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The *rhd6* Mutation of *Arabidopsis thaliana* Alters Root-Hair Initiation through an Auxin- and Ethylene-Associated Process[1]

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Root-hair initiation in *Arabidopsis thaliana* provides a model for studying cell polarity and its role in plant morphogenesis. Root hairs normally emerge at the apical end of root epidermal cells, implying that these cells are polarized. We have identified a mutant, *rhd6*, that displays three defects: (a) a reduction in the number of root hairs, (b) an overall basal shift in the site of root-hair emergence, and (c) a relatively high frequency of epidermal cells with multiple root hairs. These defects implicate the *RHD6* gene in root-hair initiation and indicate that *RHD6* is normally associated with the establishment of, or response to, root epidermal cell polarity. Similar alterations in the site of root-hair emergence, although less extreme, were also discovered in roots of the auxin-, ethylene-, abscisic acid-resistant mutant *axr2* and the ethylene-resistant mutant *etr1*. All three *rhd6* mutant phenotypes were rescued when either auxin (indoleacetic acid) or an ethylene precursor (1-aminocyclopropane-1-carboxylic acid) was included in the growth medium. The *rhd6* root phenotypes could be phenocopied by treating wild-type seedlings with an inhibitor of the ethylene pathway (aminoethoxyvinylglycine). These results indicate that *RHD6* is normally involved in directing the selection or assembly of the root-hair initiation site through a process involving auxin and ethylene.

Polarity is a fundamental property of developing organisms and plays a major role in influencing morphogenesis (Schnepf, 1986; Sachs, 1991). The molecular and genetic control of cell polarity is understood best in the fission yeast during budding (Drubin, 1991). *Saccharomyces cerevisiae* grow asymmetrically and bud at specific locations within the cell. Molecular genetic studies have defined genes that affect each of the four processes involved in bud formation: polarity establishment, bud site assembly, cytoskeletal assembly, and polarized cell growth (Drubin, 1991).

The fucoid algae, because of their developmental simplicity, have been used to study fundamental aspects of cell polarity in the plant kingdom (Kropf, 1992; Goodner and Quatrano, 1993). The fucoid zygote becomes polarized upon perceiving a wide array of environmental gradients such as light, electric current, heat, and ions. One proposed mechanism is that environmental gradients result in the asymmetric distribution of ion channels within the zygote membrane, creating an internal ion gradient that serves to initiate polarization of the zygote (Kropf, 1992; Goodner and Quatrano, 1993).

In higher plants, relatively little is known about the establishment of polarity and its effect on morphogenesis. Some notable exceptions are developmental processes that are affected by the polarized transport of auxin, including the determination of the polarized vascular tissue and the regeneration of tissue with proper polarity in the absence of environmental cues (Sachs, 1984, 1991). Although these processes are known to be affected by auxin, the molecular mechanisms involved in the control of polarity and morphogenesis have not been determined.

We are using root-hair formation in *Arabidopsis thaliana* as a model to study cell polarity and morphogenesis in plants. Root hairs are long, tubular extensions of root epidermal cells. The initiation of a root hair requires that a new site of cell expansion be established within the epidermal cell. In *Arabidopsis*, root hairs emerge from the apical end of epidermal cells (the end closest to the root meristem; Schiefelbein and Somerville, 1990), implying that these cells are polarized. The overall process of root-hair initiation is thought to involve the establishment of polarity within the epidermal cell, the selection of the initiation site, and the directed transport of secretory Golgi vesicles containing hydrolytic enzymes and cell wall components to the initiation site (Sievers and Schnepf, 1981; Schnepf, 1986).

In this report, we describe the identification and characterization of the *rhd6* (root hair defective) mutant, which defines a locus involved in root-hair initiation. Roots homozygous for the *rhd6* allele possess fewer root hairs than wild-type plants, exhibit a significant basal shift in the site of root-hair emergence, and possess many cells with multiple root hairs. Similar phenotypes were discovered in the auxin-, ethylene-, ABA-resistant mutant *axr2* and the ethylene-resistant mutant *etr1*, and the *rhd6* mutant phenotype was rescued when either auxin or an ethylene precursor was included in the growth media. The *rhd6* mutant phenotype was completely phenocopied when wild-type roots were treated with an inhibitor of ethylene biosynthesis (AVG). We conclude that *RHD6* affects root-hair initiation through a process involving auxin and ethylene and, therefore, should be useful to study the role of cell polarity in morphogenesis.

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Abbreviations: APW, artificial pond water; AVG, L-α-(2-aminoethoxyvinyl)-glycine; NPA, naphthylphthalamic acid; WS, Wassilewskija.

MATERIALS AND METHODS

Genetic Stocks

The *rhd6* mutant was isolated from a pool of T-DNA insertional mutants (Feldmann and Marks, 1987) in the ecotype WS. A homozygous mutant line was obtained that had segregated 3:1 kanamycin resistant:kanamycin sensitive and 1:3 mutant:wild-type phenotype in the F_2 generation following a cross with the Columbia wild type. The ethylene- and auxin-resistant strains were obtained from the *Arabidopsis* Biological Resource Center at The Ohio State University (Columbus, OH). The stock numbers are as follows: *etr1*, 237; *eto1*, 3072; *ein1*, 3070; *ein2*, 3071; *aux1*, 3074; *axr1*, 3075; *axr2*, 3077. The lines expressing the cDNA from the maize R locus were obtained from A. Lloyd and R. Davis (Stanford University, Stanford, CA) and were previously described (Lloyd et al., 1992).

Growth Conditions

For growth of plants in Petri plates, seeds were surface sterilized, placed on the surface of A.T. agarose medium (1% Suc, 0.6% agarose, and mineral nutrients [Estelle and Somerville, 1987]), chilled 1 to 2 d at 4°C, and incubated at 20 to 22°C in a vertical orientation as previously described (Schiefelbein and Somerville, 1990). Root-hair phenotypes were scored after 5 d of growth under these conditions unless otherwise noted. The growth of *Arabidopsis* plants in soil has been described (Schiefelbein and Somerville, 1990). A microgravity environment was mimicked by rotating germinating and growing seedlings perpendicularly to the direction of gravity at 1.2 rpm. Under these conditions, wild-type seedling roots grew randomly along the surface of the Petri dishes.

A.T. agarose media containing IAA or 2,4-D (Sigma) were prepared by diluting a 1×10^{-3} M stock solution, made in 1% ethanol, into A.T. agarose prior to autoclaving. A.T. agarose medium containing ACC (Sigma) was made by diluting a 0.5 M ACC solution, made in water, into cooled, autoclaved A.T. agarose. Similarly, A.T. agarose media containing GA_3 (Sigma), kinetin (Sigma), NPA (Uniroyal Chemical, Navgatuck, CT), or AVG (MAAG Agrochemicals, Vero Beach, FL) were made from a 30-mM GA_3 solution made in 25% ethanol, from a 1×10^{-3} M kinetin stock made in 1% ethanol, from a 0.1 M NPA stock made in water, or from a 0.1 M AVG stock made in water, respectively.

Genetic Mapping of *rhd6*

The *rhd6* mutant was crossed to the mapping strain W100 (Koornneef et al., 1987), and the F_1 progeny were allowed to self-pollinate. The F_2 progeny were scored for the various mutant phenotypes, and a linkage to the *ap1* locus was noted. F_3 seeds from F_2 plants that were homozygous or heterozygous for *rhd6* were grown and scored for the *ap1* phenotype. Five recombination events were detected out of 110 chromosomes (55 families) scored.

Transverse Sections and Environmental Scanning EM

Five-day-old seedlings were placed in molten 3% agarose made with APW (Schiefelbein et al., 1992). After solidification, sections were cut using a double-edged razor blade and were stained with a 1:20 dilution of 0.05% toluidine blue (made in 1 mM Mes, pH 6.0) in APW (pH 6.0). Environmental scanning EM was done as described by Schiefelbein et al. (1993).

Determination of Root-Hair Number and Epidermal Cell Length

Root hairs were counted over a 2-mm section of root from 5-d-old seedlings grown on vertically oriented A.T. agarose medium using a dissecting microscope (Wild M420 Makroskop). To determine epidermal cell length, 5-d-old seedlings were placed in APW and observed under a Leitz Laborlux S microscope. The lengths of five mature, hair-bearing cells per root were determined. Not all cells scored from the *rhd6* mutant possessed root hairs. Values from 10 roots were used to determine the average cell length unless otherwise noted.

Classification of Roots as Apical, Intermediate, or Basal

Because root-hair emergence from different cells within one root cannot be assumed to be independent events, whole roots were classified as either apical, intermediate, or basal using the following criteria. Five or more mature root hairs from epidermal cells at the apical end of a 5-d-old seedling root (unless otherwise noted) were scored for their position within the epidermal cell. The sites of hair emergence were defined as follows: position 0, root hair emerges from the apical-most end of the cell; position 1, root hair emerges just basal to the apical end, such that the lateral cell wall, apical to the root hair, is sloping; position 2, root hair emerges, such that the lateral cell wall, apical to the hair, is flat but less than one root-hair width in length; position 3, root hair emerges, such that the lateral wall, apical to the root hair, is greater than one root-hair width but less than two root-hair widths in length; position 4, root hair emerges, such that the lateral wall, apical to the root hair, is greater than two root-hair widths in length.

The average position of the root hairs from cells on each root was then determined. If the average value was between 0 and 1, the root was classified as apical. If the average root-hair position was between 1 and 2, the root was classified as intermediate. If the average hair position was 2 or greater, the root was classified as basal. For these observations seedlings were placed in APW and examined with a Leitz Laborlux S microscope. Statistical comparisons were carried out by the SAS/STAT computer program (SAS Institute, Inc., Cary, NC) using the Fisher's exact test.

RESULTS

Isolation and Genetic Analysis of the *rhd6* Mutant

The *rhd6* mutant was identified by visually screening seedling roots from pools of seed generated by T-DNA insertional mutagenesis (Feldmann and Marks, 1987) for individuals with root-hair abnormalities. As shown in Figure 1 and Table I, *rhd6* mutant seedlings grown on vertically oriented Petri plates with the standard growth medium (A.T. agarose, see "Materials and Methods") displayed a reduced number of

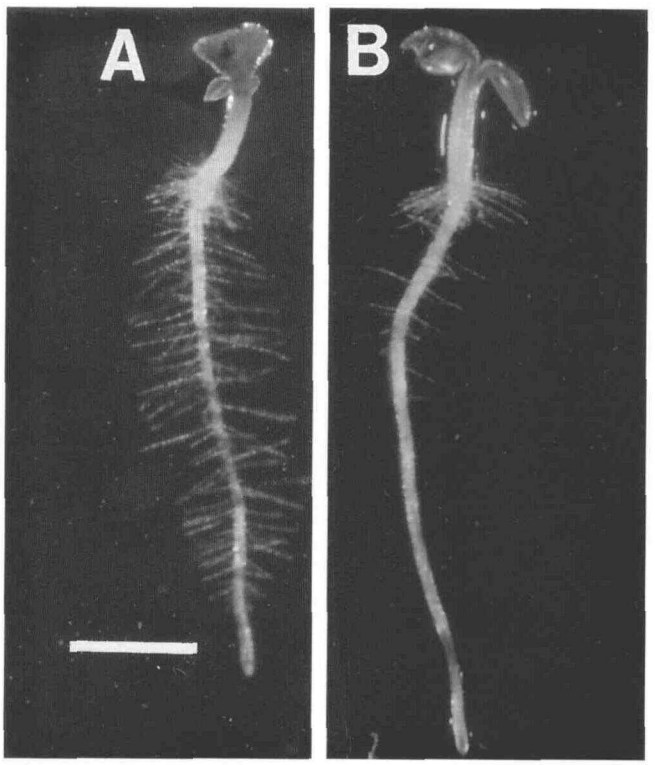

Figure 1. The *rhd6* mutant phenotype. A, Wild-type (Columbia) root with normal root-hair density and morphology. B, A typical *rhd6* mutant root showing a reduction in root-hair density. Bar represents 1 mm.

root hairs. This phenotype was variable; the root-hair density ranged from 0 to 60% of the wild type. The average length of *rhd6* root epidermal cells did not differ from the wild-type WS but was slightly greater than the wild-type Columbia (Table I). After normalizing for the cell length differences, we determined that *rhd6* mutant roots produced approximately 15 to 17% of the wild-type number of root hairs (Table I). Except for defects in the root epidermis, no other morphological abnormalities were observed in the *rhd6* mutant plants.

To examine the nature of the *rhd6* mutation, *rhd6* plants were crossed to wild-type (Columbia) plants. The progeny plants, heterozygous for the *rhd6* mutation (*rhd6*/+), displayed normal root-hair characteristics. The F_2 plants segregated 462:137 wild type:mutant, indicating that the mutation is nuclear and recessive. The *RHD6* locus was mapped to chromosome 1, approximately 5 centimorgans from the *AP1* locus, by analyzing F_2 and F_3 plants from a cross with the W100 mapping line (see "Materials and Methods"). The *rhd6* mutation was able to complement the mutations in the *rhd1*, *rhd2*, *rhd3*, and *rhd4* mutant lines (Schiefelbein and Somerville, 1990), as well as other root-hair morphology mutants (J.D. Masucci and J.W. Schiefelbein, unpublished results), indicating that the *rhd6* line possesses the only known mutant allele of the *RHD6* locus.

The *rhd6* Mutation Alters Root-Hair Initiation

Scanning EM and light microscopy were used to examine the morphology of the *rhd6* mutant roots and root hairs. As shown in Figure 2A, large portions of the root possessed epidermal cells that lacked root hairs and displayed no indication of root-hair initiation. In addition, no evidence for root-hair growth was detected along these hairless cells when

Table I. *Root-hair density, epidermal cell length, and relative root-hair number of Columbia wild-type and rhd6 mutant roots grown on various media*

Genotype	Media Supplement	Root-Hair Density[a]	Cell Length[b]	Relative Hair Number[c]
			mm	
Columbia	None[d]	30 ± 5 (37)	0.18 ± 0.01	5.4
WS	None	28 ± 4 (20)	0.21 ± 0.01	5.9
rhd6	None	4 ± 4 (56)	0.23 ± 0.02	0.9
Columbia	IAA (1×10^{-8} M)	47 ± 12 (56)	0.16 ± 0.03	7.5
rhd6	IAA (1×10^{-8} M)	19 ± 14 (29)	0.20 ± 0.04	3.8
Columbia	IAA (3×10^{-8} M)	71 ± 9 (20)	0.09 ± 0.04	6.4
rhd6	IAA (3×10^{-8} M)	70 ± 18 (20)	0.08 ± 0.04	5.6
Columbia	ACC (5×10^{-7} M)	39 ± 12 (38)	0.17 ± 0.02	6.6
rhd6	ACC (5×10^{-7} M)	24 ± 14 (67)	0.17 ± 0.05	4.1
Columbia	ACC (5×10^{-6} M)	49 ± 12 (35)	0.14 ± 0.02	6.9
rhd6	ACC (5×10^{-6} M)	32 ± 10 (22)	0.17 ± 0.04	5.4
Columbia	NPA	36 ± 9 (29)	0.15 ± 0.02	5.4
Columbia	AVG (10^{-6} M)	12 ± 7 (30)	0.18 ± 0.02	2.2
Columbia	AVG + ACC[e]	41 ± 8 (29)	0.15 ± 0.04	6.2

[a] Values represent the mean numbers ± SD of root hairs per millimeter; the number of roots scored is shown in parentheses. [b] Values represent the mean ± SD lengths of root-epidermal cells. [c] Values represent the products of root hair density and cell length. [d] Unsupplemented A.T. agarose. [e] 10^{-6} M AVG plus 5×10^{-6} M ACC.

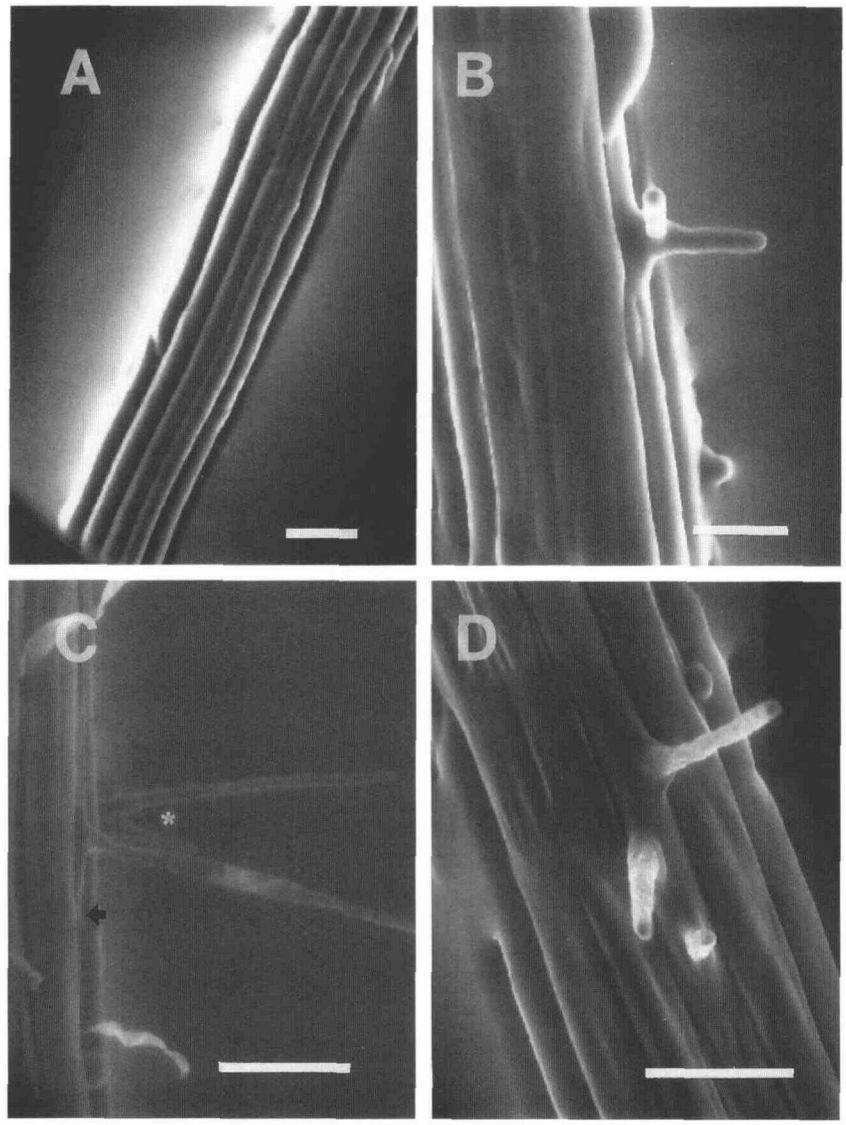

Figure 2. Root-hair initiation defects associated with the *rhd6* mutation. Environmental scanning electron micrographs of *rhd6* mutant roots. A, Section of mature root showing no sign of root-hair initiation. B, Epidermal cell with two root hairs emerging from a single initiation site. C, Background, Two hairs protruding from adjacent initiation sites (asterisk). Foreground, A single hair emerging from a basal position (arrow indicates apical cell wall). D, Trichoblast with three root-hair initiation sites. Bars represent 50 μm.

mutant roots were stained with fluorescent brightener 28 (equivalent to Calcofluor white M2R; Sigma). Fluorescent brightener 28 allows detection of areas of cell wall synthesis (Fowke et al., 1983; Hahne and Hoffmann, 1985; Galway and Hardham, 1986).

Most of the root hairs that form in *rhd6* mutant seedlings have normal morphology. However, in some instances various defects in root-hair formation were observed (Fig. 2, B–D). Occasionally, a single initiation site produced two root hairs (Fig. 2B). More often (60 of 469 scored hair-bearing cells, 13%), mutant epidermal cells possessed two or more independent hairs. These root hairs emerged from two adjacent sites (background Fig. 2C) or from distinctly separate sites (Fig. 2D). Epidermal cells possessing multiple hairs were not detected in either Columbia (597 hair-bearing cells scored) or WS (200 hair-bearing cells scored) wild-type roots. Another defect detected in *rhd6* was a general shift in the site of root-hair emergence toward the basal end of the cell. This difference was observed in epidermal cells containing multiple hairs (Fig. 2D) and single hairs (foreground, Fig. 2C). Neither changes in growing temperature (4 or 30°C rather than 22°C) nor growth in a microgravity environment (see "Materials and Methods") had an effect on the *rhd6* mutant phenotypes (data not shown). The above phenotypes show that the *rhd6* mutation alters the ability of epidermal cells to initiate root-hair formation.

The cellular organization of *rhd6* mutant roots was analyzed by examining hand-cut transverse sections. The *rhd6* roots possessed 8 endodermal cells, 8 cortical cells, and an average of 18 epidermal cells (ranging from 16–22), which concur with the numbers reported for wild-type *Arabidopsis* roots (Dolan et al., 1993). These results indicate that the cellular organization of the mature root is not affected by the *rhd6* mutation.

In *Arabidopsis*, only epidermal cells in specific files produce root hairs, whereas cells in other files remain hairless. The

position of the cells with respect to the underlying cortex appears to determine what files produce hairs. The fate of the cells in the two types of files can be deduced prior to root-hair formation, because trichoblasts (future hair-bearing cells) exhibit a delay in cellular vacuolation relative to atrichoblasts (Galway et al., 1994). To determine whether the *rhd6* mutation affects epidermal cell fate, cell vacuolation was examined in the developing epidermis of *rhd6* mutant seedlings. In the mutant roots, a delay in cell vacuolation of trichoblasts was observed, although it did not persist as long as in wild-type roots (data not shown). We conclude, therefore, that the *rhd6* mutation does not affect epidermal cell fate specification but alters trichoblast differentiation at the later stage of root-hair initiation.

The *rhd6* Mutant Differs in the Site of Root-Hair Emergence

In wild-type *Arabidopsis* plants, root hairs emerge from the apical end of epidermal cells (that end nearest the root meristem) (Schiefelbein and Somerville, 1990). Analysis of *rhd6* mutants showed that root hairs appeared to emerge more basally on the epidermal cells than in wild-type cells. To quantify the shift in the site of root-hair emergence, five possible sites of root-hair emergence, apical to basal, were defined (Fig. 3), and individual roots were classified as either apical, intermediate, or basal, depending on the average site of root hairs on their epidermal cells (see "Materials and Methods"). The distribution of wild-type roots (Fig. 4A) indicates that wild-type root-hair initiation occurs near, but not immediately at, the apical end of the epidermal cells (between sites 1 and 2 as shown in Fig. 3). Neither a microgravity environment nor changes in temperature caused a significant difference in the distribution of roots (data not shown).

The site of root-hair emergence in the *rhd6* mutant was analyzed and compared to that of the wild type. The *rhd6* line used was derived from a cross of the initial mutant (generated in the WS background) with Columbia. Because the distributions of WS and Columbia roots did not vary significantly ($P > 0.36$), Columbia was chosen as the wild-type control. In Figure 4B, root-hair cells were scored throughout the root rather than near the apical end of the root (as in Fig. 4A). This was necessary because *rhd6* mutant roots produce a small number of root hairs. For the Columbia wild type, 14% of the roots were classified as apical, 86% were classified as intermediate, and none were classified as basal. For the *rhd6* mutant, 4% of the roots were classified as apical, 21% were classified as intermediate, and 74% were classified as basal. The distribution of *rhd6* mutant roots is significantly different from the wild type ($P < 0.01$). The more basal site of *rhd6* root hairs is not likely to be due to greater cell expansion at the apical end of the cell after root-hair initiation because (a) cells with root hairs in different sites (sites 1–4) were the same length and (b) initiating root hairs were observed in basal sites (sites 3 and 4) within the cell (data not shown). These results indicate that the *rhd6* mutation alters the apical-basal positioning of root-hair emergence in epidermal cells.

To determine whether the site of root-hair emergence is related to the age of the root or the distance of the epidermal cell from the shoot, root hairs from either the apical or basal end of roots from 5-d-old wild-type seedlings were scored. As shown in Figure 4C, a significant difference ($P < 0.01$) in the root distribution was obtained when root hairs were scored at different ends of the root. In roots in which hair emergence was scored in epidermal cells close to the shoot, a large fraction was classified as apical. When root-hair emergence was scored in epidermal cells close to the root meristem, a large fraction of roots was classified as basal. These results indicate that differentiating epidermal cells of younger (shorter) roots may experience a stronger polarity signal than cells of older roots. This is consistent with a shoot-derived gradient involved in establishing the site of root-hair emergence that is attenuated as the root lengthens and ages.

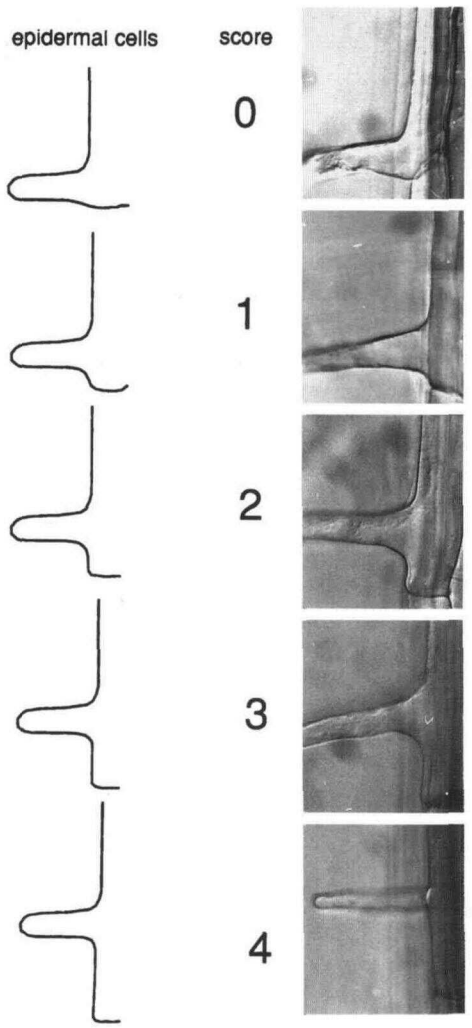

Figure 3. Sites of root-hair emergence used to classify roots. In the left column are schematic drawings of root epidermal cells showing possible sites of root-hair emergence arranged (top to bottom) from most apical to most basal with respect to the root apical meristem. In the center column is the arbitrary score assigned to each of those sites. At right are photographs of trichoblasts showing root hairs emerging from each of the five sites.

Auxin and Ethylene Mutants Differ in the Site of Root-Hair Emergence

Auxin is thought to affect polarity in plants, and the hormone ethylene has been linked to auxin action in some instances (Morgan and Gausman, 1966; Buchner and Pilet, 1983; Wood, 1985; Rengel and Kordan, 1987). Therefore, we wanted to determine whether auxin and/or ethylene affected the site of root-hair emergence. In the first set of experiments, roots from various auxin- and ethylene-resistant mutants were examined. Figure 5A shows that seedlings homozygous for the auxin-, ethylene-, ABA-insensitive mutant *axr2* (Wilson et al., 1990) produced a basally shifted distribution of roots that was significantly different from the wild-type distribution ($P < 0.01$). Interestingly, *axr2* mutants also exhibit a reduced number of root hairs (Wilson et al., 1990; Table II), and cells with multiple root hairs were observed in 4 of 98 (4%) scored trichoblasts. As shown in Figure 5B, *etr1* (Bleecker et al., 1988) also displayed a significant shift in the distribution of roots toward the basal classification ($P < 0.01$). The distribution of roots from the ethylene-insensitive mutants *ein1* or *ein2* (Guzman and Ecker, 1990), the auxin- and ethylene-insensitive mutant *aux1* (Pickett et al., 1990), and the auxin-insensitive mutant *axr1* (Lincoln and Estelle, 1990) did not vary significantly from wild type (data not shown).

To examine the effects of excess ethylene on root-hair emergence, the *eto1* mutant, which overproduces ethylene (Guzman and Ecker, 1990), was analyzed. The *eto1* mutation may be expected to cause an apical shift in the site of root-hair emergence because mutations causing an insensitivity to ethylene shift the site of hair emergence basally. As shown in Figure 5C, *eto1* mutant seedlings did display a significant apical shift in root distribution compared to the wild-type Columbia ($P < 0.01$). Taken together, these data implicate auxin and ethylene in the selection of the root-hair initiation site.

To further investigate the role of auxin and ethylene in root-hair formation, the effects of an inhibitor of ethylene biosynthesis, AVG (Zimmerman et al., 1977; Edwards et al., 1983; Geneve et al., 1989), and an inhibitor of auxin transport, NPA (Keitt and Baker, 1966; Beyer, 1972), on wild-type roots were tested. Growth of wild-type seedlings on medium containing AVG completely phenocopied the *rhd6* mutant phenotype. As shown in Table I, 10^{-6} M AVG led to a significant reduction in root-hair formation in wild-type roots. As shown in Figure 6A, 10^{-6} M AVG also caused a basal shift in the position of root hairs such that the distribution of wild-type roots grown on AVG did not vary significantly from the distribution of *rhd6* mutant roots grown on A.T. agarose ($P > 0.56$). In addition, epidermal cells possessing multiple hairs were observed in 10% of the scored epidermal cells (19 of 181). Although AVG blocks ethylene biosynthesis, it does not affect the conversion of ACC to ethylene (Adams and Yang, 1979). None of the defects associated with growth on AVG were detected in seedlings grown on medium containing both 10^{-6} M AVG and 5×10^{-6} M ACC (Table I, data not shown). The auxin transport inhibitor, NPA, however, had little effect on root hair formation. As shown in Table I, wild-type seedlings grown on 10^{-5} M NPA had the normal density of root hairs, even though this

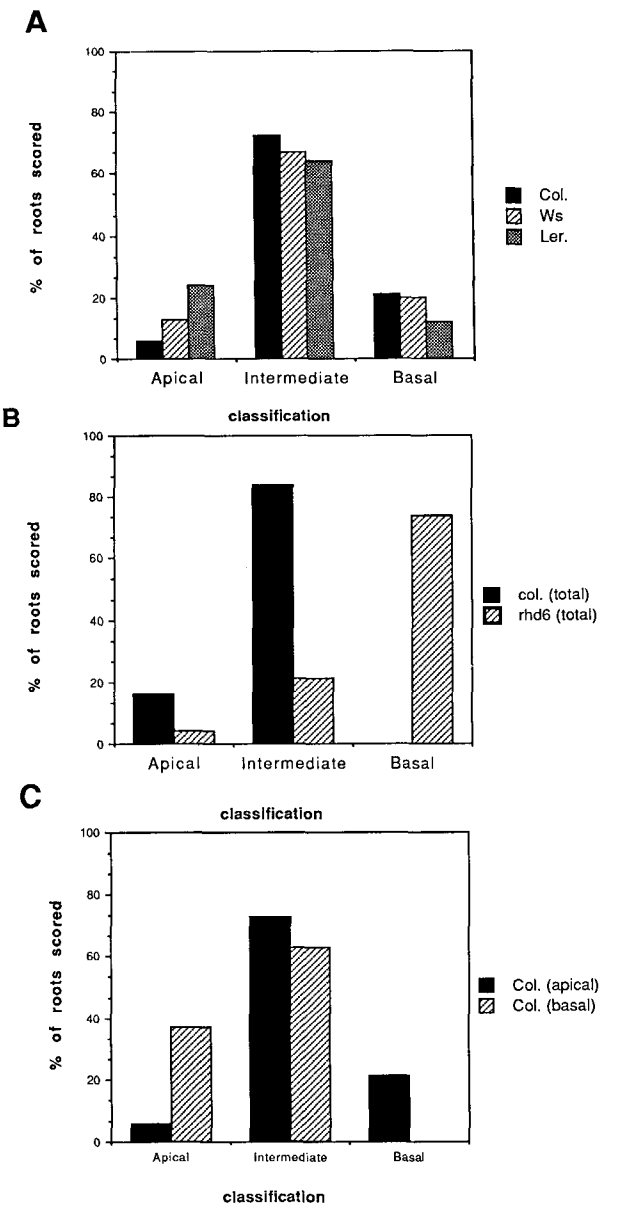

Figure 4. Distribution of roots classified according to average score for root-hair emergence for wild type and *rhd6* mutants. A, The distribution of roots from 5-d-old wild-type seedlings of the Columbia (Col., $n = 99$), WS (Ws, $n = 45$), and Landsburg erecta (Ler., $n = 25$) ecotypes classified as apical, intermediate, or basal, with respect to the average site of root-hair emergence in epidermal cells. B, The distribution of roots from 5-d-old Columbia (Col., filled bars, $n = 37$) or *rhd6* (rhd6, hatched bars, $n = 70$) seedlings, classified as apical, intermediate, or basal, with respect to average site of root-hair emergence in epidermal cells. Root hairs throughout the length of the roots were scored. The distribution of *rhd6* mutant roots is significantly different from the Columbia root distribution ($P < 0.01$). C, The distribution of roots classified for the average site of root-hair emergence in two different regions of the roots in 5-d-old Columbia seedlings is shown. Root hairs from cells near the apical end of the root [Col. (apical), filled bars, $n = 99$] or basal end of the root [Col. (basal), hatched bars, $n = 30$] were scored. The two distributions are significantly different ($P < 0.01$).

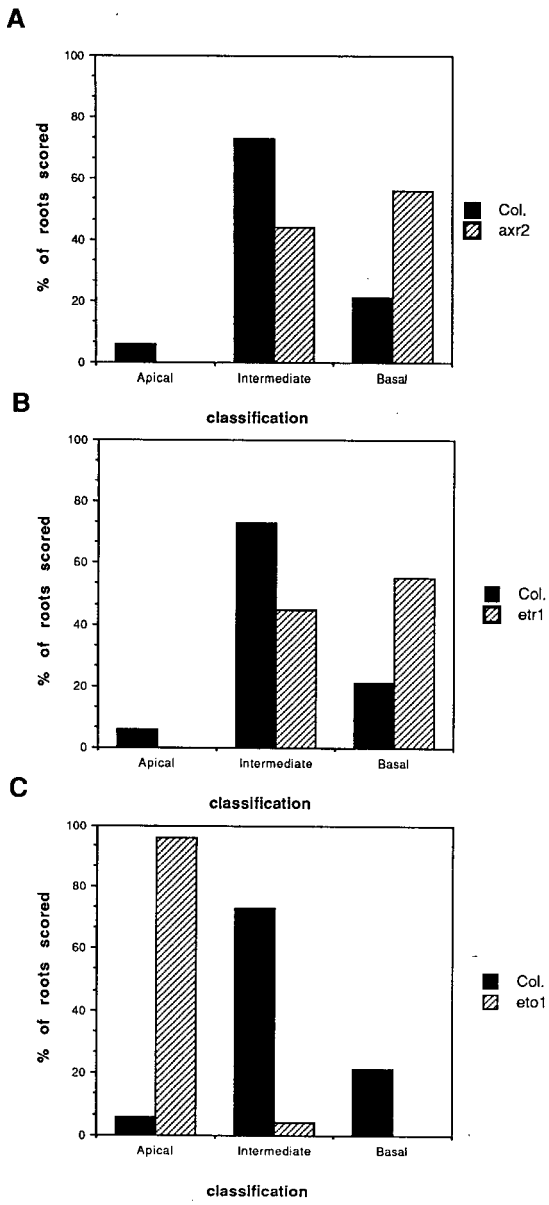

Figure 5. Distribution of roots classified according to average site of root-hair emergence for Columbia wild type and *axr2*, *etr1*, and *eto1* mutants. A, The distribution of roots of 5-d-old Columbia (Col., filled bars, $n = 99$) or *axr2* mutant seedlings (axr2, hatched bars, $n = 25$) is shown. The distribution of mutant roots is significantly different from the Columbia distribution ($P < 0.01$). B, The distribution of roots of 5-d-old Columbia (Col., filled bars, $n = 99$) or *etr1* mutant seedlings (etr1, hatched bars, $n = 22$) is shown. The distribution of *etr1* mutant roots is significantly different from the Columbia distribution ($P < 0.01$). C, The distribution of roots of 5-d-old Columbia (Col., filled bars, $n = 99$) or *eto1* mutant seedlings (eto1, hatched bars, $n = 28$) is shown. The distribution of *eto1* mutant roots is significantly different from the Columbia distribution ($P < 0.01$).

concentration of NPA was sufficient to perturb gravitropic growth. As shown in Figure 6B, growth on NPA did alter the distribution of roots classified with respect to root-hair emergence ($P < 0.01$), but the altered distribution was not clearly a basal shift. These results indicate that ethylene is required for normal root-hair production and probably acts in a process similar to the *RHD6* gene product, but the polar transport of auxin may have little effect on root-hair formation.

The *rhd6* Mutant Can Perceive and Respond to Externally Applied Auxin and ACC

Because the *rhd6* mutant and the hormone-resistant mutants *axr2*, *eto1*, and *etr1* alter the site of root-hair emergence and AVG can phenocopy the *rhd6* mutant phenotype, it is possible that the *rhd6* mutation impairs the cells' ability to respond to auxin and/or ethylene. To test this, *rhd6* mutants were assayed for their response to the exogenously applied auxins IAA and 2,4-D and the ethylene precursor ACC (Adams and Yang, 1979). Seedlings were grown on A.T. agarose plates for 3 d and then transferred to A.T. agarose plates containing various concentrations of auxin or ACC. The increase in root length was measured after 2 d on the supplemented media and compared to seedlings transferred to nonsupplemented medium. As shown in Figure 7, IAA and ACC inhibited the growth of *rhd6* mutant roots and Columbia wild-type roots to a similar extent. (2,4-D gave similar results but these are not shown.) The *rhd6* mutants also exhibited normal sensitivity to ethylene (A. Bleecker, personal communication). Therefore, the *rhd6* mutation has no discernible effect on the root's ability to perceive and respond to exogenously applied auxins, ACC, or ethylene.

Auxin and ACC Rescue the *rhd6* Mutant Phenotypes

During the course of the hormone-response experiments described above, both auxin and ACC were found to rescue the *rhd6* root-hair-density phenotype. As shown in Table I, *rhd6* mutant plants grown on A.T. agarose supplemented with 1×10^{-8} M IAA produced 51% of the relative number of root hairs as wild-type plants grown under the same conditions (compared to 18% of wild type when grown on unsupplemented A.T. agarose). Complete rescue of the root-

Table II. *Root-hair production in axr2 mutant and R-expressing roots grown on A.T. agarose with or without IAA or ACC*

Values represent the mean ± SD numbers of root hairs per millimeter, and the number of roots scored is shown in parentheses.

Genotype	Media Supplement		
	None[a]	3×10^{-8} M IAA	5×10^{-6} M ACC
axr2	14 ± 7 (43)	12 ± 7 (44)	19 ± 6 (24)
906[b]	2 ± 6 (42)	9 ± 8 (29)	18 ± 17 (5)
1411[b]	1.5 ± 2 (28)	1.5 ± 2 (23)	1 ± 1 (14)
1434[b]	0 ± 1 (44)	0 ± 1 (33)	3 ± 4 (11)

[a] Unsupplemented A.T. agarose. [b] *Arabidopsis* lines expressing the maize R cDNA (Lloyd et al., 1992).

hair-density phenotype occurred when the IAA concentration was increased to 3×10^{-8} M (Table I). When ACC was supplemented at 5×10^{-7} M, the relative number of root hairs from *rhd6* mutant seedlings was 62% of the number from wild type grown under the same conditions. Increasing the ACC concentration to 5×10^{-6} M increased the root-hair production in mutant seedlings to 78% of wild type. The root hairs produced in each of these experiments exhibited a normal morphology. These results imply that the application of auxin or ethylene can suppress the root-hair formation defects of *rhd6*.

It was possible that IAA and ACC suppressed the *rhd6* phenotype by causing epidermal cells that would normally be hairless to produce root hairs (e.g. ectopic hair production). To examine this, transverse sections of seedling roots were analyzed. As shown in Table III, hair-bearing cells from both mutant and wild-type roots grown on A.T. agarose supplemented with 3×10^{-8} M IAA or 5×10^{-6} M ACC were located in the appropriate positions (over radial cortical cell walls).

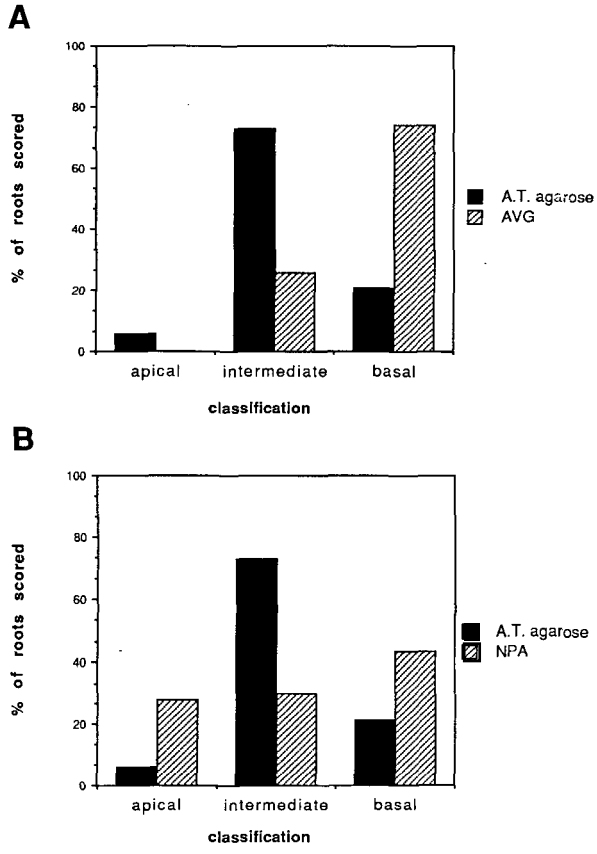

Figure 6. The distribution of roots classified according to average site of root-hair emergence for Columbia wild-type grown on medium containing NPA or AVG. A, The distribution of roots of 5-d-old Columbia seedlings grown on A.T. agarose (n = 99) or A.T. agarose supplemented with 10^{-6} M AVG (n = 39). The distributions of roots grown under these conditions are significantly different ($P < 0.01$). B, The distribution of roots of 5-d-old Columbia seedlings grown on A.T. agarose (n = 99) or A.T. agarose supplemented with 10^{-5} M NPA (n = 40). The distributions of roots grown under these conditions are significantly different ($P < 0.01$).

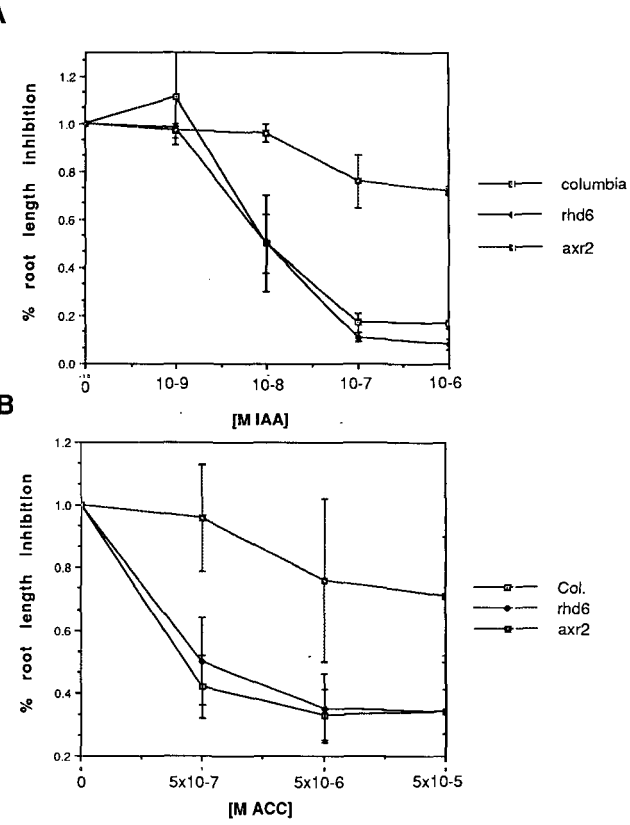

Figure 7. Response of *rhd6* mutant plants to auxin and the ethylene precursor ACC. Seedlings were grown on A.T. agarose plates for 3 d and then transferred to A.T. agarose plates containing various concentrations of auxin (A) or ACC (B). The increase in root length was measured 2 d after transfer to the supplemented media and compared to growth when transferred to unsupplemented medium. The root length inhibition equals the increase in root length measured on supplemented A.T. agarose divided by the increase in root length measured on A.T. agarose. Open boxes represent the Columbia wild type, black diamonds represent the *rhd6* mutants, and the closed boxes represent the *axr2* mutants. The data points are the averages of two trials with the end points of the error bars representing the value points for each trial. Approximately 10 seedlings were scored for each trial.

In addition, the hair-bearing cells on roots from the ethylene overproducing mutant *eto1* were present in normal positions. These results indicate that, at the concentrations used, auxin and ACC do not affect the normal positional controls governing epidermal cell fate, but they do suppress the root-hair production defect caused by the *rhd6* mutation.

The ability of IAA and ACC to rescue the other *rhd6* phenotypes, abnormal sites of root-hair emergence and multiple initiation sites per cell, were examined. These phenotypes were assayed using concentrations of IAA and ACC that did not significantly reduce epidermal cell length. Both IAA and ACC led to an apical shift in the site of root-hair emergence in both wild-type and *rhd6* mutant seedlings (Fig. 8). The apical shift caused by 1×10^{-8} M IAA resulted in distributions on wild-type and mutant roots that did not differ significantly. In addition, multiple hairs were observed

Table III. *The position of trichoblasts with respect to the underlying cortical cells*

Genotype	Media Supplement	Trichoblast Position	
		Normal[a]	Abnormal[b]
Columbia	3×10^{-8} M IAA	120	9 (11)
rhd6	3×10^{-8} M IAA	121	6 (11)
Columbia	5×10^{-6} M ACC	48	2 (7)
rhd6	5×10^{-6} M ACC	63	2 (8)
eto1	None[c]	96	3 (5)

[a] Trichoblasts observed over radial cortical cell walls. [b] Trichoblasts observed over transverse cortical cell walls; the number of roots examined is in parentheses. [c] Unsupplemented A.T. agarose.

in only 4 of 154 (3%) trichoblasts in *rhd6* mutant plants grown on 1×10^{-8} M IAA. Figure 8B shows that 5×10^{-7} M ACC is as effective ($P > 0.33$) as 1×10^{-8} M IAA in altering the site of root-hair emergence in wild-type seedlings but had less of an effect on the *rhd6* mutant. Only a small but significant ($P = 0.014$) apical shift in the distribution of *rhd6* mutant roots was detected in the presence of 5×10^{-7} M ACC compared to *rhd6* seedlings grown on unsupplemented media (cf. Fig. 8B to Fig. 4B). The addition of ACC to the medium also reduced the number of *rhd6* trichoblasts with multiple hairs from 13 to 6% (12 of 194 scored trichoblasts). The ability of auxin and ACC to rescue the various *rhd6* mutant phenotypes is consistent with the hypothesis that *RHD6* acts in an auxin- and ethylene-associated process to establish and/or respond to cell polarity during root-hair initiation.

To determine whether *rhd6* is rescued specifically by IAA and ACC, *rhd6* mutant seedlings were grown in the presence of other plant hormones. The presence of GA_3 at concentrations up to 1.2×10^{-4} M had no significant effect on root-hair production in *rhd6* mutant seedlings (Table IV). A small effect on root-hair production was observed with kinetin, but a 100-fold increase in kinetin concentration only increased the relative *rhd6* root-hair number from 30% of wild type to 50% of wild type (Table IV). Because cytokinin can induce the production of ethylene (Lau and Yang, 1973) and can act synergistically with auxin to increase ethylene levels (Kondo et al., 1975; Yu et al., 1981), it is possible that the kinetin-induced suppression of the *rhd6* phenotype is indirect.

Plants bearing other mutations that affect root-hair formation were grown in the presence of IAA and ACC to determine whether these hormones can rescue the defects in other root-hair mutants. The *axr2* mutation, which confers resistance to ethylene, auxin, and ABA (Wilson et al., 1990), blocks root-hair formation. Transgenic *Arabidopsis* seedlings expressing the maize R cDNA (Lloyd et al., 1992) also lack root hairs (Galway et al., 1994). When *axr2* mutant seedlings or seedlings harboring the R-expressing transgene were grown on medium supplemented with either 3×10^{-8} M IAA or 5×10^{-6} M ACC, root-hair production was not enhanced (Table II). Therefore, it appears that IAA and ACC specifically rescue the *rhd6* phenotype and do not affect root-hair production in other mutants.

DISCUSSION

The initiation of root hairs is similar in many respects to budding of the yeast *S. cerevisiae*. In yeast, the bud site is determined, the components necessary for restricting growth are assembled at the bud site, cytoskeletal elements are organized at the bud site, and then polar growth is initiated. Mutations affecting each process cause a different mutant phenotype. Mutations in genes affecting bud site determination, which involves the perception of and response to cell polarity, result in a normal number of buds but in random places (Chant and Herskowitz, 1991). Blocks in bud site assembly result in the lack of buds or misplaced buds (Sloat et al., 1981; Adams and Pringle, 1984; Adams et al., 1990). Mutations altering cytoskeletal components produce variable phenotypes resulting from delocalized growth, disrupted mitotic spindles, or aberrant organelle transport. For example, mutations in the yeast actin and myosin genes, which are thought to be involved in organelle transport, lead to the

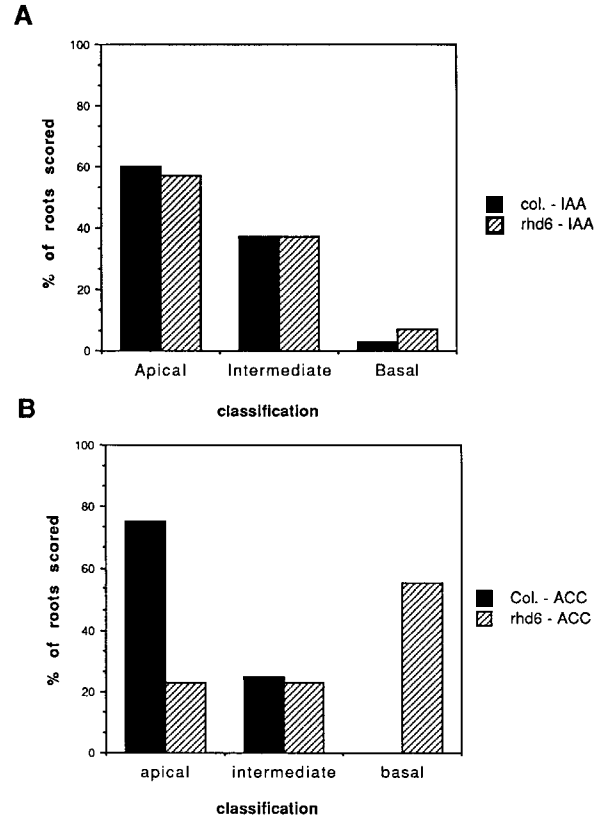

Figure 8. Distribution of roots classified according to average site of root hair emergence for Columbia wild type and *rhd6* mutants grown on IAA or ACC supplemented medium. A, The distribution of roots of 5-d-old Columbia (col., filled bars, $n = 30$) or *rhd6* mutant seedlings (rhd6, hatched bars, $n = 30$) grown on A.T. agarose supplemented with 1×10^{-8} M IAA is shown. There was no significant difference between the distribution of roots from the two genotypes. B, The distribution of roots of 5-d-old Columbia (Col., filled bars, $n = 32$) or *rhd6* mutant seedlings (rhd6, hatched bars, $n = 40$) grown on A.T. agarose supplemented with 5×10^{-7} M ACC is shown. See text for statistical comparisons.

Table IV. *Root-hair density, epidermal cell length, and relative root-hair number of Columbia wild-type and rhd6 mutant roots grown on various media*

Genotype	Media Supplement	Root-Hair Density[a]	Cell Length[b]	Relative Hair Number[c]
			mm	
Columbia	kin[d] (1×10^{-7} M)	43 ± 11 (15)	0.12 ± 0.01	5.2
rhd6	kin (1×10^{-7} M)	11 ± 8 (27)	0.19 ± 0.03	2.1
Columbia	kin (1×10^{-5} M)	41 ± 10 (30)	0.10 ± 0.04	4.1
rhd6	kin (1×10^{-5} M)	13 ± 8 (34)	0.16 ± 0.04	2.1
Columbia	GA_3 (3×10^{-5} M)	30 ± 5 (16)	0.20 ± 0.03	6.0
rhd6	GA_3 (3×10^{-5} M)	3 ± 3 (27)	0.19 ± 0.03	0.6
Columbia	GA_3 (1.2×10^{-4} M)	36 ± 7 (15)	0.18 ± 0.02	6.5
rhd6	GA_3 (1.2×10^{-4} M)	4 ± 4 (30)	0.20 ± 0.03	0.8

[a] Values represent the mean ± SD numbers of root hairs per millimeter; the number of roots scored is shown in parentheses. [b] Values represent the mean ± SD lengths of root-epidermal cells. [c] Values represent the products of root hair density and cell length. [d] kin, Kinetin.

formation of large, unbudded cells (Novick and Botstein, 1985; Johnston et al., 1991).

Predictions may be made regarding the phenotypes of mutant plants with defects at various stages of root-hair initiation using yeast budding as a model. Plants with mutations affecting the determination of the initiation site might be expected to have a normal number of root hairs that are randomly placed along the epidermal cells. Mutations affecting the assembly of initiation components might cause a reduced number of root hairs, and those root hairs that form might be misplaced. Mutations affecting cytoskeletal components may be expected to affect root-hair number and enlargement and, therefore, cause shortened or malformed root hairs.

The *rhd6* mutant phenotype reported here most closely resembles that predicted for mutations affecting the assembly of initiation components. Roots of *rhd6* plants produce a reduced number of root hairs, and those formed generally initiate at more basal sites than wild-type root hairs. In addition, mutant epidermal cells frequently initiate multiple hairs, an event that rarely occurs in wild type. Root hairs are not produced at random, however, indicating that cell polarity still influences *rhd6* root-hair initiation. Also, the *rhd6* mutation does not affect the morphogenesis of the root hair itself because initiated root hairs develop normally, and the *rhd6* mutation does not alter the basic mechanism specifying cell fate in the root epidermis. Therefore, *RHD6* is most likely required for the assembly of the cellular components at the root-hair initiation site in differentiating trichoblasts.

Our results show that ethylene and auxin are involved in root-hair formation in wild-type *Arabidopsis*. First, *axr2* and *etr1* mutant plants exhibit a basal shift in the site of root-hair emergence and *eto1* mutant plants exhibit an apical shift in root-hair emergence. Second, the addition of either auxin or the ethylene precursor ACC to the growth medium rescues the *rhd6* mutant phenotypes. Finally, wild-type seedlings grown on medium containing AVG, an inhibitor of ethylene biosynthesis, exhibit the same phenotypes as the *rhd6* mutants grown on unsupplemented medium.

In previous studies, ethylene has been shown to promote root-hair formation in pea, faba bean, and lupine (Borgstrom, 1939, as referenced by Abeles et al., 1992), as well as in tulip, which normally lacks root hairs (De Munk and De Rooy, 1971). At the concentrations used in this study, ACC did not enhance root-hair formation in wild-type *Arabidopsis* plants, although it did influence the site of root-hair initiation. Thus, ethylene may normally serve to regulate root-hair formation in many plant species. The phenocopy of the *rhd6* mutant phenotype by AVG-treated wild-type seedlings is consistent with a role for ethylene in the assembly of the root-hair initiation complex. Although the precise role of auxin in root-hair initiation is not clear, auxin has been implicated in the loosening of cell walls (Vanderhoef and Dute, 1981), the altered expression of cell wall components (Nishitani and Masuda, 1983; Ray, 1985), and the polarization of tissues (Sachs, 1984, 1991).

The plant hormones affect a variety of developmental processes and have been the subject of years of research. However, the mechanisms by which they act are still understood poorly. The apparent association among auxin, ethylene, and the newly described *rhd6* mutation indicates that the *RHD6* gene product may be useful as a tool to probe the mechanism of hormone action. In addition, the *rhd6* mutation will be beneficial for the study of root epidermal cell differentiation at both the molecular and genetic levels. An important future goal will be to determine whether the *RHD6* gene product affects the levels of auxin and/or ethylene, affects the ability of the epidermal cells to respond to auxin and/or ethylene, or acts in a pathway that is parallel to an auxin and/or ethylene pathway in the root epidermis.

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LITERATURE CITED

Abeles FB, Morgan PW, Saltveit ME (1992) Ethylene in Plant Biology, Ed 2. Academic Press, New York

Adams A, Pringle J (1984) Localization of actin and tubulin in wild type and morphogenic-mutant *Saccharomyces cerevisiae*. J Cell Biol **98:** 934–945

Adams DO, Yang SF (1979) Ethylene biosynthesis: identification of ACC as an intermediate in the conversion of methionine to ethylene. Proc Natl Acad Sci USA **76:** 170–174

Adams WEM, Johnson DI, Longnecker RM, Sloat BF, Pringle JR (1990) CDC42 and CDC43, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. J Cell Biol **111:** 131–142

Beyer EM Jr (1972) Auxin transport. A new synthetic inhibitor. Plant Physiol **50:** 322–327

Bleecker AB, Estelle MA, Somerville C, Kende H (1988) Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. Science **241:** 1086–1089

Borgstrom G (1939) Theoretical suggestions regarding the ethylene responses of plants and observations on the influence of apple-emanations. Kungl Fsiografiska Sallskapets I Lund Forhandlingar **9:** 135–174

Buchner D, Pilet PE (1983) Auxin effects on root growth and ethylene production. Experientia **39:** 493–494

Chant J, Herskowitz I (1991) Genetic control of bud site selection in yeast by a set of gene products that constitute a morphogenetic pathway. Cell **65:** 1203–1212

De Munk WJ, De Rooy M (1971) The influence of ethylene on the defelement of 5C-precooled Apeldorn tulips during forcing. Hortscience **6:** 40–41

Dolan L, Janmaat K, Willemsen V, Linstead P, Poethig S, Roberts K, Scheres B (1993) Cellular organisation of the *Arabidopsis thaliana* root. Development **119:** 71–84

Drubin DG (1991) Development of polarity in budding yeast. Cell **65:** 1093–1096

Edwards JI, Saltveit ME, Henderson WR (1983) Inhibition of lycopene synthesis in tomato pericarp tissue by inhibitors of ethylene biosynthesis and reversal with applied ethylene. J Am Soc Hortic Sci **108:** 512–514

Estelle MA, Somerville C (1987) Auxin-resistant mutants of *Arabidopsis thaliana* with an altered morphology. Mol Gen Genet **206:** 200–206

Feldmann KA, Marks MD (1987) *Agrobacterium*-mediated transformation of germinating seeds of *Arabidopsis thaliana*: a non-tissue culture approach. Mol Gen Genet **208:** 1–9

Fowke LC, Rennie PJ, Constabel F (1983) Organelles associated with the plasma membrane of tobacco leaf protoplasts. Plant Cell Rep **2:** 292–295

Galway ME, Hardham AR (1986) Microtubule reorganization, cell wall synthesis, and establishment of the axis of elongation in regenerating protoplasts of the alga *Mougeotia*. Protoplasma **135:** 130–143

Galway ME, Masucci JD, Lloyd AM, Walbot V, Davis RW, Schiefelbein JW (1994) The TTG gene is required to specify epidermal cell fate and cell patterning in the *Arabidopsis* root. Dev Biol **166:** (in press)

Geneve RL, Hackett WP, Swanson BT (1989) Root initiation in debladed petioles from juvenile and mature English ivy in response to inhibitors of ethylene biosynthesis and action. J Am Soc Hortic Sci **115:** 128–131

Goodner B, Quatrano RS (1993) *Fucus* embryogenesis: a model to study the establishment of polarity. Plant Cell **5:** 1471–1481

Guzman P, Ecker JR (1990) Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. Plant Cell **2:** 513–523

Hahne G, Hoffmann F (1985) Cortical microtubular lattices: absent from mature mesophyll and necessary for cell division? Planta **166:** 309–313

Johnston GC, Prendergast JA, Singer RA (1991) The *Saccharomyces cerevisiae* MYO_2 gene encodes an essential myosin for vectorial transport of vesicles. J Cell Biol **13:** 539–551

Keitt GW, Baker RA (1966) Auxin activity of substituted benzoic acids and their effect on polar auxin transport. Plant Physiol **41:** 1561–1569

Kondo K, Watanabe A, Imaseki H (1975) Relationships in actions of indoleacetic acid, benzyladenine, and abscisic acid in ethylene production. Plant Cell Physiol **16:** 1001–1007

Koornneef M, Hanhart CJ, Van Loenen Martinet EP, Ban Der Been J H (1987) A marker line that allows the detection of linkage on all *Arabidopsis* chromosomes. Arabidopsis Inf Serv **24:** 46–50

Kropf DL (1992) Establishment and expression of cellular polarity in Fucoid zygotes. Microbiol Rev **56:** 316–339

Lau O-L, Yang SF (1973) Mechanism of a synergistic effect of kinetin on auxin-induced ethylene production. Suppression of auxin conjugation. Plant Physiol **51:** 1011–1014

Lloyd AM, Walbot V, Davis RW (1992) *Arabidopsis* and *Nicotiana* anthocyanin production activated by maize regulators R and C1. Science **258:** 1773–1775

Lincoln C, Estelle M (1990) Growth and development of the *axr1* mutant of *Arabidopsis*. Plant Cell **2:** 1071–1080

Morgan PW, Gausman HW (1966) Effects of ethylene on auxin transport. Plant Physiol **41:** 45–52

Nishitani K, Masuda Y (1983) Auxin-induced changes in cell wall xyloglucans. Plant Cell Physiol **24:** 345–355

Novick P, Botstein D (1985) Phenotypic analysis of temperature-sensitive yeast actin mutants. Cell **40:** 405–416

Pickett FB, Wilson AK, Estelle M (1990) The *aux1* mutation of *Arabidopsis* confers both auxin and ethylene resistance. Plant Physiol **94:** 1462–1466

Ray PM (1985) Auxin and fusicoccin enhancement of β-glucan synthase in peas. An intracellular enzyme activity apparently modulated by proton extrusion. Plant Physiol **78:** 466–472

Rengel Z, Kordan HA (1987) Effects of growth regulators on light-dependent anthocyanin production in *Zea mays* seedlings. Physiol Plant **69:** 511–516

Sachs T (1984) Axiality and polarity in vascular plants. *In* PW Barlow, DJ Carr, eds, Positional Controls in Plant Development. Cambridge University Press, Cambridge, UK, pp 193–224

Sachs T (1991) Cell polarity and tissue patterning in plants. Development **1** (Suppl): 83–93

Schiefelbein JW, Galway ME, Masucci JD, Ford SK (1993) Pollen tube and root-hair tip growth is disrupted in a mutant of *Arabidopsis thaliana* Plant Physiol **103:** 979–985

Schiefelbein JW, Shipley A, Rowse P (1992) Calcium influx at the tip of growing root-hair cells of *Arabidopsis thaliana*. Planta **187:** 455–459

Schiefelbein JW, Somerville C (1990) Genetic control of root hair development in *Arabidopsis thaliana*. Plant Cell **2:** 235–243

Schnepf E (1986) Cellular polarity. Annu Rev Plant Physiol **37:** 23–47

Sievers A, Schnepf E (1981) Morphogenesis and polarity of tubular cells with tip growth. Cell Biol Monogr **8:** 265–299

Sloat BF, Adams A, Pringle J (1981) Roles of the CDC24 gene

product in cellular morphogenesis during the *Saccharomyces cerevisiae* cell cycle. J Cell Biol **89:** 395–405

Vanderhoef LN, Dute RR (1981) Auxin-regulated wall loosening and sustained growth in elongation. Plant Physiol **67:** 146–149

Wilson AK, Pickett FB, Turner JC, Estelle M (1990) A dominant mutation in *Arabidopsis* confers resistance to auxin, ethylene and abscisic acid. Mol Gen Genet **222:** 377–383

Wood BW (1985) Effect of ethephon on IAA transport, IAA conjugation, and antidotal action of NAA in relation to leaf abscission of pecan. J Am Soc Hortic Sci **110:** 340–343

Yu Y-B, Yang SF, Corse J, Kuhnle JA, Hua SS (1981) Structures of cytokinins influence synergistic production of ethylene. Phytochemistry **20:** 1191–1195

Zimmerman PW, Lieberman M, Broome OC (1977) Inhibitory effect of a rhizobitoxine analog on bud growth after release from dormancy. Plant Physiol **59:** 158–160