

# Auxin and ethylene promote root hair elongation in *Arabidopsis*

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## Summary

Genetic and physiological studies implicate the phytohormones auxin and ethylene in root hair development. To learn more about the role of these compounds, we have examined the root hair phenotype of a number of auxin- and ethylene-related mutants. In a previous study, Masucci and Schiefelbein (1996) showed that neither the auxin response mutations *aux1* and *axr1* nor the ethylene response mutations *etr1* and *ein2* have a significant effect on root hair initiation. In this study, we found that mutants deficient in either auxin or ethylene response have a pronounced effect on root hair length. Treatment of wild-type, *axr1* and *etr1* seedlings with the synthetic auxin, 2,4-D, or the ethylene precursor ACC, led to the development of longer root hairs than untreated seedlings. Furthermore, *axr1* seedlings grown in the presence of ACC produce ectopic root hairs and an unusual pattern of long root hairs followed by regions that completely lack root hairs. These studies indicate that both auxin and ethylene are required for normal root hair elongation.

## Introduction

Root hair development has been the topic of much study in recent years. In *Arabidopsis*, root anatomy has been well characterized and the pattern and course of root hair development has been documented (Dolan *et al.*, 1993; Galway *et al.*, 1994, 1995). Root hairs are tubular projections which grow out of a specialized subset of epidermal cells called trichoblasts. (Peterson and Farquhar, 1996). Positional information is important for controlling epidermal cell fate. As epidermal cells proceed through the elongation zone of the growing root, cells that lie over the anticlinal junction between two underlying cortical cells assume the characteristics of trichoblasts while cells that lie above the periclinal surface of a single cortical cell become atrichoblasts (van den Ber *et al.*, 1995; Masucci *et al.*, 1996; Tanimoto *et al.*, 1995). This developmental regularity, combined with the defined radial pattern of root tissues and the stacked columnar nature of cells along the length of

the root, give rise to an ordered pattern of root hair cell files spaced between non-root hair cell files on the surface of the *Arabidopsis* root.

Many factors, both genetic and environmental, contribute to the wild-type pattern of root hair development (Bates and Lynch, 1996; for review see Dolan and Roberts, 1995; Ridge, 1995). Several genes have been defined in *Arabidopsis* by mutational analysis that function in root hair development. The best characterized of these genes are *TTG* and *GL2*. Both are shoot and root epidermal developmental regulators (Koornneef, 1981; Rerie *et al.*, 1994). They are likely to function as early negative regulators of trichoblast development or, alternatively, as positive regulators of atrichoblast development since mutations in these genes allow almost all root epidermal cells to develop hairs (Galway *et al.*, 1994; Masucci *et al.*, 1996; Masucci and Schiefelbein, 1996). The *ttg* mutation abolishes the earliest cytological distinctions between trichoblasts and atrichoblasts while *gl2* mutants retain these distinctions. (Galway *et al.*, 1994; Masucci *et al.*, 1996). Furthermore, *GL2* expression is reduced in a *ttg* background indicating that *TTG* acts earlier in the development of the epidermis and may directly regulate *GL2* expression. Both *GL2*, a protein that contains homeodomain and leucine zipper motifs (DiCristina *et al.*, 1996), and *TTG* are likely to act as transcription factors.

Several other *Arabidopsis* mutants with abnormal root hair phenotypes have been isolated. One group includes *cow1*, *cpc1*, *rhd1*, *rhd2*, *rhd3* and *rhd4*, and is characterized by defects in root hair shape and number (Galway *et al.*, 1997; Grierson *et al.*, 1997; Masucci and Schiefelbein, 1994; Schiefelbein and Somerville, 1990; Wada *et al.*, 1997). Three root hairless (*rhl*) mutants have also been found as well as three mutants producing ectopic root hairs (*erh*) (Schneider *et al.*, 1997). Notably, the *rhl* mutants also lack root hairs in the collet region, a phenotype not observed in other root hair mutants (Schneider *et al.*, 1997). The *RHD3* and *CPC1* genes have been cloned and encode putative GTP-binding and Myb-like DNA binding proteins, respectively. (Wada *et al.*, 1997; Wang *et al.*, 1997). In addition, the *RHL1* gene was recently cloned and shown to encode a novel nuclear protein (Schneider *et al.*, 1998). Isolating the remaining genes and characterizing them on the molecular level will certainly provide further insight into the root hair development pathway from initiation to elongation.

Another class of mutants, those involved in auxin and ethylene responses, also affect root hair development and have been implicated both by genetic and physiological studies. The auxin response mutations *aux1*, *axr1*, *axr2*

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and *axr3* all affect root hair growth (Leyser *et al.*, 1996; Lincoln *et al.*, 1990; Okada and Shimura, 1994; Wilson *et al.*, 1990). The ethylene response mutant, *ctr1*, has ectopic root hairs (Tanimoto *et al.*, 1995). Moreover, the ethylene precursor, aminocyclopropane carboxylic acid (ACC), induces ectopic root hair formation in wild-type seedlings while the ethylene biosynthesis inhibitor, aminovinylglycine (AVG), abolishes root hairs (Masucci and Schiefelbein, 1996; Tanimoto *et al.*, 1995). These observations have led to the hypothesis that ACC or ethylene act as a diffusible signal directing root hair production in those epidermal cells supertending the boundary between two underlying cortical cells (Tanimoto *et al.*, 1995). Interestingly, the application of 2,4-D or IAA does not cause ectopic root hair development despite the fact that both hormones induce ethylene biosynthesis in roots (Masucci and Schiefelbein, 1996). In addition, neither the *etr1* nor *ein2* mutants have reduced numbers of root hairs, suggesting that ethylene is not required for initiation of hair growth (Masucci and Schiefelbein, 1996).

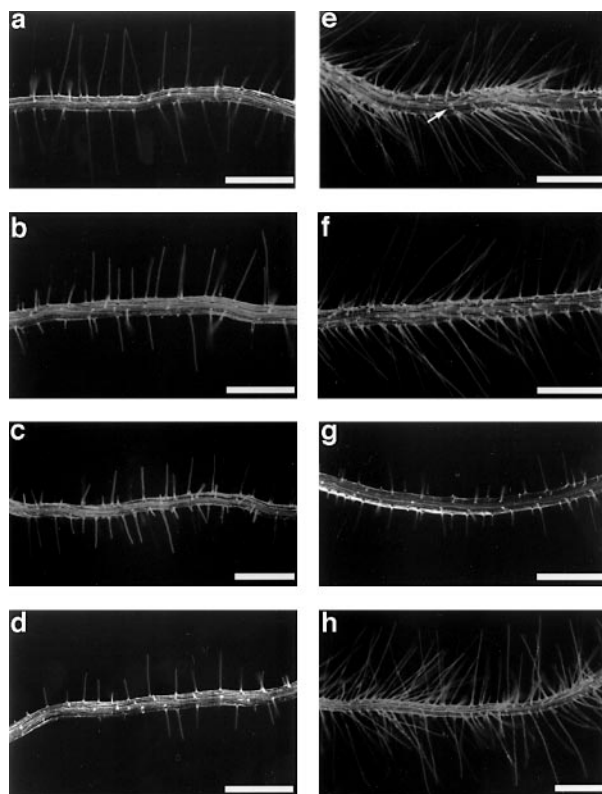
We are interested in understanding the mechanisms by which auxin and ethylene act to control root hair development. Toward that goal we have conducted morphological analyses of root hairs in wild-type and phytohormone mutant seedlings of *Arabidopsis thaliana*. The mutants *axr1*, *aux1*, *etr1*, *ein2* and *eto1* were all found to have abnormalities in root hair elongation. We have also examined the effects of auxin and ethylene on root hair length by growing seedlings on 2,4-D or ACC supplemented media. Furthermore, we describe the effect of the *sar1* mutation on root hair development in the auxin mutants. The results of our studies indicate that auxin and ethylene are both positive regulators of root hair elongation in *Arabidopsis*.

## Results

### Effects of auxin response mutations on root hair development

Genetic studies in *Arabidopsis* have implicated auxin in the process of root hair development (Cernac *et al.*, 1997; Masucci and Schiefelbein, 1996; Wilson *et al.*, 1990). Cernac *et al.* (1997) showed that the *axr1* mutants have reduced numbers of root hairs. However, it was not clear from their data if the mutant was deficient in root hair initiation or elongation. In an earlier study, Masucci and Schiefelbein (1996) examined the weak *axr1-3* allele and found a normal number of root hairs, suggesting that the apparent reduction observed by Cernac *et al.* (1997) was due to a defect in elongation. To distinguish between these possibilities, we measured root hair numbers and lengths in a large sample of *axr1* seedlings.

When viewed with a dissecting microscope, the strong

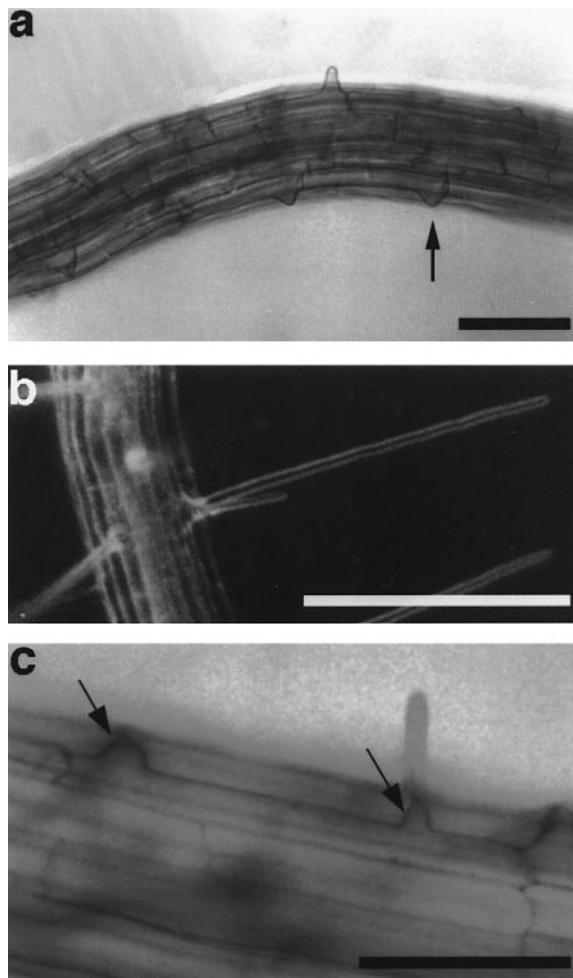


**Figure 1.** Roots of 7-day-old *Arabidopsis* seedlings grown on unsupplemented nutrient media.

The photographs show wild-type root hairs compared with various hormone mutants. (a) Columbia wild-type; (b) *axr1-12*; (c) *aux1-7*; (d) *etr1*; (e) *sar1-1*; (f) *axr1-12 sar1-1*; (g) *aux1-7 sar1-1*; (h) *eto1-1*. Scale bars = 500  $\mu$ m.

allele *axr1-12* has less than half the number of root hairs per millimeter as wild-type roots (Cernac *et al.*, 1997). However, when viewed at a 400  $\times$  magnification,  $46.5\% \pm 2.8$  of the *axr1-12* root epidermal cells had initiated a root hair compared to  $50.0\% \pm 2.3$  in wild type. These results confirm that the *axr1* mutations have little effect on root hair initiation. In contrast, *axr1-12* had a dramatic effect on root hair length (Figures 1 and 2, Table 1). The root hairs on the mutant were significantly shorter than wild-type root hairs and much more variable in length. When plotted as a histogram, the lengths of wild-type root hairs resemble a typical Gaussian distribution with values centered around a predominant mean value, whilst *axr1-12* root hairs display a much flatter distribution (Figure 3). The difference between the mean root hair length values for wild-type and *axr1-12* is significant. We conclude, therefore, that *axr1-12* seedlings are severely deficient in root hair elongation. The *axr1* phenotype is not allele specific as the same defects are seen in *axr1-3*, a weak allele (data not shown, Lincoln *et al.*, 1990).

The *sar1-1* mutant of *Arabidopsis* suppresses nearly every aspect of the *axr1* phenotype (Cernac *et al.*, 1997). Based on double mutant analysis, *SAR1* functions after *AXR1* in auxin response (Cernac *et al.*, 1997). One of the



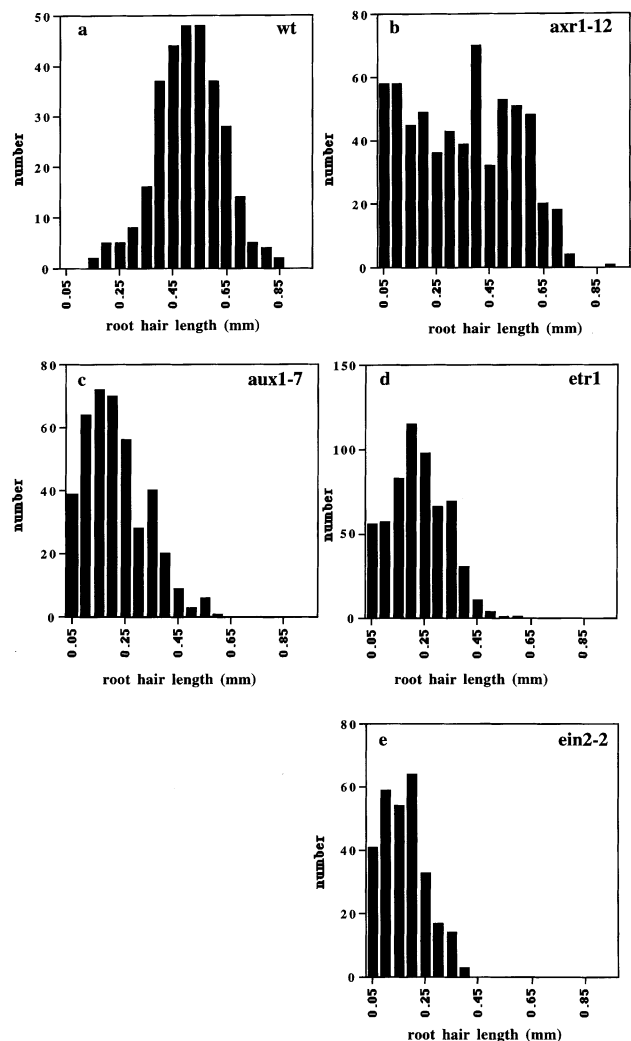
**Figure 2.** Root hair phenotypes of hormone response mutants. (a) Photomicrograph of a 10-day-old *axr1-12* root grown on unsupplemented media. Arrow indicates a trichoblast located above the elongation zone with an initiated, non-elongated root hair. Scale bar = 100 µm. (b) Photomicrograph of a bifurcated (twin) root hair of a 7-day-old *aux1-7* seedling grown on unsupplemented media. Scale bar = 250 µm. (c) Photomicrograph of a 7-day-old *aux1-7 sar1-1* seedling grown on unsupplemented media. Arrows indicate two root hairs initiating from the same trichoblast. Scale bar = 50 µm.

**Table 1.** Root hair length in wild-type and mutant seedlings

Genotype	Root hair length (µm) ± SE
wild-type	499 ± 5.5 (427) <sup>a</sup>
<i>axr1-12</i>	310 ± 8.3 (575)
<i>aux1-7</i>	201 ± 4.4 (764)
<i>sar1-1</i>	500 ± 5.9 (595)
<i>aux1-7 sar1-1</i>	246 ± 4.9 (796)
<i>axr1-12 sar1-1</i>	592 ± 6.2 (592)
<i>etr1</i>	203 ± 4.1 (659)
<i>eto1-1</i>	752 ± 9.1 (295)

<sup>a</sup>Number of root hairs measured.

phenotypes that *sar1* suppresses is the failure of *axr1-12* seedlings to elongate root hairs (Cernac *et al.*, 1997). We performed a detailed analysis of the effects of the *sar1-1*



**Figure 3.** Distributions of root hair lengths in 7-day-old *Arabidopsis* seedlings grown on unsupplemented media. (a) Columbia; (b) *axr1-12*; (c) *aux1-7*; (d) *etr1*; (e) *ein2-2*.

mutation on root hair development in the *axr1-12* and *aux1-7* mutants.

The lengths (Table 1) and distribution (data not shown) of *sar1-1* root hairs closely resembles that of wild-type seedlings. Occasionally, we observed an ectopic root hair in *sar1-1* seedlings (arrow, Figure 1e). Similar to the study by Cernac *et al.* (1997), the double mutant *axr1-12 sar1-1* has a root hair phenotype resembling that of the *sar1-1* single mutant. The distribution of root hair lengths is like that of either *sar1-1* or wild type (data not shown). Surprisingly, we found that the length of hairs was significantly greater in *axr1-12 sar1-1* seedlings than in either wild type or *sar1-1*.

The auxin resistant mutant, *aux1-7*, has also been reported to have shorter root hairs than wild-type seedlings (Okada and Shimura, 1994). Unlike *axr1-12*, *aux1-7* root hairs assume a more normal length distribution (Figure 3).

*aux1-7* root hairs did not elongate to the maximum of about 0.75 mm that wild-type or *axr1-12* achieve on unsupplemented media (Figure 3). Both *axr1-12* and *aux1-7* produce some bifurcated or 'twin hairs' which originate from the same cell and have split into two distinct shafts very near the base of the hair (Figure 2d). The *AUX1* gene product is related to transmembrane amino acid transporters and has been proposed to function as an auxin influx carrier in *Arabidopsis* roots (Bennett *et al.*, 1996). Thus the *aux1-7* phenotype might be explained as the result of loss of auxin redistribution into trichoblasts where it is required for normal root hair elongation.

We found that the *sar1-1* mutation has some interesting effects on root hair development in the *aux1-7* background. Root hairs in the double mutant are significantly longer than *aux1-7* and there is an increase in the number of root hairs per millimeter compared with *aux1-7* (data not shown). At least part of the increase in hair number is due to ectopic root hair formation in the double mutant (i.e. root hairs initiating in several adjacent files, Figure 1g) as well as the occasional initiation of more than one root hair within the same trichoblast (Figure 2c). The data suggest that *sar1-1* is able to partially suppress the *aux1-7* reduction in root hair numbers and root hair length.

#### Effects of ethylene mutants on root hair development

Although the plant hormone ethylene has been strongly implicated in *Arabidopsis* root hair development, a root hair defect in the ethylene response mutants *etr1* and *ein2* has not been described. Both mutants are completely resistant to ethylene treatment with respect to all phenotypes observed including hypocotyl and root growth inhibition, leaf chlorophyll loss, ethylene biosynthesis and induction of seed germination (Bleecker *et al.*, 1988; Roman *et al.*, 1995). We analyzed the root hair morphology of these two mutants, as well as the ethylene overproducing mutant *eto1-1* (Guzmán and Ecker, 1990). *etr1* has significantly reduced root hair lengths compared to wild-type seedlings while still displaying a normal root hair length distribution pattern (Table 1, Figure 3). *etr1* trichoblasts occasionally fail to initiate a root hair (see Figure 1c) and the hairs do not elongate past a maximum of about 0.5 mm (Figure 3). *ein2-1* seedlings have an average root hair length and distribution pattern similar to that of *etr1* (Figure 3). These results indicate that ethylene contributes to hair elongation.

In contrast to the *etr1* phenotype, *eto1-1* trichoblasts are significantly longer than wild-type, achieving an average length of just over 0.75 mm compared to 0.5 mm for wild-type (data not shown). The distribution of root hair lengths is also normal in *eto1-1* seedlings (data not shown).

#### Effects of hormone treatments on wild-type and mutant phenotypes

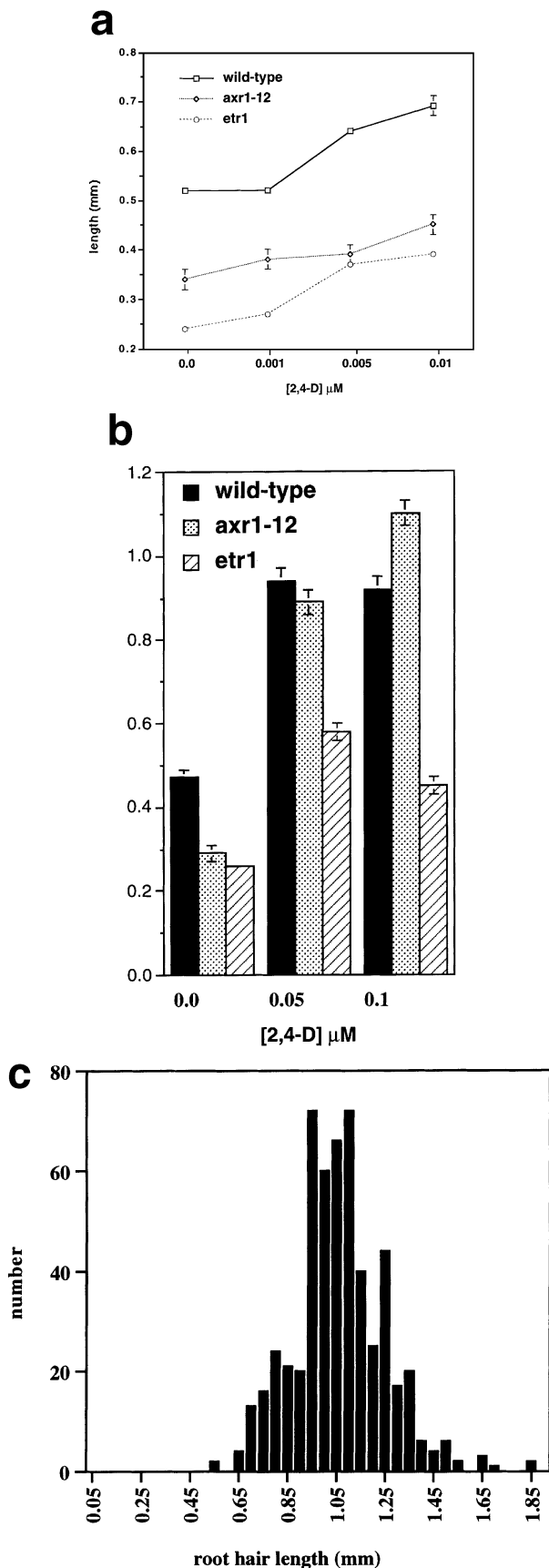
Because both auxin and ethylene response mutants have root hair elongation defects, we reasoned that the application of either 2,4-D or ACC to seedlings might also affect root hair elongation. Okada and Shimura (1994) have observed increased root hair lengths when seedlings were transferred to media containing auxin or auxin transport inhibitors. Our results again quantitatively agree with these observations. Figure 4(a) shows the result of treating wild-type, *axr1-12* and *etr1* seedlings with increasing amounts of 2,4-D. All three genotypes show a modest increase in root hair length in response to low concentrations of 2,4-D. We wished to look at the effects of higher concentrations of 2,4-D on root hair elongation but wild-type and *etr1* seedlings roots do not elongate when germinated on high 2,4-D concentrations. We therefore transferred 7-day-old wild-type and *etr1* seedlings to media containing 0.05  $\mu$ M or 0.10  $\mu$ M 2,4-D. Figure 4(b) shows that each genotype responds dramatically to higher levels of 2,4-D. Wild-type and *etr1* root hairs nearly double in average length while *axr1-12* root hairs are over four times as long (Figure 4b). Furthermore, the root hair length distribution of *axr1-12* is restored to normal on higher concentrations of 2,4-D, while the distributions of wild-type and *etr1* remain normal (Figure 4c, data not shown).

Treatment of seedlings with ACC also had a dramatic effect on root hair length. As shown in Figure 5(a), increasing concentrations of ACC lead to increased root hair lengths in all three genotypes. 5  $\mu$ M ACC caused an increase in root hair length of > 80% in wild-type seedlings and 200% in *axr1-12* seedlings (Figure 4a). In this trial, *etr1* root hair length increased slightly on 5  $\mu$ M ACC (Figure 4a), but in other trials root hair lengths were increased by as much as 100% to a maximum of approximately 0.4 mm (data not shown).

The *axr1-12* seedlings displayed a novel response to 5  $\mu$ M ACC. Some regions of the root have very long root hairs while other regions completely lack hairs (Figure 5c). This phenotype was not observed in 2,4-D treated *axr1-12* seedlings. Thus, *axr1-12* root hair initiation and elongation were effectively rescued by 2,4-D but not by ACC treatments. *axr1-12* roots also showed ectopic root hair formation on 5  $\mu$ M ACC whereas wild-type and *etr1* seedlings did not (Figure 5d).

#### Discussion

During the last 5 years significant progress has been made in our understanding of the genetic regulation of root hair development in *Arabidopsis*. Still, little is known about either the signaling processes which direct epidermal cell differentiation or the specific effects of hormones on its



later stages. Auxin and ethylene are both positive regulators of root hair development that exert their influence on root hair development after the *TTG/GL2* pathway has established the early differentiation events (Masucci and Shiefelbein, 1996). Unlike ethylene, the importance of auxin in the process has been questioned because applying auxin to seedlings does not cause ectopic root hair formation (Masucci and Shiefelbein, 1996; Tanimoto *et al.*, 1995; this study). However, the observations that both *axr1* and *aux1* have shorter hairs, as well as the effects of exogenous auxin on hair length, suggest that auxin is necessary for normal root hair elongation.

Interactions among hormone response pathways complicate the interpretation of the data. The *rhd6* root hair deficiency is rescued by treatment with either auxin or ACC (Masucci and Schiefelbein, 1994). Furthermore, *aux1* is ethylene resistant and some aspects of the *aux1* phenotype may be a consequence of reduced ethylene response (Pickett *et al.*, 1990). It is also possible that seedling responses to 2,4-D treatment are indirect since 2,4-D stimulates ethylene production.

In previous reports, a model has been presented in which ethylene is required for initiation of root hairs in trichoblast files. This model is based on the production of ectopic root hairs in the *ctr1* mutant and pharmacological studies using inhibitors of ethylene synthesis or action. Our results present the first clear genetic evidence that ethylene response is required for normal root hair elongation. However, it is striking that both the *ein2* and *etr1* mutants do produce many normal root hairs, despite the fact that both mutants are insensitive to ethylene. There are several possible explanations for this result. One possibility is that the mutants retain a residual ethylene response sufficient for some root hair development. The fact that in some trials *etr1* seedlings responded to ACC supports this view. In addition, treatment of wild-type seedlings with AVG, an inhibitor of ethylene biosynthesis, results in the loss of root hairs (Masucci and Schiefelbein, 1996; Tanimoto *et al.*, 1995).

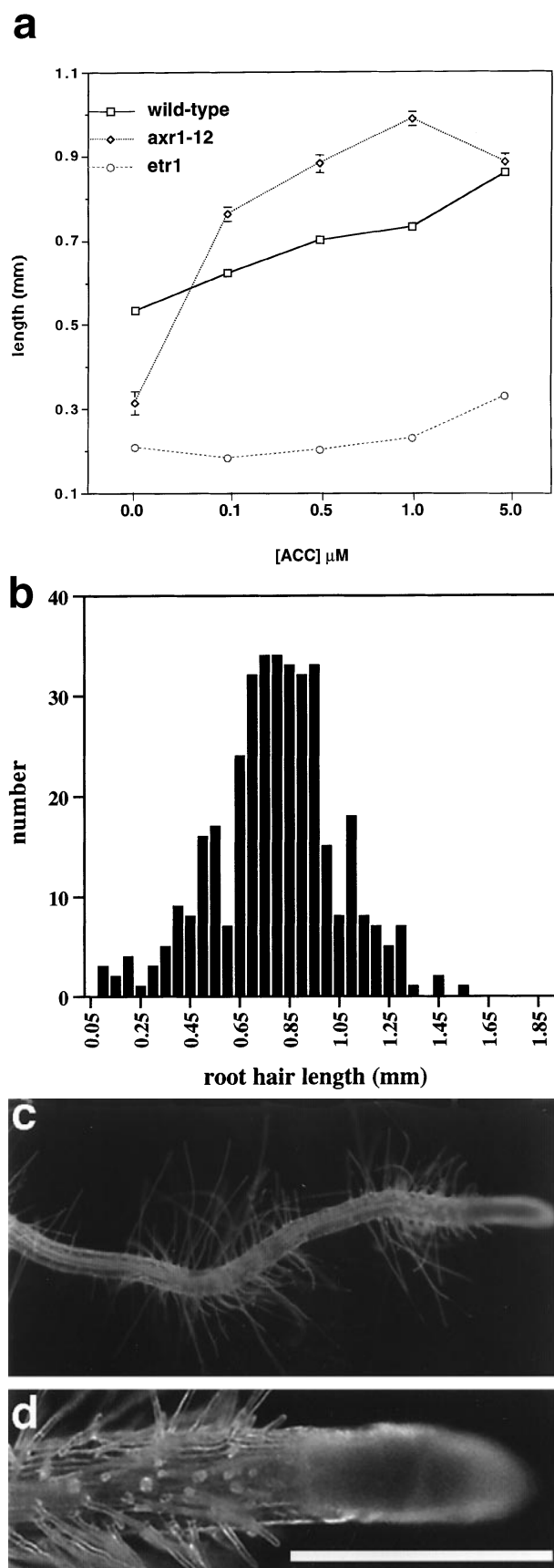
The *axr1* phenotype is a challenge to explain. Some root hairs were as long as wild-type whilst most were extremely short. One explanation for this result is that with respect to root hair elongation, the *axr1* mutation is leaky and that

**Figure 4.** Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on root hair lengths.

(a) Wild-type (solid line), *axr1-12* (dotted line) and *etr1* (dashed line) seeds were germinated on unsupplemented media or supplemented with increasing concentrations of 2,4-D. Data points represent the average length of 75–100 root hairs plus and minus standard error.

(b) Graph shows the effect of higher concentrations of 2,4-D on root hair elongation. Each bar represents the average length of 75–100 root hairs plus standard error. Wild-type (solid bars), *axr1-12* (shaded bars), and *etr1* (dashed bars). Note that y-axis scales are different in (a) and (b).

(c) Distribution of root hair lengths of 7-day-old *axr1-12* seedlings grown on 0.1 μM 2,4-D.



some function is retained in the cells where root hairs have elongated. Alternatively, the loss or partial loss of the auxin response pathway in the *axr1* mutant may cause the cells to be much more sensitive to subtle differences in auxin levels or other environmental factors. If individual cells are subjected to a changing environment or are exposed to different amounts of auxin as the root grows, then root hair elongation might be different in trichoblasts within the same cell file.

The differences in the *aux1* and *axr1* phenotypes probably reflect the difference in the function of the respective proteins. *AUX1* encodes a putative auxin transport protein that may be required to redistribute auxin during differentiation of the epidermis and root hair elongation. *AXR1* encodes a protein with similarity to the N-terminal half of ubiquitin-activating enzyme, E1, and is required, in combination with a second protein called ECR1, for the activation of an *Arabidopsis* ubiquitin-related protein called RUB1 (del Pozo *et al.*, 1998; Leyser *et al.*, 1993). An analogous protein modification functions in yeast to promote progression through the cell cycle via an *AXR1* homologue, *ENR2* (Lammer *et al.*, 1998). Immunolocalization studies indicate that *AXR1* is localized to the nucleus suggesting that the targets of RUB modification are nuclear proteins. Thus, RUB modification of nuclear proteins in the root hair appears to be required for root hair growth (Gray and Estelle, 1998). The *aux1* mutants, on the other hand, may simply fail to distribute auxin into trichoblasts where it is required to promote root hair elongation via the *AXR1* and possibly other pathways.

*axr1-12* is also hypersensitive to the effects of ACC on root hair development, displaying an exaggeration of the phenotype of seedlings grown on unsupplemented medium (Figure 5c). For some trichoblasts, ACC promotes elongation of root hairs to about twice that of wild-type roots while others completely lack hairs (Figure 5c). This suggests that auxin and ethylene are interacting to promote root hair elongation. While it can be argued that root hair elongation in the presence of hormones is a stress response of the root system, these data in combination with mutant analyses indicate that auxin and ethylene promote root hair elongation. Future studies, including those that focus

**Figure 5.** Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) on root hair length.

(a) Wild-type (solid line), *axr1-12* (dotted line) and *etr1* (dashed line) seeds were germinated on unsupplemented media or supplemented with increasing concentrations of ACC. Data points represent the average length of 75–100 root hairs plus and minus standard error.

(b) Distribution of root hair lengths of 7-day-old *axr1-12* seedlings grown on 5  $\mu\text{M}$  ACC.

(c) Photograph of a 7-day-old *axr1-12* seedling root grown on 5  $\mu\text{M}$  ACC. Patches of long root hairs followed by areas devoid of hairs are observed. Scale bar = 500  $\mu\text{m}$ .

(d) Photograph of the root tip of a 7-day-old *axr1-12* seedling grown on 5  $\mu\text{M}$  ACC. Root hairs can be seen initiating in all cell files. Scale bar = 500  $\mu\text{m}$ .

on the cellular responses to auxin and ethylene, may help clarify the role of these compounds in root hair development.

## Experimental procedures

### Plant materials and growth conditions

Wild-type or mutant *Arabidopsis thaliana* (ecotype Columbia) seeds were surface sterilized in 10% bleach for 15 min and rinsed three times with sterile distilled water. Seeds were dispersed onto Petri plates containing nutrient medium (Wilson *et al.*, 1990) containing 1% agarose and 1% sucrose. Seeds were cold treated at 4°C for 2–4 days and grown vertically in an incubator at 20°C under 16 h illumination.

### Morphometric analysis

Seedlings were grown vertically as described above for 7 days. Seedlings were stained with dilute toluidine blue (< 0.05%) and placed onto a glass microscope slide under a cover slip. The number of root hairs in a 2 mm section at the approximate midpoint of the root were counted under a dissecting microscope. Trichoblasts (from the stained seedlings) located above the elongation zone of the root were measured using an ocular micrometer with a Zeiss phase microscope at 500× magnification. Root hairs that grew along the surface, but not penetrating, agarose nutrient media were photographed at 70× magnification at the midpoint of the root using an Olympus SZH10 stereo dissecting microscope equipped with an Olympus 35 mm camera (model PM-C35B) and Kodak black and white ASA125 film. Root hairs from the photographs were measured with a ruler and their lengths in µm determined by comparison with an ocular micrometer photographed at the same magnification. Odd root hair phenotypes were photographed using an Olympus dissecting scope as described above or by using a Zeiss Axioplan microscope.

### Significance tests

Significance tests based on normal distributions comparing the means of two large samples ( $n > 30$ ) were carried out using the following equation:  $d = \frac{\text{mean}_1 - \text{mean}_2}{\sqrt{(s_1^2/n_1 + s_2^2/n_2)}}$  where  $n$  = number of measurements, mean = sum of individual measurements/ $n$ , and  $s$  = standard deviation for each sample. When  $d > 2.576$  then the null hypothesis (no difference in sample means) was rejected at the 99% confidence level. When  $n < 30$ , Student  $t$ -tests were carried out. In each case the  $t$ -test result agreed with the original 'd' test. All differences were significant to at least a value of  $P < 1\%$ . This indicates that the probability of the differences in the two sample means was due to chance alone was less than 1%. A detailed description of each test can be found in Bailey (1995).

### Distribution of root hair lengths

Root hair length measurements from three separate trials on unsupplemented media were compiled for all genotypes measured. Measurements for four trials of *axr1-12* on ACC and for three trials on 2,4-D were compiled.

### Hormone treatments

Seeds of each genotype were germinated and grown vertically in an incubator for 7 days on unsupplemented nutrient media as described above, or under the same conditions on nutrient media supplemented with 2,4-D or ACC. For 0.1 µM 2,4-D study, wild-type and *etr1* seedlings were grown for 7 days on unsupplemented nutrient media as described above then under sterile conditions were transferred to the same nutrient media supplemented with 2,4-D and grown for another 7 days. Root hairs were photographed and measured as described above. Data were plotted using Cricket Graph 1.6 software.

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