.5	7.81 ± 2.89	1.44 ± 0.43	11.01 ± 0.27
7.0	6.91 ± 0.57	0.90 ± 0.45	7.15 ± 0.20

of current flow. The outward current associated with the root cap was not investigated in this study and the "outward current" mentioned here refers specifically to that associated with the mature cells of the main root body. The electrical activity associated with georeacting maize root caps has already been reported in detail (3). It should be borne in mind that the circulating current described here is strictly a property of an intact root system, since inward current cannot occur without counterbalancing outward current. The root must therefore be considered as an electrically contiguous, spatially differentiated tissue. This points to the likelihood that the electrophysiological properties of root segments will be different from the properties of the same cells prior to their excision.

Ionic Composition of Current

It was apparent that the removal of Na^+, K^+, and Cl^- from the measuring medium had little effect on the inward or
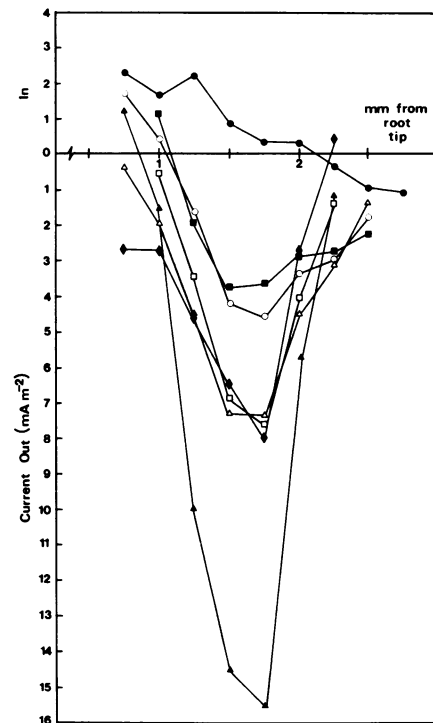

Figure 7. Root-generated current profiles were mapped at the current cross-over region resulting from the following sequence of media substitutions: APW control ($\bullet$), APW + IAA ($\blacksquare$), APW − Na^+ + IAA ($\bigcirc$), APW − K^+ + IAA ($\triangle$), APW − Cl^- + IAA ($\square$), APW − Ca^{2+} + IAA ($\blacktriangle$) and after Ca^{2+} ions were restored by flushing the measuring chamber through for a second time with APW + IAA ($\blacklozenge$). Six-day old. *Z. mays* seedlings were utilized with all media adjusted to pH 7.0. IAA was used at a concentration of 10^{-4} M.

outward current densities, or the position where inward current reversed to outward current. Deletion of Ca^{2+} resulted in a slight increase in the inward current density without changing the position of the current cross-over point. This increase in current density is unlikely to result from Ca^{2+} ions carrying a component of the current, since the removal of Ca^{2+} stimulated the current as opposed to reducing it. Instead it is suggested that Ca^{2+} may in some way regulate or modulate the proton current loop. Removal of Ca^{2+} from the bathing medium of the freshwater alga *Chara corallina* results in a depolarization of internodal cells and an increase in membrane conductance (2). That Ca^{2+} might regulate transcellular currents, while not being a major current carrying ion, has already been suggested with reference to germinating *Lilium longiflorum* pollen tubes (35). Raising the pH of the bathing medium resulted in an instantaneous reduction of the inward current density and an acropetal compression of the inward current zone, while lowering the pH increased the inward current density and extended basipetally the inward current zone. A similar effect was reported from *H. vulgare* (36).

These results suggest that H^+ (or OH^-) ions are the main component of the inward current which enters the elongating tissues of the root tip, as well as being the most probable carrier of the outward current which leaves from mature root regions. This pattern, with current entering growing, and leaving from nongrowing tissues, is in accord with previous

root examinations made with vibrating electrodes (1, 21, 22, 36). The suggestion that H^+ ions may be a major component carrying the endogenous current supports earlier reports where protons have been identified as the major current-carrying ion in both freshwater and terrestrial plants (4, 8, 36). It should, however, be borne in mind that the techniques employed in these studies do not permit readily a distinction between H^+ influx and OH^- efflux. In addition, at external pH values less than 6 bicarbonate ion efflux might also be considered to be almost equivalent to H^+ efflux (see discussion by Weisenseel et al. [36]). Weisenseel et al. calculated that the H^+ ion influx required to overcome the CO_2 efflux for H. vulgare roots growing in APW represented approximately 50% of the total inward H^+ current. It must also be stressed that the composition of the current is made by inference only since the vibrating probe measures only the net movement of electrical charge, not the fluxes of particular ionic species. We have shown that roots of Z. mays decolorize bromocresol agar around the zone of presumed cell division and extension indicating local extracellular alkalinity (our unpublished observations). This is compatible with proton uptake at this location and may be another manifestation of an inwardly driven proton current. This again falls short of proving that the electrical current is due to protons since local alkalinity could be generated by ammonium release or electroneutral excretion of acidic metabolites. The most likely interpretation of the results however is that most of the electrical current is due to spatially segregated electrogenic transport of protons.

Current Generation, pH, FC, IAA, and the Acid Growth Hypothesis

The influx of H^+ ions into the root apex of Z. mays would appear to be at odds with the acid growth hypothesis when applied to developing roots in general and corn roots in particular. There is evidence that both maize root-tip segments (9) and intact root tips (23) acidify their bathing medium. There has, however, been a recent report showing that H^+ ions flow into the apex of Z. mays roots as far back as 3 mm from the apex (3). This zone of current influx can be considered to be well within the elongation zone (26). The pH pattern reported by Bjorkman and Leopold (3) (their Fig. 2) derived from roots growing in APW at pH 6.0, reflects qualitatively the current profile we illustrate in Figure 1. The proposition that roots generate a region of high pH around their growing tips is not new (36), and such alkaline environments have been suggested to correspond to the region of maximum cell elongation of H. vulgare roots (24). It is significant that in all cases where an alkalinization of the growing tip environment has been reported, intact plants were used as experimental material. In the case of our own experiments great care was taken to ensure vibrating probe examinations were carried out during steady-state root growth of intact plants rather than on excised tissues recovering from preparation and handling stress. In some experiments activation of growth in root segments by low pH and low pH-inducing factors (i.e. IAA and FC) may have resulted from accelerated recovery from excision or abrasion injury (13). There is little doubt that a low pH environment in the vicinity of cell walls can, over the short term, stimulate strongly

elongation in root segments (9). It is still not clear whether this has any significance to the long term growth of intact roots in response to auxin or low pH (5, 13).

It is, however, difficult to reconcile the proposition that FC enhances a proton influx with more than a decade of work suggesting it stimulates energy-linked net H^+ efflux (19). It is again worth pointing out that our results were derived from intact root tissues, whereas the majority of published data relate to the effects of FC on root segments. Some concern has been expressed about the validity of comparing results obtained from exposing to FC excised tissues, to those derived from intact tissues (12). In addition, little consideration has been given to the effect of FC on tissues in different developmental states. Where this has been taken into account, it would appear that the capacity of root tissues to acidify their bathing medium increases with the age and the differentiation of the root (11). Pitman et al. (29) also indicated that that the stimulation of H^+ efflux and K^+ influx by FC in intact H. vulgare roots was a property of the mature nongrowing tissues as well as the immature regions. Although we detected no FC-induced stimulation of outward current in the apical 5 mm of roots (the development of root hairs beyond this point prevents accurate current mapping), this represents only a small portion of the total root surface area of a 6 d old seedling. We are now attempting to investigate the effects of FC along the whole length of the mature root in Z. mays We would propose at this stage that FC may indeed stimulate H^+ efflux from mature root tissues, and that this might be balanced by an increased H^+ influx into the region of the root tip in which cell division and elongation takes place. The FC-induced current pattern we have reported may, therefore, be incomplete and represent only one limb of an enhanced ion flux. Integration of the effects of FC into a spatially separated yet balanced flux may explain the observed lack of cytosolic pH perturbation experienced in the presence of FC (32). The effectiveness of FC in stimulating inward current was related to the pH of the bathing medium: greater stimulation of high pH, less at low pH. There may be a physiological limit to H^+-influx which is reduced at higher values in the presence of FC.

The stimulated inward current in the presence of FC was shown clearly not to be due to increased K^+ uptake (19), as removal of K^+ ions from the measuring medium had no significant effect on the enhanced current density. We cannot as yet say what effect the removal of K^+ in the presence of FC has on the outward current, but it has been suggested that the stimulation of K^+ influx by FC may be a property of mature nongrowing root tissues (29).

The presence of exogenous IAA caused a reduction in the inward current density at all pH values examined. A similar reaction was reported by Brawley et al. (4) when examining the effects of IAA on the current generated by the developing embryo of Daucus carota. Our data would also suggest there was some relationship between the effects of IAA on current density, and the pH of the experimental medium, i.e. the effectiveness of IAA in reducing inward current density falls with decreasing pH. IAA also caused an acropetal shift of the point where inward current switches to outward current. It had, therefore, the dual effect of reducing the current density,

and restricting the volume of tissue into which inward current flows, both of which were pH dependent.

Current Generation and Root Growth

Factors which promote (low pH and FC) or reduce (high pH and IAA) the elongation of intact primary roots of Z. *mays* increase or decrease, respectively the density of current flowing into meristematic and elongating tissues. The enhancement of maize root segment (9) and intact root (23) elongation by low pH is well documented.

The effective stimulation of elongation by FC does not show any linear pattern as pH is reduced from pH 7.0 to 3.0. The largest FC-induced stimulation of elongation occurred at pH 6.0, and fell as the pH was raised or lowered to pH 7.0 or 3.0, respectively. The effect of FC on the inward current densities did, however, show a trend across this pH range: the amount of FC-induced current enhancement fell as pH was reduced. Compared to APW controls, FC induced an 8-fold stimulation of the current density at pH 7.0, whereas stimulation of the elongation rate was minimal. At pH 6.0, FC induced a 3-fold stimulation of inward current and more than a doubling of elongation. Similarly, Gabella and Pilet (11) reported that the acidification response of maize root segments to FC was linear, but its maximum did not coincide with the maximal elongation. It has also been shown that increasing the osmotic pressure of the medium bathing maize root segments progressively inhibited segment elongation without affecting the membrane potential changes that were induced by FC (7). There would appear, therefore, to be a complex relationship between applied FC and root segment metabolism (including growth). These are complicated further when considering intact roots since it is clear that the growth responses to FC are connected to the age of the root tissues and their morphological characteristics (11, 29). Thus, it would appear that the effect of FC does not depend on the increase of cell elongation.

What effect exogenous IAA has on intact roots may depend both on the concentration of IAA and on the initial elongation rate of the root. Pilet and Saugy (27) have reported that slow growing roots were all inhibited by exogenous IAA (at any concentration), whereas the elongation of fast growing roots was promoted by IAA at low concentrations. It has also been reported that the uptake of IAA (and therefore its cytosolic concentration) may be pH dependent (20). There would appear, therefore, to be a complex relationship between external pH, IAA-uptake, and root elongation. This relates to our IAA growth results presented in Table I. At pH 7.0, when the roots were growing slowly the effect of 10^{-4} M IAA was to reduce the elongation rate of the root by some 90%. At pH 3.0, however, IAA had a negligible effect on cell elongation. Whether this is due to the initial elongation rates of the root as influenced by the pH of the bathing medium, the effect of pH on the uptake kinetics of IAA, or a combination of both, we cannot clearly say. What is suggested, is the inhibitory effects of IAA are swamped by the short-term stimulatory effects of low pH.

CONCLUSIONS

Studies using a vibrating microelectrode and ion-substitution experiments suggest that the root-generated electrical current of Z. *mays* is due primarily to a circulation of protons. The extension rate of the root correlated with the density of the proton current entering actively dividing and elongating cells. Factors which promoted (low pH and FC) or restricted (high pH and IAA) root growth, enhanced or reduced the root-generated current, respectively.

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