In the Early Response of Arabidopsis Roots to Ethylene, Cell Elongation Is Up- and Down-Regulated and Uncoupled from Differentiation[1]

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Slight changes in the concentration of ethylene in the environment modulate the elongation of target cells in the root epidermis of Arabidopsis. The response is immediate, concentration dependent, and reversible on root base but irreversible on cell base, whereas cell differentiation is not affected. We suggest that in natura ethylene is a means of fine and fast tuning of root elongation.

In Arabidopsis, root elongation is reduced in a concentration-dependent way and radial expansion is stimulated when plants are exposed to ethylene or 1-aminocyclopropane-1-carboxylic acid (ACC), the precursor of ethylene (Jackson, 1991; Dolan, 1997; Smalle and Van Der Straeten, 1997). In addition, root hair growth is promoted by ACC. Anatomical and clonal analysis revealed that the root epidermis is composed of two types of cells occurring in parallel files along the root: trichoblasts and atrichoblasts (Dolan et al., 1993, 1994; Galway et al., 1994; Schneider et al., 1997). Ethylene can also affect differentiation patterns by inducing ectopic root hair formation in atrichoblast cell files (Tanimoto et al., 1995; Masucci and Schiefelbein, 1996; Pitts et al., 1998). However, the precise mechanisms governing these ethylene-induced changes in cell development are not fully understood.

The effect of ethylene on root development usually is scored after long incubation times (many hours to days) of the plants, often using high concentrations of ethylene or ACC. Such reports can be useful from the standpoint of a bioassay, but have much less potential in supporting theories pertaining to root development. Only a limited number of reports refer to short-term responses of roots (Jackson et al., 1981; Whalen and Feldman, 1988). We analyzed the effect of ethylene on the primary root of Arabidopsis, focusing on the very early responses (within minutes) of the epidermis. For this purpose, we used a confocal microscope to monitor minute changes in the elongation of individual cells in intact growing roots.

In Arabidopsis roots, mature cell length normally was used to analyze the effect of different mutations or hormone-treated seedlings on a cellular basis (Masucci and Schiefelbein, 1996; Rahman et al., 2000), but this parameter is not suitable to record fast responses and fine dose-dependent regulation of cell elongation. In wild-type roots, grown on hormone-free Murashige and Skoog medium, fast-cell elongation occurs between 250 and 950 μm from the root tip (Beemster and Baskin, 1998; Mullen et al., 1998). Within this zone, the cells elongate along the root growth axis from 15 to 130 μm. The formation of a root hair bulge is often taken as a marker for the switch from elongation to differentiation. Epidermal cells do not cease elongation immediately, however. They elongate further during 1 to 1.5 h until they reach the mature length of 150 to 200 μm. Mature cell size can thus only be evaluated in single still images taken far from the root tip; this implies the loss of information on early responses to ethylene. Therefore, we introduce the length of the first epidermal cell with visible root hair bulge (LEH), counting from the root tip, as a new parameter of root development. It marks the onset of differentiation and the end of rapid elongation (Fig. 1). The movie (http://www.uia.ac.be/bio/fymo/lejie/root.html) illustrates the rapid elongation of cells shorter than LEH, and the slow elongation in cells longer than LEH. Root hair initiation or bulging is strictly regulated in time and space and happens every 27 min in each trichoblast cell line. As shown in the movie, LEH can be easily recognized and measured. In seedlings grown on a medium containing ACC, the value of LEH is reduced with a dose-response ratio similar to that of total root length (Fig. 2). Therefore, a specific level of ethylene (or ACC) defines a specific LEH, the cell size reached at the onset of differentiation.

When a growing plant root is suddenly exposed to ethylene (1 μL L^{-1}), the effect on cell elongation is dual. In the elongation zone the epidermis cells having a length greater than or equal to the LEH corresponding to the given ethylene concentration immediately stop their elongation. Cells closer to the root tip with a length less than LEH elongate further until

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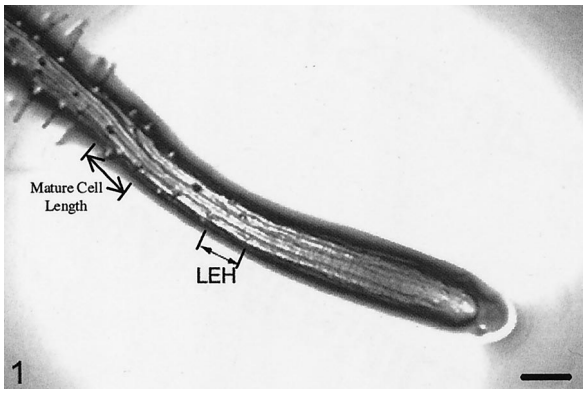

Figure 1. Root elongation and root hair initiation in wild-type Arabidopsis plants, grown on the surface of Murashige and Skoog medium (see movie at http://www.uia.ac.be/bio/fymo/lejie/root.html). The first root hair bulges appear on trichoblasts with a length of approximately 130 μm; this length is defined as LEH. In these cells, elongation still goes on for a while (1–1.5 h). Scale bar = 100 μm.

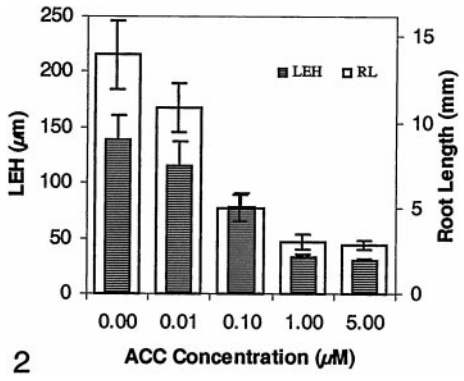

Figure 2. LEH is a reliable parameter for monitoring root elongation in Arabidopsis. Root length (RL) and LEH have a similar dose-response ratio for ACC. Wild-type (Col-0) plants were grown for 4 d on Murashige and Skoog medium and Murashige and Skoog medium supplemented with 0.01, 0.1, 1, and 5 μM ACC. White bars indicate root length and hatched bars indicate LEH. The values indicate means with SD.

they reach the LEH set by the ethylene concentration, and then stop to elongate. The effect is measurable within 20 min; it is illustrated in Figure 3, A through C, and in the movie (http://www.uia.ac.be/bio/fymo/lejie/eth1.html). Cell differentiation is not affected because root hairs are still initiated at the same time intervals. In combination with the effect on elongation this leads to the typical hairy root appearance.

This early ethylene response is not only concentration dependent and immediate, but on root base it is also reversible, as illustrated in Figure 3, D through F, showing the same root as in Figure 3, A through C, but after the removal of ethylene. It is very clearly demonstrated by the movie (http://www.uia.ac.be/bio/fymo/lejie/eth2.html). The LEH of new cells entering the elongation zone increases again to the control value. However, the older cells, locked in their ethylene-specified LEH, cannot react. Thus a temporary application of ethylene leads to a root with only a dense bush of root hairs locally.

Similar to the effect of ethylene, application of ACC induces a decline in LEH detectable within 20 min. After 180 min, root hair bulges appeared from trichoblasts with the "specified" LEH (refer to Fig. 2). This is exemplified for a treatment with 5 μM ACC where the LEH is decreased from 135 to 35 μm (Fig. 4A).

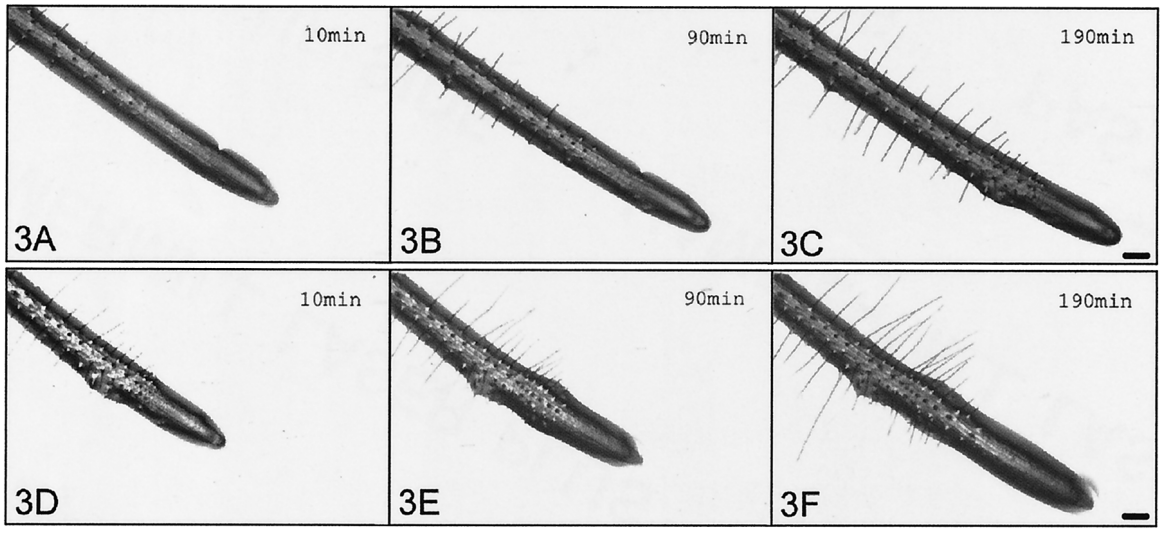

Figure 3. The early response to ethylene of an Arabidopsis root and the recovery from ethylene exposure. A through C, The early response of the root to ethylene (1 μL L^{-1}) during the first 190 min. The live movie (http://www.uia.ac.be/bio/fymo/lejie/eth1.html) demonstrates that cells longer than 40 μm (the LEH specified by this ethylene concentration) stop elongation instantaneously (within 20 min). Cells shorter than 40 μm elongate until they reach the LEH. The timing of root hair bulging is not affected by ethylene. D through F, The recovery of cell elongation when the root is exposed to normal air. The live movie is linked at http://www.uia.ac.be/bio/fymo/lejie/eth2.html. Scale bar = 100 μm. Please note that the apparent swelling of the root in C through F is an optical artifact due to the water level along the root changing with root hair density.

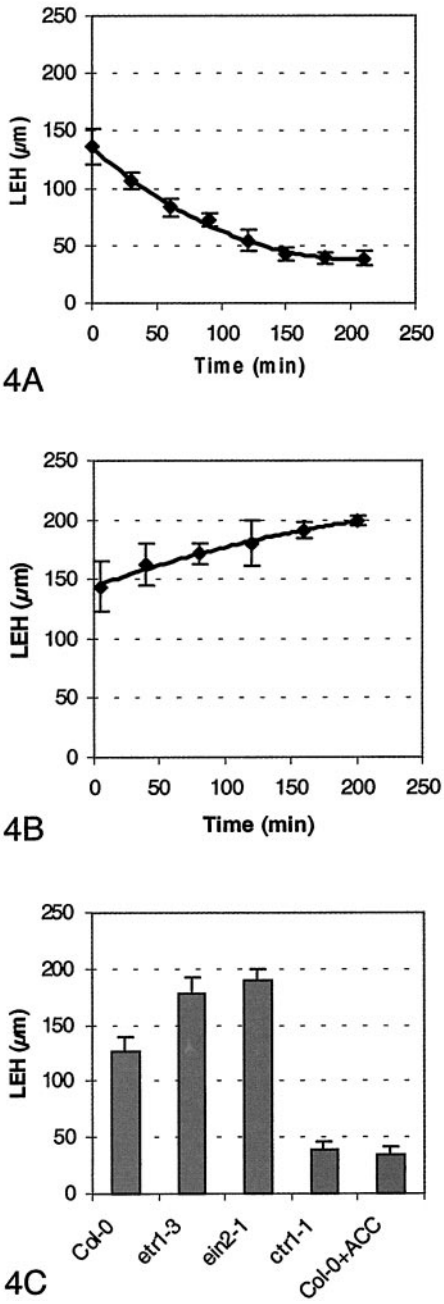

Figure 4. A, Inhibition of cell elongation by ACC: 4-d-old seedlings grown in Murashige and Skoog medium were transferred to Murashige and Skoog medium supplemented with 5 μM ACC. The decrease of LEH was already detectable 20 min after the transfer. After 180 min, root hairs emerge from short trichoblasts, with a length specified by the ACC concentration (see Fig. 2). B, The stimulation of cell elongation by 2-aminoethoxyvinyl-Gly (AVG). Seedlings grown for 4 d in Murashige and Skoog medium were transferred to medium supplemented with 5 μM AVG. The increase of LEH over the course of time is illustrated. C, LEHs of ethylene mutants and ACC-treated wild-type seedlings. Ethylene-insensitive mutants *etr1–3* and *ein2–1* have longer LEH than wild type. The short LEH of *ctr1–1* can be phenocopied in wild type by application of 5 μM ACC in the medium. The values always indicate means with SD.

Moreover, when the endogenous ethylene level is reduced by treating the plant with the ethylene synthesis inhibitor AVG, the epidermis cells elongate more than in control plants and consequently also have a higher LEH value (Fig. 4B).

When analyzed for LEH, the phenotypes of ethylene mutants support the data obtained about wild-type plants with and without ACC (Fig. 4C). The *etr1–3* and *ein2–1* mutants are ethylene insensitive (Bleecker et al., 1988; Guzmán and Ecker, 1990; Roman et al., 1995). Both have long roots and also possess elevated LEH values as compared with the wild type. The short roots of the *ctr1–1* mutant, a constitutive ethylene triple response mutant (Kieber et al., 1993), can be phenocopied by subjecting wild-type plants to ACC concentrations above 5 μM. The high density of root hairs on ACC-treated roots correlates with the LEH that is reduced from 130 to 35 μm.

Ectopic root hair formation did not occur during the experiment because it needs longer incubation times (more than 6 h) for its initiation and only occurs at ACC concentrations of 5 μM or higher (data not shown).

Thus there are three sequential responses of Arabidopsis roots to ethylene: a fast down regulation of cell elongation, the induction of ectopic root hairs, and an increase in width of the root. Of these responses, the early response has not been documented in detail before. However, it is the most interesting response from the perspective of plant growth in natura. Slight changes in the endogenous ethylene concentration of the growing root can up- or down-regulate the elongation of individual cells, without affecting differentiation and its timing, as is the case with AVG and ACC or ethylene, respectively, in our experiments. Short exposures to increased ethylene levels (very likely reflective of normal developmental conditions in nature) thus lead to reversible and root zone-specific effects. This early and reversible response can be the basis of a new model for dynamics of root growth and environmental adaptation.

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