Gradients in maize roots: local elongation and pH

Jean-Marc Versel and Guy Mayor
Institut de Biologie et de Physiologie Végétales, Bâtiment de Biologie, Université de Lausanne, CH-1015 Lausanne, Switzerland

Abstract. The elongation rate, the gradient of the local elongation rate and the surface pH of maize roots were measured over 12 h. A data bank was constituted by storing these values. By sorting these results on the basis of different elongation rates, different classes of root were obtained. Two classes were chosen: the low-growth roots and the high-growth roots. The mean growth of these two root classes was stable with time and differed significantly from one another. The surface pH of the elongation zone was the same for the roots of these two classes, but the roots selected for their higher growth rate had a larger acid efflux in this zone.

Key words: Acid efflux and growth – Elongation growth-pH (root surface) – Root growth – Zea (root growth).

Introduction

The analysis of root growth (frequently considered as axial elongation) has been studied for many years (Sachs 1873; Goodwin and Stepka 1945; Erikson and Goddard 1951; Gandar 1983). Ink marks were commonly made along the root allowing analysis of its local growth for several hours (Pilet 1960, 1961; Erikson 1976). Alternatively, Heinowicz et al. (1977) and Franssen et al. (1981, 1982) used resin beads as markers to measure growth gradients.

The localization of the largest contribution to axial growth varies from one author to another (Erikson 1976; Pilet and Senn 1980) because of the use of different materials, methods of measurement and expressions of data. It should be pointed out that the influence of different growth rates was generally neglected in the comparison of results.

The experimental study of “acid growth” (Rayle and Cleland 1970; Hager et al. 1971) led to considerable progress in the understanding of cell elongation mechanisms. Later, several authors presented evidences for “acid growth” in roots (Edward and Scott 1974; Gabella and Pilet 1978; Moloney et al. 1981, 1982). The primary root, because of its definable functional zones (Pilet and Senn 1980) appears to be a promising material for such studies.

Several authors used roots growing on agar plates containing a pH indicator to demonstrate an acid efflux by the colour changes of the dye (Weisenseel et al. 1979; Mulkey et al. 1981, 1983; Marschner et al. 1982). Such an approach is essentially qualitative and static, which is a considerable limitation. Recently Pilet et al. (1983) using Sephadex beads containing pH indicator compared the gradients of growth and pH changes along maize roots; the colour variation of these separated beads was considered as a “surface pH probe”.

The aim of this paper is to analyse the local growth and – by using a new microdensitometric method – the local surface pH for two classes of maize roots characterized by different elongation rates. The evolution with time of their growth and surface pH will be used to establish whether roots having a higher elongation rate develop a more intense acidification on their surface.

Material and method

The germination and selection of Zea mays caryopses (cv. LG.-11; Ass. Suisse des sélectionneurs, Lausanne, Switzerland) has already been described (Pilet 1977). Primary roots were grown in the presence of CaSO₄ (0.5 mM) and selected on the basis of their length (15 ± 3 mm) for all experiments.

Selected Sephadex beads (G 25 coarse, diameter 0.4 ± 0.1 mm; Pharmacia Fine Chemicals AB, Uppsala, Sweden) were soaked in an aqueous solution of bromocresol purple.
(0.71 mM) with the pH adjusted to 5.8 using NaOH (0.1 N).
The beads were placed on the roots in two opposite rows at regular intervals (Pilet et al. 1983). The roots were then set vertically in special boxes (100% relative humidity, 22° C) and placed in white light (0.90 ± 0.06 W m⁻²; Pilet 1979). Their growth was recorded by taking photographs (Kodak Ektachrome tungsten 160, Vivitar filter FLB, Olympus OM 2 N) every hour for 12 h, without modification of the experimental conditions.

The slides were reproduced on paper, using a microfilm enlarger (x 10). The distance separating two consecutive beads (d_i,i-1) was measured on the photocopies, using a digitalizing table (HIPAD; Houston Instrument, Austin, Tex., USA) interfaced with a microcomputer (ABC 80; Luxor AB, Motala, Sweden).

From these data, the elongation rate of each root, R (Eqn. 1), and the gradient of their local elongation rate, grad r (Eqn. 2), were calculated.

\[ R = \frac{(L_{n_i} - L_{n_i-1})(t_{n_i} - t_{n_i-1})^{-1}}{t_{n_i} - t_{n_i-1}} \]  
(Eqn. 1)

where \( L_{n_i} \) is the root length at the time \( t_{n_i} \).

\[ \text{grad } r = 2 \cdot 10^2 \cdot (d_{i,i-1})^{-1} \cdot (r_i - r_{i-1})^{-1} \]  
(Eqn. 2)

where \( d_{i,i-1} \) is the distance between two neighbouring beads at the time \( t_i, \).

This gradient which is, by definition, the derivative of the local elongation rate along the root axis, was generally calculated by using Eqn. (3) (Erikson 1976). But the mathematical expressions 2 and 3 are equivalent.

\[ \text{grad } r = 2 \cdot 10^2 \cdot (r_i - r_{i-1})^{-1} \cdot (L_{n_i} - L_{n_i-1})^{-1} \]  
(Eqn. 3)

where \( r_i \) is the local elongation rate, i.e. the displacement rate of the bead \( i \) relative to the root tip in the mean time \( t_{n_i-1} \) to \( t_{n_i} \) and \( L_{n_i} \) is the distance from the bead \( i \) to the root tip, at the time \( t_{n_i} \).

Each gradient value (grad r) was both referred to the intermediate time between two consecutive measurements and to the mean distance from the root tip to the middle of the interval between the two corresponding beads.

The transmittance of each bead was measured on the slides by using a microdensitometer (Leitz, Wetzlar, FRG; MPV 3) at 450 and 550 nm (bandwidth: 20 nm). These two values were selected since they coincide with the maximal absorbance of two pigments (yellow and magenta) of the film used, and since the transmittance at these wavelengths shows the greatest changes with pH variations. The pH of the bead (pH_{i,i}) was obtained by comparing the ratio of these two transmittance values to a standard curve (with a resolution of ± 0.1 between pH 4.5 and pH 7.0) obtained by using beads placed in buffers of different pH and photographed in the same conditions.

From these data, a flux \( \Phi \) (Eqn. 4) and a mean pH (mean of the pH of one bead at two consecutive times of measurement) were calculated for each bead.

\[ \Phi = 10^{-\text{pH}_{i,i}} - 10^{-\text{pH}_{i,i-1}} \]  
(Eqn. 4)

pH and flux values were referred to the corresponding mean time and to the mean distance from the corresponding beads to the root tip.

Results and discussion

Elongation rate. Data were sorted into two classes based on the elongation rate (R) of the roots 5.5 h after the beginning of the experiment. The two classes contained roots having elongation rates (R) of 0.1-0.7 mm h⁻¹ or 0.8-1.5 mm h⁻¹. These limits of selection were chosen in order to have approximately the same number of roots in each class.

The mean elongation rates of these two types of roots were significantly different from one another throughout the experiment (Fig. 1). Moreover, they remained stable with time except during the first hours when the roots underwent an adaptation to the new growth conditions (Beffa and Pilet 1982). Thus, it was possible by sorting data to obtain classes whose roots rapidly reached a specific "steady state" of growth.

Gradient of the local elongation rate. Data for the gradient values (grad r) were divided according to the same criteria and the same limits of selection as those previously chosen. However, they were sorted for each period of time analysed in order to obtain roots having the same mean elongation rate at any moment of the incubation.

The pattern of the growth gradient along the root axis did not vary significantly during the experiment (Fig. 2, only one class being analysed). The decrease of the highest gradient value between 1.5 and 11 h (6.3 ± 2% at 3.9 ± 0.3 mm from the tip) resulted from the five fold increase of distances between the consecutive beads defining this gradient value during this period. Indeed, a similar decrease of the peak value (7.2 ± 1.5% at