Plant Physiol. (1988) 87, 874–877
0032-0889/88/87/0874/04/$01.00/0

Effect of a Longitudinally Applied Voltage Upon the Growth of *Zea mays* Seedlings[1]

Received for publication July 20, 1987 and in revised form January 18, 1988

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ABSTRACT

The electrical parameters that affect young seedling growth were investigated. Voltages ranging from 5 to 40 volts were applied longitudinally along the mesocotyl region of 4-day old *Zea mays* L. (cv Silver Queen) seedlings for periods of 3 or 4 hours. It was determined that: (a) making the tips of the seedlings electrically positive relative to the base strongly inhibited shoot growth at 5 volts, whereas the reverse polarity had no effect; (b) at higher voltages, making the tip of the seedlings negative caused less growth inhibition than the reverse polarity at each voltage level; (c) the higher the applied voltage the greater the degree of inhibition; and, (d) the more growth inhibition experienced by the plants the poorer, and slower, their recovery. Previous observations of a relationship between the amount of free indole-3-acetic acid in the mesocotyl cortex and the growth rate of the mesocotyl and of gravitropism-induced movement of labeled indole-3-acetic acid from the seed to the shoot lead to the prediction of a voltage-dependent gating of the movement of indole-3-acetic acid from the stele to the cortex. This provided the basis for attempting to alter the growth rate of seedlings by means of an applied voltage.

This laboratory has developed a working theory to explain how an asymmetric distribution of solutes such as indole-3-acetic acid (IAA), IAA-esters, gibberellins, and calcium is attained in a plant stem following a tropic stimulus (4). The theory presents a mechanism for lateral transport, the central postulate being that solutes are selectively leaked from the vascular tissue into the surrounding cortical tissues through voltage-gated channels (2). The channels, presumably portions of the plasmodesmata, would be analogous to the voltage-gated gap junctions of animal tissues (20). There is support for the theory since membrane depolarization is an early event following a tropic stimulus (6, 7), and regulated movement of IAA from stele to cortex has been observed (5). Also, a protein immunologically related to the animal gap junction protein, as well as a voltage-regulated channel (29, 34), has been found in plants (28).

A prediction of the theory is that an applied potential should affect hormone distribution, and thus, growth. Growth is easier to measure, and, in the case of mesocotyl tissue of *Zea mays*, may predict the amount of IAA (8, 24, 32). In this work, we have established the electrical conditions for altering plant growth and for producing sufficient quantities of cortical and stele tissue for subsequent physico-chemical analyses of hormone amounts (13).

Voltages ranging from 5 to 40 V were applied longitudinally along the mesocotyl region for periods of 3 or 4 h. We demonstrated that: (a) making the tip of the plant positive relative to the base strongly inhibited growth at 5 V whereas the reverse polarity had no effect; (b) making the tip of the plant negative caused less growth inhibition than making the tip of the plant positive at each voltage level; (c) the higher the applied voltage, the greater the degree of inhibition; and, (d) the more growth inhibition experienced by the plants the poorer, and slower, the recovery after cessation of the applied voltage.

The literature reporting effects of an applied potential on plant growth has been reviewed (14, 15, 17, 33). Our work confirms previous studies showing that setting the tip at a negative potential is less injurious, or even stimulatory, as compared to placing the tip at a positive potential (11, 12). The chemical basis for these effects is not known, but it is of interest that growth of tissue cultures is stimulated when the tissue is made negative relative to the media (19) and that this stimulation is eliminated by the auxin transport inhibitor, 2,3,5-triiodobenzoic acid (18, 19). Since the vascular stele and cortical tissues of the *Zea mays* mesocotyl are readily separated, it should be possible in future work to determine how the movement of solutes from stele to cortex might be regulated and, ultimately, to better understand the chemical basis for the observed growth changes.

MATERIALS AND METHODS

Plant Material. Corn kernels, *Zea mays* L. cv Silver Queen, were sterilized in a 1:20 dilution of commercial (NaOCl) bleach for 10 min and then imbibed for 20 h in cold running tap water. The seeds were germinated in rolled moistened paper towels and grown for 4 d in the dark at 25°C in a room with 85% relative humidity. Handling of the plant material was in the dark or under a green safe light that did not induce phototropic curvature during an 18 h test period. Under these conditions, 90% of the growth occurs in the mesocotyl region of the shoot and so would be below the contact of the top electrode.

Electrolyte Solution. The electrolyte was Shive's nutrient solution. It consisted of 4.5 mM $Ca(NO_3)_2$, 2.3 mM KH_2PO_4, 2.2 mM $MgSO_4$, 0.68 mM $(NH_4)_2SO_4$, and 0.02 mM $FeSO_4 \cdot 7H_2O$. The use of a nutrient solution instead of single salt solution as an electrolyte introduces some additional complexity in the experiment, but its use avoids grossly altering the electrical behavior of the membranes in contact with the electrolyte (23).

Electrical Potential Application Apparatus. The apparatus, constructed from a plastic test tube rack, permitted application of an electrical potential along the length of 10, 4-d-old, vertically oriented seedlings (Fig. 1). The seedlings were placed in the apparatus with kernels resting on the bottom tier and the roots dangling into the electrolyte solution in contact with a platinum plate of 38 cm^2 area. The top electrode consisted of an electrolyte-

[1] Supported by a National Aeronautic and Space Administration Research Associate Award to M. F. D. and by Research Grant Awards to R. S. B., Space Biology, NASA-NAGW-97, the Shuttle Flight Program NAG-2-362, and Metabolic Biology, National Science Foundation DMB-8504231.

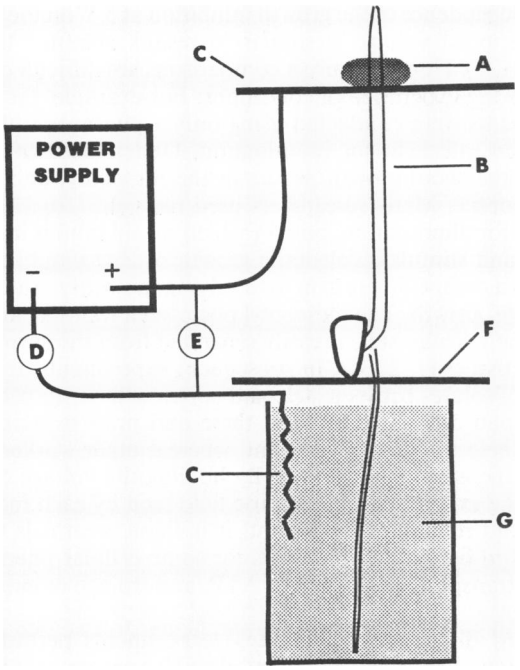

FIG. 1. Diagram view of the electric-application apparatus arranged for top positive experiment. The coleoptile, B, is supported by the bottom tier of the test tube rack, F. The current path is from the D.C. power supply to the platinum foil, C, through the elctrolyte saturated foam collar, A, into the top of the seedling. After traversing the seedling, it returns to the power supply from the platinum electrode, C, and electrolyte solution, G. The voltage and resultant current from the dc power supply are measured by the ampere meter, D, and the voltage meter, E.

saturated foam collar of 3 mm thickness loosely surrounding the coleoptile at approximately 5 mm below the tip and resting on platinum foil. The electrical path length through the plant was approximately 8 cm. The platinum strips were connected to a DC power supply while the current and voltage were monitored by digital multimeters.

Procedure. The seedlings were placed in the apparatus with the electrodes attached and were allowed to become acclimated. The top foam electrodes were rinsed at half-hour intervals during the course of the experiments and were monitored for electrically induced pH changes. Changes in pH were not observed, except for a barely detectable shift at 40 V, the highest voltage used. Shoot growth was recorded by photographing the shoots against a millimeter-ruled background using the green safelight for illumination. Growth, during each time interval, was measured from the film negatives to the nearest 0.2 mm with the aid of a dissecting microscope. Thirty plants were used for each experiment: 10 plants for the control (no voltage), 10 plants for the tip at positive potential, and 10 plants for the tip at negative potential. The three samples were run simultaneously in separate copies of the apparatus. Data from the individual measurements were averaged to reduce the variability encountered when examining individual plants. The statistical methods used to determine significant differences ($P = 0.01$) were Student's t test and analysis of variance.

RESULTS

Figure 2 shows the effect of an applied voltage on the growth of the shoots. For these experiments, the seedlings were allowed to acclimate for 1 h after being placed in the apparatus; the voltage was then applied for 3 h; and finally the seedlings were permitted to recover for 2 h. Each graph shows the cumulative growth of control seedlings and of seedlings exposed to the

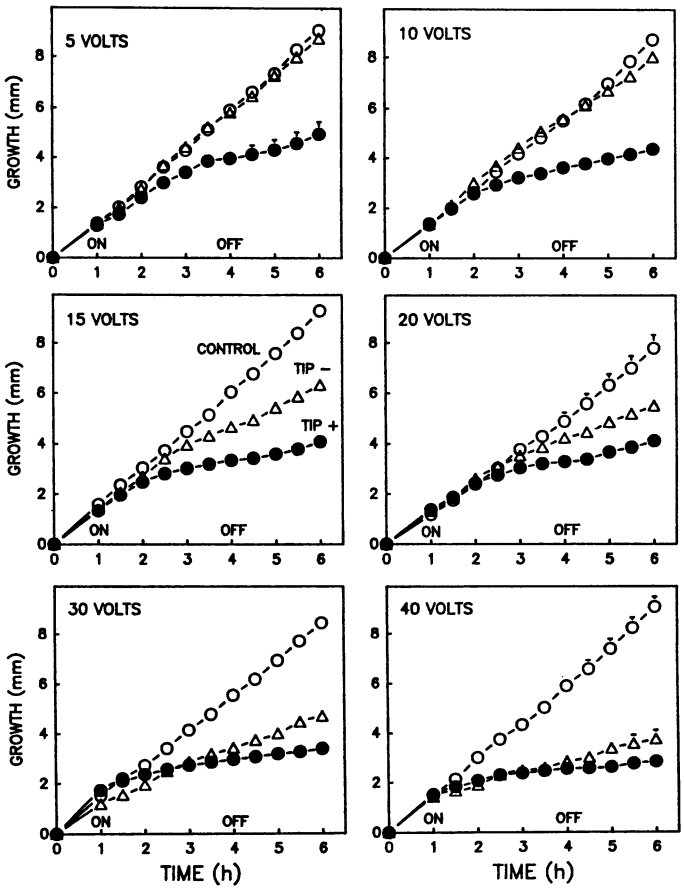

FIG. 2. Cumulative growth in millimeters of 4-d, dark-grown corn seedlings' shoots as a function of the magnitude and polarity of an applied voltage. The electrical potential was applied between 1 and 4 h to the shoots with the apical tip at negative (△) or positive (●) potential with respect to the base of the shoot or with no potential applied, control (○). Each point is the average of 20 independent measurements, and the error bars represent the standard error.

indicated voltage with either a top negative or top positive polarity. The controls exhibited an average growth rate of 1.45 mm/h. Growth stimulation above the control was not observed for any of the applied voltages. The amount of inhibition was dependent on both the polarity and the magnitude of the applied voltage. The current flow through the seedlings was proportional to the applied voltage but independent of the polarity. The current per plant ranged from an average of 6.7 μamps at 5 V to 40 μamps at 40 V. When the applied voltage was 15 V or less, the current was constant during the experiment. When the applied voltage was greater than 15 V, the current decreased slightly during the experiment.

A second set of experiments with a longer time course was done to examine better the recovery of the plants after the electrical potential application. In these experiments, seedling growth was monitored during a 2-h acclimation period, and 4 h of electrical stimulation were then given followed by an 18-h recovery time. The resultant growth rates for the control and two polarities, at either 5 or 10 V of applied potential, are shown in Figure 3. As can be seen, there was no, or almost no, inhibition of growth rate for seedlings having a 5 V tip-negative potential. Further, these seedlings recovered 89% of the control growth rate 18 h after cessation of the treatment. By contrast, seedlings having a 5 V tip-positive potential showed a 60% inhibition of growth rate 2 h after initiating treatment, and this increased to 90% inhibition at 2 to 4 h. Nonetheless, the tip-positive, 5 V

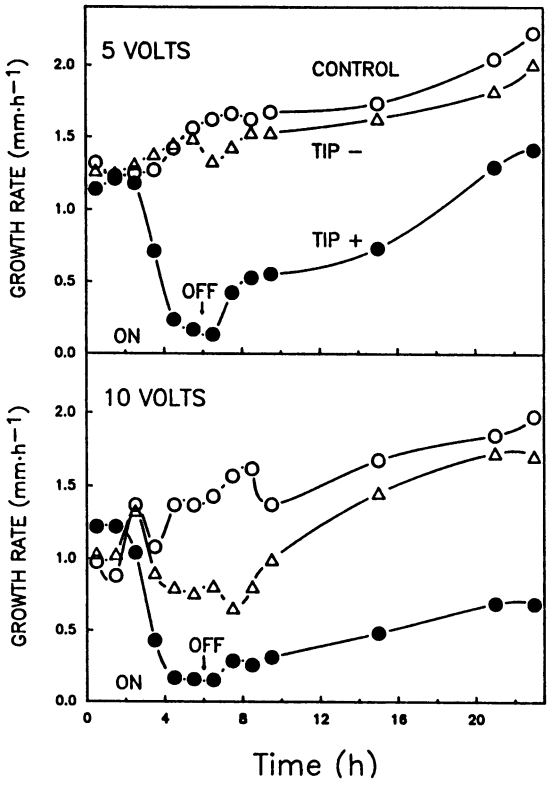

FIG. 3. Growth rate in millimeters per hour of 4-d, dark-grown corn seedlings' shoots as a function of time and of the magnitude and polarity of a voltage applied for 4 h. The electrical potential was applied between 2 and 6 h to the shoots with the apical tip at negative (△) or positive (●) potential with respect to the base of the shoot or with no potential applied, control (○). Each point is the average of 30 independent measurements.

seedlings recovered to 64% of the control growth rate within 18 h. At 10 V, even the tip-negative-treated seedlings exhibited a 45% inhibition of growth, and this recovered to 89% of the control rate in 18 h. At 10 V, tip-positive potential, there appears to be irreversible damage, since the growth rate fell by 89% and recovered to only 36% of the control.

There was a minimum of two repetitions of each experiment. There was no significant variation between the repetitions of experiments to a probability of 0.01, as measured by Student's *t* test or by analysis of variance. There was no visible damage to the plants subjected to electrical stimuli, nor any loss of turgor compared with the controls. The electrical resistance of plants during the experiments did not decrease with time, signifying no significant membrane damage (26).

DISCUSSION

Four conclusions may be drawn from the data of this paper. First, the higher the voltage the greater the degree of shoot growth inhibition, once a threshold value was passed. The threshold voltage was less than 5 V for tip-positive plants and above 5 V for tip-negative plants. The lowest voltage tested was 5 V applied along the length of an approximately 8 cm long section of seedling shoot. This would amount to an electric field of 0.6 mV per 10 μm, the length of a typical cell, a small potential relative to the potential across the plasma membrane. Second, growth inhibition was greater when the tip of the shoot was positive relative to the roots than the reverse polarity. Third, the larger the applied voltage, the earlier the onset of growth inhibition. Fourth, the more inhibition, the poorer, and slower, the recovery.

The dependence of the growth inhibition at 5 V on the polarity of the applied voltage is similar to results obtained by other workers (25). However, direct comparisons are difficult owing to the differing experimental conditions. For example, the present experiments were conducted using only a phototropically inactive green safe light for illumination. Under these conditions, 90% of the shoot growth occurs in the mesocotyl and 10% in the coleoptile. Many researchers used red light (10, 12) or day light (9) for illumination, both of which would inhibit mesocotyl growth and stimulate coleoptile growth of *Zea mays* seedlings. There is a compelling reason for studying mesocotyl, rather than coleoptile, growth. The mesocotyl possesses a root-like anatomy. Thus, the vascular stele is easily separated from the surrounding cortical tissues (1, 2, 31). In subsequent experiments, the distribution of solutes between stele and cortex will be studied.

A second difference between these and prior experiments is that we held the voltage constant, whereas other workers maintained a constant current (12). By holding the voltage constant during the experiment, the electric field seen by each individual cell should remain constant, and it is these potentials that are believed to be the driving forces for some cellular phenomena, such as transport, generation of ATP, and storage of energy (21, 23).

A third difference is that these experiments permitted the application of an electrical potential to 10 or more seedlings at a time. Thus, it was possible to produce gram quantities of voltage-treated stele, or cortical tissues, for subsequent chemical analysis. Furthermore, better statistics are obtained since many seedlings may be observed at one time. A shortcoming of this procedure and of the photographic method used to measure growth changes is the lesser sensitivity. They are less capable of detecting rate changes than the angular transducers used by other workers for growth measurements (3).

To the best of our knowledge, the reason for the marked inhibition of growth observed when the tip of the plant is made positive relative to the roots is unknown. The current flow is the same at either polarity, and the electric field is only 0.6 to 5 mV/10 μm. Jaffe and Niccitelli (23) separated the effects of small applied potentials on plasma membranes into two categories. The first category, electric fields of the order of 0.2 to 0.4 mV/10 μm, was thought to be sufficient to cause migration of mobile plasma membrane components within the plane of the plasma membrane (22). A second category, cells in a medium electric field (3–6 mV/10 μm), might exhibit changes in transmembrane potential, possibly altering ionic flux through the membranes (30) and the regulation of voltage-dependent channels in the plant membranes (34). The system developed in the present work produces sufficient vascular and cortical tissue to permit measuring the distribution of solutes between these two tissues.

A connection between the effects of an applied electrical potential on growth and on growth hormones is tenuous but not unreasonable. Auxin is known to influence the membrane potential of tissues and protoplasts. Etherton (16) reports that the addition of indole-3-acetic acid at concentrations that enhance tissue elongation will hyperpolarize the membrane potential of oat coleoptile cells. Marre *et al.* (27) observed hyperpolarization of the transmembrane potential and cell enlargement with the addition of physiological levels of auxin. They attributed the increase of the negative transmembrane potential to a metabolism-dependent, proton-extrusion mechanism. Vorobiev and Manusadzhanas (35, 36) state that the type of membrane-potential response (hyperpolarization, depolarization, or no change in membrane potential) in response to IAA addition depends on the initial level of the membrane potential. However, a 50% increase in membrane resistance occurred after the addition of IAA regardless of membrane potential shifts.

In this laboratory, we have observed a direct relationship

between the amount of free IAA in the mesocotyl cortex and the rate of growth of the mesocotyl (32). In addition, based upon tropism-induced changes in the movement of labeled IAA from seed to shoot (1), we predicted a voltage-dependent gating of the movement of IAA from stele to cortex (4). These observations provided the basis for attempting to alter the growth rate of the seedling by means of an applied potential and then to determine if the growth change was concomitant with endogenous hormone distribution. Specifically, we plan to examine the polarity-dependent growth inhibition at 5 V. Using this voltage, we can possibly separate effects on the plant that are due to current from effects that are due to the current's direction. In subsequent studies, we hope to determine if the effects of an applied potential on growth can be correlated with changes in the distribution of small solute molecules in the tissue, such as IAA, gibberellins, or calcium.

There is an apparent interaction between endogenous electric fields and plant growth. It would be unreasonable to expect that application of electric fields would consistently stimulate growth in differentiated tissue, since the endogenous electric fields are probably optimized for maximum growth, and any disruption would result in growth inhibition. However, in undifferentiated tissue, such as a callus, an electric field dramatically stimulates both growth and differentiation (19). Thus, the endogenous field may either mirror or determine the direction and polarity of plant growth. The hormone auxin would then initiate the biochemical machinery that makes growth possible.

Acknowledgments—We thank Dr. Bernard Epel and Dr. Dennis Reinecke for their assistance in manuscript preparation.

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