Influence of a Weak DC Electric Field on Root Meristem Architecture

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• Background and Aims Electric fields are an important environmental factor that can influence the development of plants organs. Such a field can either inhibit or stimulate root growth, and may also affect the direction of growth. Many developmental processes directly or indirectly depend upon the activity of the root apical meristem (RAM). The aim of this work was to examine the effects of a weak electric field on the organization of the RAM.
• Methods Roots of *Zea mays* seedlings, grown in liquid medium, were exposed to DC electric fields of different strengths from 0.5 to 1.5 V cm$^{-1}$, with a frequency of 50 Hz, for 3 h. The roots were sampled for anatomical observation immediately after the treatment, and after 24 and 48 h of further undisturbed growth.
• Key Results DC fields of 1 and 1.5 V cm$^{-1}$ resulted in noticeable changes in the cellular pattern of the RAM. The electric field activated the quiescent centre (QC): the cells of the QC penetrated the root cap junction, disturbing the organization of the closed meristem and changing it temporarily into the open type.
• Conclusions Even a weak electric field disturbs the pattern of cell divisions in plant root meristem. This in turn changes the global organization of the RAM. A field of slightly higher strength also damages root cap initials, terminating their division.

Key words: Electric field, root apical meristem, quiescent centre, polar auxin transport, *Zea mays*.

INTRODUCTION

Electromagnetic fields and living organisms

Low frequency electromagnetic fields (EMF) are a ubiquitous factor in the Earth’s environment. They originate from various sources: geomagnetic fields, electric potential in the atmosphere and cosmic radiation (Feynman et al., 2001). All living organisms, including plants, have been exposed to the Earth’s natural EMF from the beginning of life. They have adapted to it, over 2–3 billion years of their evolution. Numerous studies show an association of the internal EMF of plants with many physiological processes. For example, the electric current is involved in graviperception (Collings et al., 1992) and root growth (Souda et al., 1990). Plants use their internal EMF for determining and controlling their physiological polarity (Jaffe and Nuccitelli, 1977) and, specifically, for setting their biological rhythms (Carre, 1996). There are also many reports describing biological effects of animal and plant exposure to the external EMF (Blank, 1995; Rajendra et al., 2004). In plants it usually causes multiple effects, observed on different levels of plant organization: changes in growth direction (Wang et al., 1989; Stenz and Weisenseel, 1991; Malhó et al., 1992) and rates of cell division (Goldsworthy and Rathore, 1985; Rech et al., 1987), enhancement or inhibition of flowering (Filek et al., 2003, 2006) and stimulation of embryogenesis (Dijak et al., 1986).

It was Elfving who, as early as 1882 (cf. Schrank, 1959), carried out the first experiments with roots growing in an electric field. He observed that the roots of *Lepidium*, *Sinapis* and *Raphanus* curved toward the anode, but the roots of *Brassica* curved towards the cathode. The electric current used in his experiments was so strong that it usually caused injury to the roots; thus these reactions were aberrant rather than typical. More recently it has again been demonstrated that the roots of *Zea mays* and *Lepidium sativum* respond to an electric field and grow toward the cathode when the electric current is 1 V cm$^{-1}$ (Stenz and Weisenseel, 1991), and toward the anode when the field is 8 V cm$^{-1}$ (Stenz and Weisenseel, 1993). These authors suggested that the latter effect, i.e. growth toward the anode, might result from damage caused by a field higher than 1 V cm$^{-1}$. They concluded that the typical response of uninjured roots was growth toward the cathode. The more precise observations of root electrotropic response showed that root curvature occurred in two different regions: in the central elongation zone (CEZ) and in the distal elongation zone (DEZ) (Wolverton et al., 2000). Since then, it is known that the electrotropic reaction occurs in the elongation zone. However, the question remains of what happens in the most sensitive, meristematic region of the root, when the field is applied.

One answer to this question was given recently in a paper by Bitonti et al. (2006). They observed a reduction in size of the quiescent centre (QC) and some disorder in root cap (RC) cells of the roots of *Z. mays* exposed to a continuous EMF for 30 h.

Root structure and its physiological basis

One of the most important and dynamic regions of the plant root is the root apical meristem (RAM), which consists of undifferentiated, rapidly dividing cells. The results of their activity are the tissues and organs of the post-embryonic plant. The meristem also plays a role as the organizing centre for plant morphogenesis (Kerk and
Feldman, 1995). Proximal to the root meristem is the RC, which protects the underlying RAM when the root traverses through the soil. In plant species such as maize, a discrete boundary exists between the RC and the RAM. This boundary is called the root cap junction (RCJ). Roots of this type, with a so-called closed meristem organization, are contrasted with roots having an open meristem organization, where there is no sharp boundary between the RC and the root proper (Clowes, 1981). The type of root architecture is specific for a given plant, suggesting a decisive role for the genetic blueprint in setting up processes resulting in an appropriate RAM cellular phenotype. In the closed type of RAM, the cells that form the RC and root body are typically produced by different tiers of cell initials; in the open type they originate from a common group of initials. The RCJ arises as the consequence of a high rate of cell division in the cap initial cells (resulting in a columnellar structure within the cap) and a low rate in the adjacent tier of cells in the QC. The QC, first discovered by Clowes (1956) with use of radiolabelled DNA autoradiography, is a distinct central region of the RAM, which consists of slowly dividing cells (Jiang and Feldman, 2005).

On the proximal edge of the QC, a group of mitotically active cells is located. These are cell initials, which form the proximal meristem (Clowes, 1956). They give rise to all cells and tissues of the primary root body (Feldman and Torrey, 1976). On the distal edge of the QC, the active cells are root cap initials (RCIs) (Baum and Rost, 1996; Barlow et al., 2001). There are two types of RCJ: cap columella initials, situated at the proximal end of central cell files forming columella; and protoderm initials encircling the columella initials (Barlow, 2003).

The status of being an initial is not permanent for a particular cell. This cell’s fate and identity may change over time, especially when the QC increases or decreases in size. Thus the cells should be regarded as initials on the basis of their position relative to the QC (Feldman and Torrey, 1976).

MATERIALS AND METHODS

Plant material and medium

All experiments were carried out with 2-d-old seedlings of *Z. mays* ‘Limko’. Maize caryopses were soaked in running tap water for 5 h. Next they were placed in Petri dishes and stacked between layers of wet paper. The Petri dishes were placed vertically in a dark chamber at 29°C. Humidity. After 48 h, seedlings with straight main roots about 10–15 mm long were used for the experiment.

Application of DC electric fields

The seedlings were placed vertically in a plastic chamber and their roots were submerged in 1 mm MES (pH 6-0) (Fig. 1). The seedlings were held in this position attached to a plastic plate located in the centre of the chamber (Fig. 1D). The medium was aerated and stirred with an aquarium pump. Seedlings were allowed to adapt to these new conditions for at least 1 h before application of the electric current. The electric field was generated by two platinum electrodes placed in the chamber on its opposite sides (Fig. 1A). The electrodes (Fig. 1B, C) were connected to a constant current source. The strengths of the field applied in variants of the experiment were different, but the frequency of 50 Hz and the timing of root exposure were always the same. The exposure time (3 h) was already known from earlier experiments by Stenz and Weissensel (1993) to be sufficient to cause visible root bending. The control seedlings were grown in the same conditions as the seedlings exposed to the EMF, but without voltage. The applied voltage was calculated according to the equation: \[ V = \frac{I}{A} = \sigma E \] where \( V \) is the current density, \( I \) is the current, \( A \) is the cross-section of the container, \( \sigma \) is the conductivity of the medium and \( E \) is the electric field strength.

Histological preparation

The roots were sampled for anatomical investigation immediately after the treatment with the electric field and after 24 or 48 h of subsequent growth. In one experiment, the roots before sampling were allowed to grow for 5 d. Roots were cut at 3–5 mm above the tip and fixed in FAA (formalin–alcohol–acetic acid) or 4% paraformaldehyde (PFA) at room temperature for 24 h. They were then dehydrated in a graded series of ethanol, and embedded in paraplast or paraffin. Root tips were sectioned at 4 and 7 µm with a Leica RM 2135 rotary microtome. The sections were stained with fuchsin [in a periodic acid–Schiff (PAS) reaction], as in Figs 3A, B and 4D, or with Safranin/Fast green (Figs 3 C, D and 4A–C) and calcofluor (Fig. 3E). Images of the sections were taken and archived with a light microscope (Olympus SZX9) and a fluorescent microscope (Olympus BX50) connected to a Silicon Graphics INDY Workstation.

RESULTS

In all experiments where the plant roots were exposed to the EMFs, the protocol of Stenz and Weissensel (1993) was
followed, with some minor modifications. MES-buffered medium, without salt, was used. It was aerated and stirred by air from the aquarium pump. Only a weak electric field was used, which did not cause injury to the roots. Only those roots which either did not show any sign of damage (as a change in colour of the root tip) or were able to grow continuously after disconnection of the field were analysed.

**Exposure of the root to a DC electric field of 0.5 V cm\(^{-1}\)**

In the first series of experiments the roots exposed to the electric field were growing, gravitropically downward. When the field was disconnected, the roots were allowed to grow further and they continued evidently undisturbed growth downward. Anatomical observations of the roots sampled immediately after the electric field had been shut off and after 24 h from that moment showed that there was no variation in the RAM cellular structure in comparison with the control (Fig. 3A, B).

**Exposure of the root to a DC electric field of 1 V cm\(^{-1}\)**

These experiments showed that almost all of the roots bent toward the cathode, clearly demonstrating an electrotropic reaction (Fig. 2A). At the end of the action of the EMF, the average curvature of the root tips was approximately 30°. After disconnecting the field, the roots continued their growth in the direction attained for 24 h. Then they started growing gravitropically, i.e. downward (Fig. 2B). The architecture of the RAM showed some changes 24 h after disconnecting the field. This resulted from periclinal divisions of the cells forming the first tier of the root body. A new layer of cells appeared between the tip of the procambial cylinder and the RCJ. At 48 h after disconnecting the field, the expansion of the root proper cells began toward the RC area (Fig. 3E). During that process, the boundary between these two regions maintained its integrity in >90% of the roots. It bent, however, resulting in formation of a very characteristic mammillary apex (Fig. 3C, D). In the remaining 10% of the roots, the integrity of the boundary was already destroyed, by a few cells of the root proper protruding actively and deeply into the RC. Cells of the cortex—epidermis complex, formerly belonging to the root proper, slowly converted into the RCIs and a new epidermis became constituted laterally from the outer layer of the former cortex. This process started slowly defining a new RCJ boundary, now located deeper within the former root body. By 48 h, the previous RCJ was faint or almost completely disappeared as a result of periclinal divisions of epidermal derivatives. The root meristem organization became at this stage clearly open (Fig. 4A). In order to see the long-term effects of RAM reconstruction, some roots were allowed to grow for 5 d after the treatment. It was observed that in these roots a new cap had been produced and the cells of the old disintegrating cap were already dead and highly compressed (Fig. 4B, D). Between the RC and root proper a new RCJ was arising, with the meristem becoming closed again. Neither of the above effects was observed in control roots.

**DISCUSSION**

In the normal gravitropic reaction, a plant root typically changes the direction of its growth but the cellular pattern of the RAM remains undisturbed. When the roots of maize in the present experiments had been stimulated by an EMF of even low strength, but sufficient to induce bending, the treatment profoundly affected the pattern of cell division, changing the global organization of the RAM. This was a somewhat unexpected result as the roots at first sight did not differ from those which had gravitropically stimulated and, most importantly, were still capable of growth. Reconstruction of the cellular pattern of the RAM
most probably was a result of a reaction or even damage in the most sensitive RCIs, as indicated by the lower rate or the entire cessation of their division. When the field action is limited in time, the changes are reversible – the RC cells became replaced by others, produced by numerous cell divisions in the activated QC. The new distinct regions of root cellular pattern, such as a new RC and new tiers of initials, emerged from deeper, more proximally located strata of cells. The sequence of changes observed in these experiments resembled reconstruction of the root cellular pattern after surgical removal of the RC (Barlow, 1974).

It is well known that RCIs are extremely sensitive to environmental factors. Increasing sensitivity of the cells, the derivatives of which are constantly discarded, is sensible from the point of view of adaptive strategies. On the other hand, the agents usually affecting rapidly dividing cells, e.g. X-irradiation, triiodobenzoic acid (TIBA) and low temperature, accelerate cell divisions in the QC, resulting in perturbation of the organization of the RAM (Clowes, 1963; Clowes and Stewart, 1967; Kerk and Feldman, 1994).

The sequence of these events and their possible causal relationship with auxin transport is not entirely clear. Kerk and Feldman (1994) suggested that all structural changes result from damage of cap initials, which secondarily disrupts the polar auxin transport. Such damage in the present experiments is suggested by the fact that the initials are pushed out and replaced by new cells adopting their function, located deeper in the region of the root proper.

Morris (1980) hypothesized, in turn, that a small DC electric current might directly inhibit polar auxin transport. When the current is switched off, freshly supplied indole acetic acid (IAA) should be again transported normally. This means that functions of auxin polar transport are not permanently inhibited. According to this view, perturbation of auxin movement alters positional signalling, i.e. changes not only the condition of the QC but also the signal, which flows from the QC and controls the activity of RCIs. This

**Fig. 3.** The response of maize root apices subjected to various treatments, as seen on longitudinal central sections. The control root, growing without application of the electric field (A), shows a closed meristem organization, with a clearly demarcated root cap junction separating the root cap and root body. A similar pattern is present in the root exposed to an electric field of 0.5 V cm \(^{-1}\) (B). Mammillary root apex developing 24 h after disconnecting the field of 1 V cm \(^{-1}\) (C and D). Formation of a new cap in the root treated with the same field strength as in C, but sampled 48 h after disconnecting the field (E). Scale bar = 100 μm (A–D), 200 μm (E).
causes aberration in root meristem organization (van der Berg et al., 1997). This hypothesis explains the cessation of cell division and, especially, accumulation of starch granules in the RCIs observed in the present experiments. These cells would not be expected to differentiate properly, as they do develop the clear phenotype of a statocyte, if lethally damaged by the field.

Thus, despite a growing body of experimental data, the first target of the action of an EMF in the RAM still remains unknown and the question of whether the changes in cell divisions are due to the damage to sensitive RCIs or to the polar auxin transport being altered by the field still awaits an answer.

LITERATURE CITED


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