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Calcium and Rhizodermal Differentiation in Primary Maize Roots

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ABSTRACT

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Rhizodermal differentiation of maize (*Zea mays* L. cv. LG 11) roots cultured in humid air was influenced by a pretreatment for 2 h in $CaCl_2$ or $CaSO_4$ solutions. This increased the number of hair-producing roots and the density of hairs. Ethylene glycol-*bis*-(β-aminoethyl ether)*N*,*N*′-tetraacetic acid (EGTA) was inhibitory. Root hairs emerged in the part of the cell nearer to the tip. Trichoblasts were shorter and elongated more slowly than atrichoblasts. The elongation of the lower part of the trichoblast was less than that of the upper part.

Key words—Cell length, cell number, hair position.

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INTRODUCTION

Cells of the rhizodermis may produce root hairs which are important in nutrient uptake from soils (Lamont, 1982). In roots grown in different nutrient solutions, the length of the hairs depends among other factors on the pH and the concentrations of Ca^{2+} and nitrate (Cormack, 1949; Ewens and Leigh, 1985). When roots are grown in solutions without added calcium, their growth is severely inhibited (Pilet and Belhanafi, 1961; Tanaka and Woods, 1972; Ferguson and Bollard, 1976; Burström, 1981; Ewens and Leigh, 1985) and the density and length of root hairs are reduced (Cormack, 1949; Burström, 1952; Tanaka and Woods, 1972; Ewens and Leigh, 1985). Nevertheless, Ekdahl (1953) reported that the concentration of exogenous calcium was not a determining factor; in moist air, without an external supply of calcium, root hairs developed very well. In maize roots cultured in humid air without applied calcium, some roots produce no hairs at all and the presence or absence of a hairy zone is not related to root growth (Jaunin and Hofer, 1986).

In most species, root hair forming cells, usually called trichoblasts, are distinguishable from hairless cells (atrichoblasts) on the basis of cell size, activities of several enzyme systems, density of the cytoplasm and the amount of RNA, nuclear DNA, histones and proteins (Avers, 1961; Cutter and Feldman, 1970; Dosier and Riopel, 1977, 1978; Harris, 1979). In *Zea mays* roots, Clarke, McCully, and Miki (1979) did not observe any cytoplasmic or nuclear differences between trichoblasts and atrichoblasts. Moreover, no definite relation was

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observed between length of the rhizodermis cells and their capacity for producing hairs (Cormack, 1949; Clarke *et al.*, 1979). In this material grown in humid air, cell divisions in the rhizodermis were asymmetrical with a short apical cell and a large basal cell (Ivanov and Filippenko, 1979).

In a study of cell polarity and the emergence of hairs in maize roots, Clarke *et al.* (1979) observed that the hairs form at the acropetal end of the rhizodermal cell. The final position of the hair on the cell depends on the growth of the different parts of the cell (Cormack, 1949).

The aim of the present paper was, first, to investigate whether calcium influences the differentiation of the rhizodermis into trichoblasts and atrichoblasts in maize roots cultured in humid air and, secondly, to determine the size of the rhizodermal cells and the position of the hair on the cell along the root axis.

MATERIALS AND METHODS

Plant material

The germination of *Zea mays* L. caryopses (cv. LG 11) has already been described (Jaunin and Hofer, 1986). After 48 h, intact seedlings with straight primary roots ($14 \pm 1{\cdot}0$ mm) were selected in dim green light [530 ± 20 nm, $1{\cdot}2 \times 10^4$ W m^{-2} (Pilet, 1979)]. As observed with the scanning electron microscope, no outgrowths due to emergence of root hairs could be detected.

Roots attached to the caryopsis were immersed vertically for 2 h in a buffered solution (1·0 mol m^{-3} 3,3-dimethylglutaric acid-NaOH, pH 6·0, 2·0 cm^3 per root) without (control) and with $CaCl_2$ (1·0 mol m^{-3} or 10 mol m^{-3}) or $CaSO_4$ (10 mol m^{-3}), or ethylene glycol-*bis*-(β-aminoethyl ether)*N*,*N'*-tetraacetic acid (EGTA) (0·1 mol m^{-3}). Roots were then rinsed for 1 s in the control solution. These solutions were prepared with double-distilled water and filter-sterilized, using 0·45 μm filters (Millipore filter unit). Then roots attached to the caryopsis were placed in a vertical position in sealed boxes for the following 20 h (dark, saturated atmosphere, 21 °C).

Growth measurements

Root growth was recorded for 20 h in humid air by taking photographs every 2 h (light provided for 7 s per photograph by white fluorescent tubes, Philips 20 W, $9{\cdot}1 \times 10^{-2}$ W m^{-2}) as already described (Jaunin and Hofer, 1986).

Location of the hairy zone

After 20 h in humid air, the roots were excised and individually fixed in 2% (v/v) glutaraldehyde (4 °C). Measurements were made, using a binocular microscope (Wild M5, ×12, ×25) with an eye-piece graticule.

Determination of cell number, cell dimensions and position of hairs on the cells

The material was fixed overnight in glutaraldehyde at 2% (4 °C). After dehydration in a series of acetone baths, roots were dried through the CO_2 critical point, sputter-coated with gold (about 70 nm) and viewed in a JEOL JSM-35 SEM at 25 kV (Centre for Electron Microscopy, University of Lausanne). Measurements were done on photographs (final enlargement 310×) in consecutive 0·75 mm segments along the root axis. This was performed between 3·0 mm and 6·75 mm counted from the tip. In regions distal to 3·0 mm, it was difficult to identify cell limits because of the homogeneity of the outer wall (Hofer and Pilet, 1986). In more basal regions, cell sizes could not be measured due to the overlapping of root hairs. Cell measurements were realized using a digitizing pad (Hi PAD, Houston Instruments, Austin, TX, U.S.A.) interfaced with a microcomputer (ABC 80, Luxor AB, Motala, Sweden). The number of cells which give rise to hairs was expressed in % of total number of cells. Cells which had an emerged root hair were counted as trichoblasts; the others were counted as atrichoblasts, even though a hair could be expected to form in some cells.

RESULTS

Calcium and formation of root hairs

The concentrations of calcium salts and of the calcium-chelating agent EGTA (Schmid and Reilley, 1957) were chosen not to affect the elongation rate of roots compared with control

TABLE 1. *Relative number (in %) of roots producing hairs*

Pretreatment	Percentage (%)
Buffer (control)	66 (56–77)
$CaCl_2$ 1·0 mol m^{-3}	83 (73–100)
$CaCl_2$ 10 mol m^{-3}	97 (92–100)
$CaSO_4$ 10 mol m^{-3}	100
EGTA 0·1 mol m^{-3}	38 (22–57)

Roots attached to the caryopsis were immersed 2 h in buffer without and with $CaCl_2$, $CaSO_4$ or EGTA and then transferred to humid air for the following 20 h.

The numbers of roots tested were 84 for the control and 30 for the other pretreatments. Confidence intervals ($P = 0{\cdot}05$) are in brackets.

specimens (pre-incubated in buffer). The elongation rate of the latter as a function of time has already been given (Jaunin and Hofer, 1986); the mean growth rate over 20 h was 0·85 mm h^{-1}.

Hairs were produced in 66% of control roots (Table 1). Although the amounts of free Ca^{2+} initially available in the solution would not be the same for $CaCl_2$ and $CaSO_4$, these two salts (at 10 mol m^{-3}) both significantly increased the percentage of roots with hairs. The difference observed between the control roots and the roots treated with 1·0 mol m^{-3} $CaCl_2$ was not significant. The calcium-chelating agent EGTA at 0·1 mol m^{-3} significantly decreased the percentage of hair-producing roots.

After 20 h, calcium salts and EGTA did not affect the length of the hairy zone, but root hairs formed nearer to the tip only when roots were pre-incubated with $CaCl_2$ at 10 mol m^{-3} (Table 2).

Since there was no significant difference between the number of hair producing roots with $CaCl_2$ and $CaSO_4$, subsequent experiments were performed with $CaCl_2$ which is almost entirely dissociated in solution at 10 mol m^{-3}.

Root hair density was studied along the root axis for material pre-incubated for 2 h in $CaCl_2$ at 10 mol m^{-3} and then transferred to humid air for the following 20 h. Results are

TABLE 2. *Length of the apical zone without hairs (zone 1) and of the hairy zone (zone 2)*

Pretreatment	Length (mm) ± s.e.	
	Zone 1	Zone 2
Buffer (control)	4·4 ± 0·3	15·0 ± 0·6
$CaCl_2$ 1·0 mol m^{-3}	3·8 ± 0·1	17·3 ± 0·8
$CaCl_2$ 10 mol m^{-3}	3·3 ± 0·1[a]	15·0 ± 0·8
EGTA 0·1 mol m^{-3}	3·8 ± 0·2	14·5 ± 1·1

Roots attached to the caryopsis were immersed 2 h in buffer without and with $CaCl_2$, or EGTA and then transferred to humid air for the following 20 h.

[a] Value significantly different from control ($P = 0{\cdot}05$).

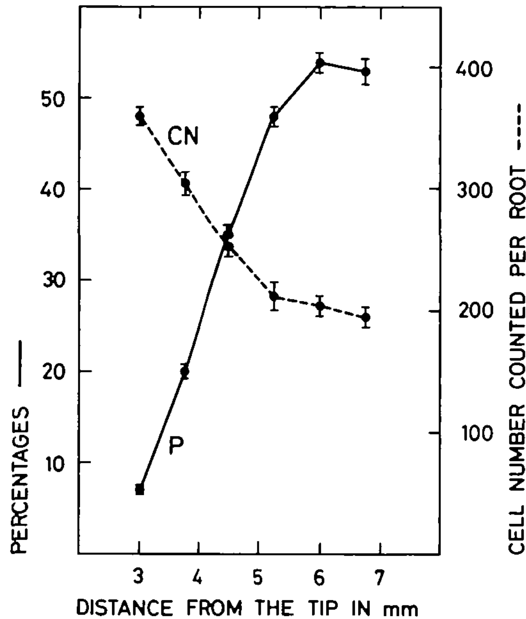

FIG. 1. Cell number (CN) counted per root (± s.e.) and percentage (P) (± s.e.) of cells which had given rise to hairs along the root. Roots attached to the caryopsis were immersed 2 h in buffer with $CaCl_2$ at 10 mol m^{-3} and then transferred to humid air for the following 20 h. Number of roots tested 10.

given as percentages of the total number of cells since cell number decreases towards the root base (Fig. 1). These values increased rapidly from 3·0 mm to 5·25 mm in the growing part of the root and reached a constant value at 6·0 mm from the tip where growth is about to cease (Pilet, Versel, and Mayor, 1983). Consequently, emergence of root hairs occurred in the extending part of the root and above this region cells cannot give rise to hairs (Jaunin and Hofer, 1986). In roots which had a hairy zone, percentages of cells which had given rise to hairs in control roots were compared with $CaCl_2$-treated and EGTA-treated roots (Table 3). This was performed 6·0 mm from the tip where the density of root hairs was highest. In this zone, in control roots, less than half of the cells formed hairs. This percentage was significantly higher for roots pre-incubated with $CaCl_2$ and significantly lower for roots pre-incubated with EGTA.

TABLE 3. *Relative number (in %) of cells which had given rise to hairs 6·0 mm from the tip*

Pretreatment	Percentage (% ± s.e.)
Buffer (control)	43 ± 1
$CaCl_2$ 10 mol m^{-3}	54 ± 1
EGTA 0·1 mol m^{-3}	30 ± 1

Roots attached to the caryopsis were immersed 2 h in buffer without and with $CaCl_2$, or EGTA and then transferred to humid air for the following 20 h.

Cell number counted: 2000 for each pretreatment.

Length of rhizodermal cells

The lengths of rhizodermal cells along the root axis were studied with scanning electron microscopy on roots pre-incubated 2 h in buffer with $CaCl_2$ at 10 mol m^{-3} and then transferred to humid air for the following 20 h (Fig. 2). Trichoblasts and atrichoblasts already showed significant differences in their length 3·0 mm from the tip, where the most apical hairs were found. The length of trichoblasts progressively increased between 3·0 mm and 5·25 mm from the tip and reached a constant value at 5·25 mm. The increase in cell length of atrichoblasts was larger and a constant value reached only at 6·0 mm. Consequently, the difference in length between trichoblasts and atrichoblasts depends on the region observed. Their extension growth between 3·0 to 6·75 mm behind the tip was 83% and 144% respectively.

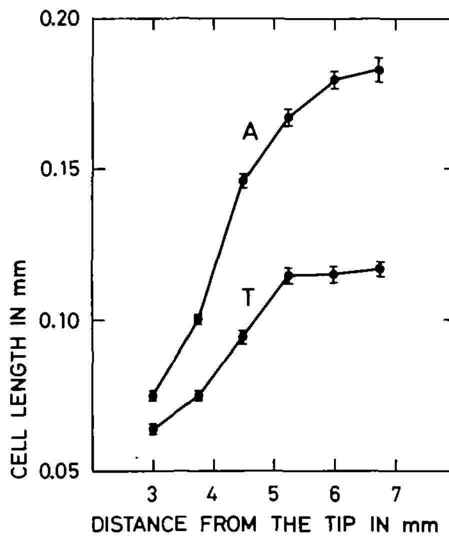

FIG. 2. Length (in mm ± s.e.) of trichoblasts (T) and atrichoblasts (A) along the root. Roots attached to the caryopsis were immersed 2 h in buffer with $CaCl_2$ at 10 mol m^{-3} and then transferred to humid air for the following 20 h. Number of roots tested 10.

Position of the hairs on trichoblasts

The position of the hair on the trichoblast is shown in Fig. 3 for roots pre-incubated in $CaCl_2$ at 10 mol m^{-3}. The distances from the apical and basal ends of the cell to the centre of the hair were measured along the root axis. At 3·0 mm from the root tip, root hairs emerge nearer the apical end of the hair-forming cell. The basal and apical parts of the cell increased in length at the same rate when less than 4·5 mm from the root tip. From 4·5 mm to 5·25 mm, the length of the basal part of the cell increased more rapidly than the apical part. Consequently, the position of the hair is even further from the middle of the cell. A constant value for both parts was reached at 5·25 mm behind the tip. The total increase was 80% for the apical part and 92% for the basal part.

DISCUSSION

The results show that under the growth conditions used, the differentiation of the rhizodermis into trichoblasts and atrichoblasts is dependent on calcium salts in maize roots

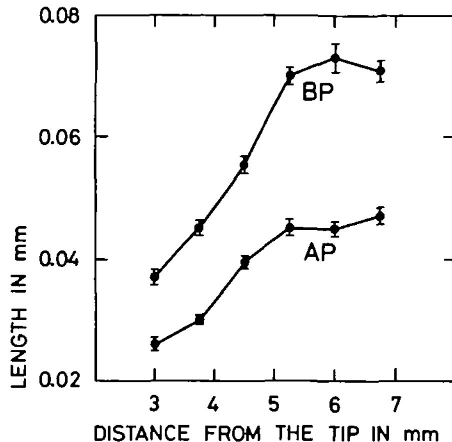

FIG. 3. Position of the hair on the trichoblast along the root axis. AP: apical part of the cell to the hair centre (in mm ± s.e.); BP: basal part of the cell to the hair centre (in mm ± s.e.). Roots attached to the caryopsis were immersed 2 h in buffer with $CaCl_2$ at 10 mol m^{-3} and then transferred to humid air for the following 20 h. Number of roots tested 10.

grown in humid air. EGTA at 0·1 mol m^{-3} did not completely inhibit rhizodermal differentiation. Perhaps this concentration does not complex all the calcium involved in differentiation. Preliminary tests have shown that higher concentrations of this calcium-chelating agent prevent root hair formation, but also affect root elongation and even seem to be toxic for the roots. Trichoblasts are shorter than atrichoblasts and length curves along the root axis are different for the two types of cells. The difference in size was observed already at 3·0 mm from the tip; this could be due to asymmetrical divisions (Ivanov and Filippenko, 1979).

Root hairs always originate nearer the apical end of the cell. Similar results were obtained by Clarke *et al.* (1979) for maize roots. Moreover, the parts of cell apical and basal to the hair have different rates of elongation. It was reported in *Gibasis geniculata* roots that the site of root hair inception might be determined by the intrinsic polarity of the cell (Nakazawa and Yamazaki, 1982). Clarke and McCully (1985) noted a structural change in rhizodermal cell walls of *Zea mays* during hair initiation and observed a strongly callose-positive papilla proximal to an emerged root hair or nearer the site where a hair would be expected to form. Moreover, callose synthesis can be regulated by calcium (Kauss, 1985). With electron probe X-ray microanalysis, a large amount of calcium was observed in the peripheral layers of *Nicotiana tabacum* roots (Scheidecker, Chevalier, Andreopoulos-Renaud, Bizid, and Boumati, 1981) and in the epidermis of *Zea mays* roots (Chino, 1981). Further studies are required to investigate the ways in which calcium influences rhizodermal differentiation.

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