

## The effects of electric field on the growth of intact seedlings and coleoptile segments of *Zea mays* L.

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### Abstract

The experiments were carried out with 96-h-old intact maize seedlings and 10 mm long coleoptile segments cut 4 mm below the tip. The electric fields were applied longitudinally along the seedlings. The electric field (15 V) caused inhibition of the elongation growth of intact seedlings which was dependent on both the polarity and the duration of the applied voltage. The growth inhibition was greater when the tip of the shoot was positive relative to the roots. The electric field also caused inhibition of indole-3-acetic acid (IAA) and fusicoccin (FC) induced growth of maize coleoptile segments excised from electrically treated seedlings. IAA-induced growth of coleoptile segments was greater when the tip of the shoot was negative to the roots (not in the case of FC-treated segments and intact seedlings). It was suggested that apart from the changes induced by electric field in transport system of auxin the electric field affected also the activity of plasmalemma proton pump.

*Key words:* elongation, fusicoccin, indole-3-acetic acid, polarity, voltage

### Introduction

The physiological effect of electric field have been extensively studied in various plant material, particularly with regard to its influence on the growth and development (for review see Scott 1967, Jaffe and Nuccitelli 1977, Robinson 1985, Nuccitelli 1988, Ezaki *et al.* 1990). For example, Black *et al.* (1971) found an increase in linear growth of 5 to 30 % when tomato plants were treated with currents of 3 to 15 mA. These findings were true for plants treated with currents negative-to-ground, but a reduction of growth was found when plants were treated with 15 mA currents positive-to-ground or with currents of either direction greater than 30 mA. More recently it was reported that the cumulative growth of *Zea mays* L. seedlings was inhibited when the voltages ranging from 15 to 40 V, independently of the polarity, were applied longitudinally along the mesocotyl region (Desrosiers and

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Bandurski 1988). Higher growth inhibition these authors observed when the tips of the seedlings were electrically positive relative to the base. In contrast to this Medvedev and Markova (1990) found stimulation of the growth rate of the *Zea mays* L. coleoptile segments when the anode was at their apical end. However, no significant change in growth rate these authors found when the direction of the current was reversed. On the other hand, Goldsworthy and Rathore (1985) reported dramatic increase in the growth of tobacco callus tissue with electric currents flowing from the medium into callus tissue, while currents with opposite direction had a slightly inhibiting effect. Also roots of many plants respond to electric field and exhibit enhanced growth either on the anodal or on the cathodal side (Moore *et al.* 1987, Marcum and Moore 1990, Ezaki *et al.* 1990, Stenz and Weisenseel 1991).

As can be easily seen from the contributions quoted above, there are many discrepancies between the results obtained by various authors and the effect of electric field and current is not well understood. Therefore the aim of the present investigations was the comparison of the effects of electric field on cumulative growth of intact maize seedlings and elongation growth of the coleoptile segments (excised from electrically treated seedlings) in the presence of either IAA or FC.

### Materials and methods

The experiments were carried out with 96-h-old intact *Zea mays* L. seedlings and 10 mm long coleoptile segments (with the first leaves removed) cut 4 mm below the tip. The maize seedlings were cultivated at high humidity in the dark at 27 °C. All manipulations and growth experiments were conducted under dim green light (irradiance 6  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) at room temperature of 24 °C and relative humidity of 85 %. The experiments with intact seedlings were done in Shive's nutrient solution with the following composition: 4.5 mM  $\text{Ca}(\text{NO}_3)_2$ , 2.3 mM  $\text{KH}_2\text{O}_4$ , 2.2 mM  $\text{MgSO}_4$ , 0.68 mM  $(\text{NH}_4)_2\text{SO}_4$  and 0.02 mM  $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ . The seedlings were positioned vertically with the roots dangling into plexiglass chamber filled with the nutrient solution. The electric field (15 V) was applied longitudinally along the intact seedlings. The top electrode consisted of a Ag/AgCl and salt bridge (the agar block saturated with 10 mM KCl) was fixed on the coleoptile at approximately 4 mm below the tip. A similar electrode of opposite polarity was in contact with the electrolyte solution surrounding the roots. When the electric field of 15 V was applied along the seedlings the current flow through the seedlings was stable (*ca.* 8  $\mu\text{A}$ ). A similar procedure for studying the effects of electric fields upon the growth of maize seedlings has been previously used by Desrosiers and Bandurski (1988).

The second part of experiments was accomplished with 10 mm long coleoptile segments excised from electrically treated maize seedlings. After excision one coleoptile segment was strung on 8 mm long stainless steel needle which has been stuck into plexiglass block. This segment was inserted vertically in a small glass vessel (photometric cuvette) filled with incubation medium of the following composition: 1 mM KCl, 0.1 mM  $\text{CaCl}_2$ , 0.1 mM NaCl. This medium is routinely used in our laboratory for studying the elongation growth of maize coleoptile

segments (Karcz and Stolarek 1988, Karcz *et al.* 1990). Elongation growth of the coleoptile segment was traced in regular intervals of 5 min by measuring the position of the apical end of segment with horizontally placed microscope, equipped with a micrometer.

Cumulative growth of the intact maize seedlings was measured each 30 min by means of a shadow-graph method under dim green light at  $21\times$  magnification. The growth regulators IAA and FC were used in a final concentrations of  $10^{-5}$  and  $10^{-6}$  M, respectively. The growth experiments were repeated at least 9 times.

## Results

Elongation growth of intact seedlings was inhibited when the electric field was applied longitudinally along the seedlings with either a top negative or top positive polarity (Fig. 1). However, the inhibition was dependent on both the polarity and the duration of the applied voltage. When the tip of the shoot was positive relative to the roots, independently of the duration of the applied voltage, the growth inhibition was greater (Fig. 1). For example, the cumulative growth of seedlings non-treated with electric field was  $13.5 \pm 0.8$  mm within 6 h, whereas that of electrically treated

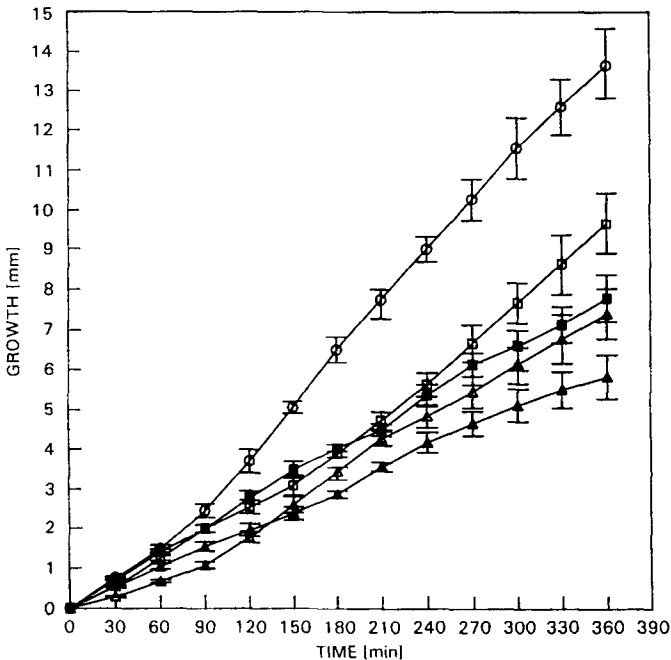


Fig. 1. The effects of electric field (15 V) on cumulative growth of intact maize seedlings. The electric field was applied after 1 h of preincubation. Control - seedlings not treated electrically (*open circles*); *open triangles and squares* - seedlings treated electrically 3 h with the apical tip at positive or negative polarity, respectively; *closed triangles and squares* - seedlings treated 5 h with the apical tip at positive or negative polarity, respectively.

seedlings for 5 h with the apical tip at positive (5+) or negative (5-) polarity was  $5.7 \pm 0.6$  mm and  $7.8 \pm 0.6$  mm, respectively. Application of the electric field within 3 h with either a top negative or top positive polarity was less injurious (ca. 10 %), as compared to 5 h period (Fig. 1).

The second set of experiments was done to examine the effects of IAA and FC on elongation growth of 10 mm long coleoptile segments excised from electrically treated maize seedlings. IAA was added at time "0" (Fig. 2) to the incubation medium 1 h after acclimation (data not shown) of the segments in measuring device. Electric field causes, similarly as for intact seedlings, inhibition of IAA induced growth of maize coleoptile segments (Fig. 2). The amount of inhibition was greater

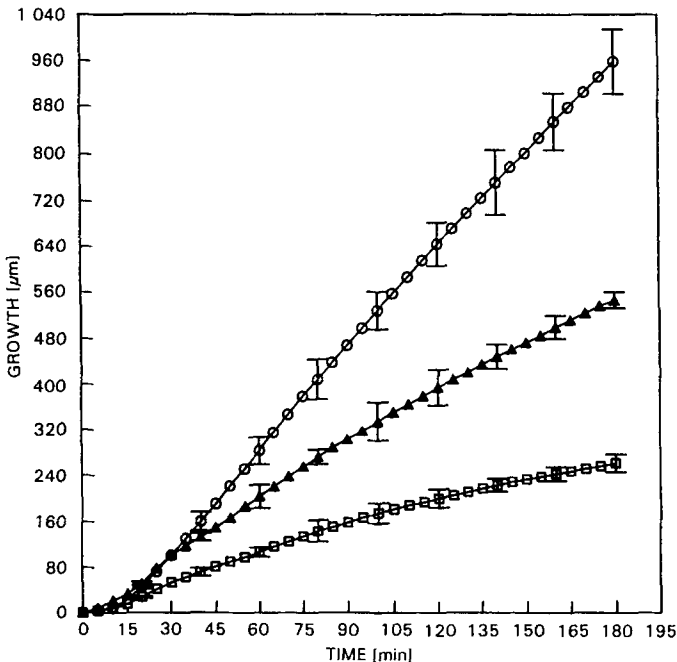


Fig. 2. The effect of IAA ( $10^{-5}$  M) on elongation growth of maize coleoptile segments excised from electrically treated (3 h) intact seedlings. IAA was added at time "0" to the incubation medium. Circles - IAA-induced growth of the coleoptile segments excised from electrically not treated maize seedlings; triangles, squares - IAA-induced growth of the coleoptile segments excised from electrically treated maize seedlings with the apical tip at positive or negative polarity, respectively.

when the coleoptile segments were excised from seedlings with a top negative polarity. For this polarity, inhibition exceeding 70 % of the control elongation growth (IAA-induced growth of electrically non-treated seedlings) was observed. It is noteworthy to point out that for IAA-induced growth of the coleoptile segments the positive polarity was less injurious as compared to the negative polarity (which is just opposite to intact seedlings). The inhibition of elongation growth of the coleoptile segments by electric field was also observed in the presence of  $10^{-6}$  M FC. In this case, however, application of the electric field with negative polarity at the top had only slight effect (inhibition ca. 10 %) on FC-induced elongation growth of

coleoptile segments. For reverse polarity (positive polarity at the top) inhibition of FC-induced growth exceeded 30 % (Fig. 3).

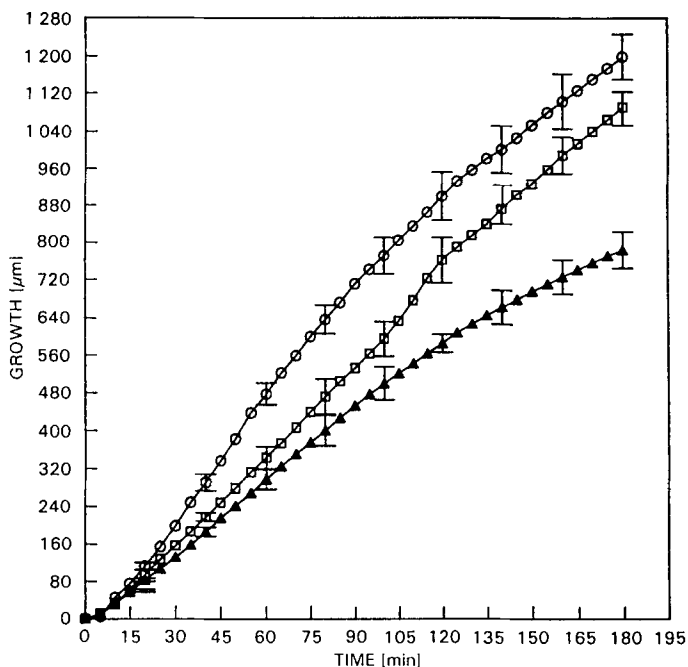


Fig. 3. The effect of FC ( $10^{-6}$  M) on elongation growth of maize coleoptile segments excised from electrically treated (within 3 h) intact seedlings. FC was added at time "0" to the incubation medium. Circles - FC-induced growth of the coleoptile segments excised from electrically not treated maize seedlings; triangles, squares - FC-induced growth of the coleoptile segments excised from electrically treated maize seedlings with the apical tip at positive or negative polarity, respectively.

## Discussion

Our results indicate that electric field caused the suppression of both the cumulative growth of intact maize seedlings and the elongation growth of the coleoptile segments (excised from electrically treated seedlings) in the presence of either IAA or FC.

The inhibitory effect of the electric field on the cumulative growth of intact seedlings observed by us generally agrees with the results reported by Desrosiers and Bandurski (1988); in both cases the electric field (15 V within 3 h) brought about *ca.* 40 and 50 % inhibition of growth for a top negative and positive polarity, respectively (Fig. 1). Desrosiers and Bandurski (1988) and (Bandurski *et al.* 1986) attempted to attribute the inhibitory effect of the electric field to the changes in the voltage-dependent gating of the movement of IAA from stele to cortex.

In the case of experiments accomplished with the coleoptile segments, we have found that electric field significantly inhibited IAA-induced growth, especially with a top negative polarity (*ca.* 70 %), whereas FC-induced growth was less inhibited. It is

noteworthy to point out that for both cumulative growth of intact seedlings and FC-induced growth of the coleoptile segments the electric field with a negative polarity at the top of the seedlings was less injurious as compared to reverse polarity.

A somewhat similar experiments with maize coleoptile segments have recently been accomplished by Medvedev and Markova (1990). These authors observed both acceleration of growth and activation of IAA transport when the apical part of the coleoptile segments was positively charged. They attributed the acceleration of growth to the changes of the basipetal auxin transport. Also, Goldsworthy and Rathore (1985) connected the dramatic increase in the growth rate of electrically stimulated tobacco callus tissue with changes in the polar uptake of auxin. Direct comparison of the experiments accomplished with coleoptile segments to the results obtained by Medvedev and Markova (1990) are difficult because of the different experimental conditions. For example, their experiments were conducted using coleoptile segments being directly under action of the electric field (without addition of IAA).

Comparison between coleoptile segments and intact seedlings is also difficult since irradiance of the intact seedlings with a phototropically inactive green safe light causes predominantly growth in the mesocotyl region (90 % of the shoot growth) and only 10 % in the coleoptile (Desrosiers and Bandurski 1988). Moreover, mesocotyl possesses a root-like anatomy (Epel *et al.* 1992).

Although further investigation are needed, it seems reasonable to assume that apart from the changes induced by electric field in transport system of auxin, the electric field may also affect the activity of plasmalemma proton pump, which in turn is involved in IAA and FC-induced growth (Kutschera and Schopfer 1985a,b, Lüthen *et al.* 1990, Hager *et al.* 1991, Rayle and Cleland 1992). This seems likely in view of the results of Miller *et al.* (1993) and Ezaki *et al.* (1990), who showed that electric field caused inhibition of electrogenic pumping in *Nitella* and asymmetrical H<sup>+</sup> redistribution around the elongation region of Adzuki beans roots, respectively.

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