Plant, Cell and Environment (2001) **24**, 459–467

Regulation of root hair density by phosphorus availability in *Arabidopsis thaliana*

Z. MA, D. G. BIELENBERG, K. M. BROWN & J. P. LYNCH

Department of Horticulture, The Pennsylvania State University, 103 Tyson Building, University Park, PA 16802, USA

ABSTRACT

We characterized the response of root hair density to phosphorus (P) availability in *Arabidopsis thaliana*. *Arabidopsis* plants were grown aseptically in growth media with varied phosphorus concentrations, ranging from 1 mmol m^{-3} to 2000 mmol m^{-3} phosphorus. Root hair density (number of root hairs per mm of root length) was analysed starting at 7 d of growth. Root hair density was highly regulated by phosphorus availability, increasing significantly in roots exposed to low-phosphorus availability. The initial root hairs produced by the radicle were not sensitive to phosphorus availability, but began to respond after 9 d of growth. Root hair density was about five times greater in low phosphorus (1 mmol m^{-3}) than in high phosphorus (1000 mmol m^{-3}) media. Root hair density decreased logarithmically in response to increasing phosphorus concentrations within that range. Root hair density also increased in response to deficiencies of several other nutrients, but not as strongly as to low phosphorus. Indoleacetic acid (IAA), the auxin transport inhibitor 2-(*p*-chlorophenoxy)-2-methylpropionic acid (CMPA), the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), and the ethylene synthesis inhibitor aminooxyacetic acid (AOA) all increased root hair density under high phosphorus but had very little effect under low phosphorus. Low phosphorus significantly changed root anatomy, causing a 9% increase in root diameter, a 31% decrease in the cross-sectional area of individual trichoblasts, a 40% decrease in the cross-sectional area of individual atrichoblasts, and 45% more cortical cells in cross-section. The larger number of cortical cells and smaller epidermal cell size in low phosphorus roots increased the number of trichoblast files from eight to 12. Two-thirds of increased root hair density in low phosphorus roots was caused by increased likelihood of trichoblasts to form hairs, and 33% of the increase was accounted for by changes in low phosphorus root anatomy resulting in an increased number of trichoblast files. These results show that phosphorus availability can fundamentally alter root anatomy, leading to changes in root hair density, which are presumably important for phosphorus acquisition.

Keyword: *Arabidopsis thaliana*; phosphorus; root hair density.

Correspondence: Dr Jonathan Lynch. E-mail: JPL4@psu.edu

INTRODUCTION

Low soil phosphorus availability is a primary constraint to plant growth on earth (Lynch & Deikman 1998). In terrestrial ecosystems, phosphorus is progressively lost from soil through weathering, and reactions with various soil constituents substantially reduce phosphorus bioavailability to plants (Frossard *et al*. 1995). Plants display an array of physiological responses to phosphorus availability (Raghothama 1999), including morphological and architectural responses of the root system that may affect phosphorus acquisition (Lynch 1995; Lynch & Beebe 1995). Responses that influence the spatial exploitation of the soil are particularly important for the acquisition of phosphorus, which is relatively immobile in most soils.

Root hairs are subcellular extensions from the root epidermis that facilitate the uptake of immobile nutrients such as phosphorus by increasing the absorptive surface area of the root and allowing the root to explore a greater soil volume. The importance of root hairs in the acquisition of phosphorus has been shown by the observation of the depletion zones of ^{32}P around root hairs (Lewis & Quirk 1967; Bhat & Nye 1974). Research shows that among barley cultivars, greater absorption of soil phosphorus is associated with longer and denser root hairs (Gahoonia & Nielsen 1997). Recent comparisons of wild-type and hairless *Arabidopsis* mutants showed the importance of hairs for phosphorus uptake (Bates & Lynch, 2000a).

Many plant species exhibit increased extension of root hairs in response to phosphorus deficiency (Foehse & Jungk 1983; Bates & Lynch 1996). The response of root hairs to low-phosphorus availability may be an important strategy for enhanced phosphorus acquisition when phosphorus availability is low. In *Arabidopsis*, root hairs grow longer and more dense under low phosphorus, and phosphorus uptake per unit root length is increased with longer root hairs (Bates 1998; Bates & Lynch, 2000b). It is not clear what changes in root anatomy are associated with these responses.

In *Arabidopsis*, the root epidermis consists of discrete files of hair-bearing and hairless cells (Dolan 1996). Epidermal cells lying over the anticlinal wall separating two cortical cells differentiate into cells that normally bear root hairs (trichoblasts), whereas those located over the outer periclinal cortical cell walls differentiate into hairless cells (atrichoblasts) (Dolan *et al*. 1994; Galway *et al*. 1994). At an early stage of epidermal development, cells in the meris-

tematic region differentiating into trichoblasts can be distinguished from those developing into atrichoblasts by their relatively dense cytoplasm. During later stages of epidermal development, the two differentiating cell types differ in their rate of vacuolation, in the extent of elongation, and eventually in the presence or absence of root hairs (Dolan *et al*. 1994; Galway *et al*. 1994).

Many factors may affect the development of cell types, including signals from the environment, internal genetic programmes, and the positions of cells relative to each other (Masucci & Schiefelbein 1996). One model suggests that epidermal cells positioned over the space between the underlying cortical cell files produce hairs because they are exposed to relatively high levels of endogenous ethylene or its precursor 1-aminocyclopropane-1-carboxylic acid (ACC), which induce root hair production (Dolan 1996). Plants grown on high concentrations of the ethylene precursor ACC develop more root hairs occurring in positions normally occupied by atrichoblasts (i.e. ectopic root hairs) (Tanimoto, Roberts & Dolan 1995), whereas plants grown in the presence of either the ethylene synthesis inhibitor (AVG) or the ethylene perception antagonist (Ag^{+}) have fewer root hairs (Masucci & Schiefelbein 1994; Tanimoto *et al*. 1995). Auxin and the ethylene precursor ACC have been shown to restore normal root hair density in the hairless mutant *rhd6* (Masucci & Schiefelbein 1994). Plant hormones may play an important role in modulating the number and the length of root hairs in response to various environmental conditions (Masucci & Schiefelbein 1996).

Arabidopsis root hairs grow longer and more dense in response to low-phosphorus availability (Bates & Lynch 1996), a response that would increase phosphorus uptake in natural soils (Bates & Lynch, 2000a; b). The mechanism by which the density increases is not known. The goals of this research were: (1) to characterize the response of root hair density to phosphorus availability in *Arabidopsis*; (2) to test the interaction of phosphorus availability with auxin and ethylene on root hair density; and (3) to investigate potential anatomical alterations associated with the change of root hair density in response to phosphorus availability.

MATERIALS AND METHODS

Plant material

Arabidopsis thaliana L. (Heynh) 'Columbia' ecotype obtained from the Ohio State University Arabidopsis Biological Resource Center was used in these experiments.

Plant culture

The growth media contained 3 mol m^{-3} KNO_3, 2 mol m^{-3} $Ca(NO_3)_2$, 0·5 mol m^{-3} $MgSO_4$, 25 mmol m^{-3} KCl, 12·5 mmol m^{-3} H_3BO_3, 1 mmol m^{-3} $MnSO_4$, 1 mmol m^{-3} $ZnSO_4$, 0·25 mmol m^{-3} $CuSO_4$, 0·25 mmol m^{-3} $(NH_4)_6Mo_7O_{24}$, 25 mmol m^{-3} Fe-EDTA; 0·55 mol m^{-3} myo-inositol, 2·5 mol m^{-3} MES, 29·2 kmol m^{-3} sucrose, and 2 g L^{-1} Phytagel (Sigma Chemical Co., St. Louis, MO, USA)(Bates & Lynch 1996). Media were prepared by adding the stock solutions of the above salts and the organic components in appropriate amounts to Millipore-filtered water. The pH of the media was adjusted to 5·7 with dilute KOH before Phytagel was added. For media of varied phosphorus concentrations, $NH_4H_2PO_4$ was added to give the targeted phosphorus concentration. For low phosphorus (1 mmol m^{-3}) media $(NH_4)_2SO_4$ was used to replace $NH_4H_2PO_4$, with no additional source of phosphorus. The Phytagel concentration used here contains approximately 1 (± 0·5) mmol m^{-3} of phosphorus in the final media as determined by the phosphomolybdate method (Watanabe & Olsen 1965).

Media for the nutrient deficiency experiments were prepared by substituting a complementary salt for the nutrient to be removed. Background concentrations of nutrients (B, Cu, Fe, K, Mg, Mn, Zn) found in Phytagel represent less than 5% of the nutrient concentrations normally added as a constituent of the nutrient media (Bates & Lynch 1996).

All growth media were autoclaved and seeds were surface-sterilized and sown on the solidified media in Petri dishes. Plates were incubated in a plant culture room with constant light (40 μmol photons m^{-2} s^{-1}) and temperature (25 °C) in a horizontal orientation for 3–4 d until the roots reached the bottom of the plates. Plates were then placed at an angle of 45°, so that subsequent root growth occurred along the bottom of the dish (Bates & Lynch 1996).

To investigate root hair response to growth regulators, plants were grown as previously described in either low- or high-phosphorus media. After 12 d of growth, 10 mL of new medium containing either indoleacetic acid (IAA), the auxin transport inhibitor 2-(*p*-chlorophenoxy)-2-methylpropionic acid (CMPA), ACC, or the ethylene synthesis inhibitor amino-oxyacetic acid (AOA) was added to the existing medium. The concentration of the added compound was calculated on the basis of a final volume of 30 mL medium. The added compounds were assumed to reach homogenous distribution within the 30 mL of solution within 15 min, as previously reported in this system (Bates & Lynch 1996).

Root hair density measurements

A time-course experiment was conducted to determine where and when reliable differences in root hair density between phosphorus treatments could be observed. Root hair density was determined as the number of hairs in each of the five apical 1 mm segments of root observed with a microscope (40×). Root hairs were counted three-dimensionally with the adjustment of the microscope's plane of focus. On the basis of the results from the time course experiment, root hair density was subsequently evaluated at 10 d after planting, except in the growth regulator and inhibitor experiment, for which root hair density was counted 3 d after the addition of desired compounds at 10 d of growth. In all experiments, six plants were measured for each treatment.

Microscopy and fluorescence

Root samples taken from plants grown under either low- or high-phosphorus conditions for 10 d were fixed and embedded for cross-sectioning. Segments 2–3 mm from root tips were excised and fixed in 4% glutaraldehyde in 0·1 M phosphate buffer (pH 7·4) for 1 h at room temperature. Roots were then washed three times for 5 min each with 0·1 M phosphate buffer (pH 7·4). Washed roots were incubated for 2 h at room temperature in 1% osmium tetroxide in 0·1 M phosphate buffer (pH 7·4) and kept in a dark box for 2 h at room temperature. Roots were again washed for 5 min in 0·1 M phosphate buffer (pH 7·4), followed by two 5 min washes in distilled and deionized water. Subsequently, the roots were dehydrated in a graded ethanol series and 100% acetone, then embedded in Spurr's resin (Hayat 1989). Cross-sections were made and dried on glass slides, stained briefly with 1% toluidine blue, washed, and observed with a microscope (Hayat 1989).

Root samples were also taken for staining with the fluorescent dye 2′,7′-*bis*(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF; Molecular Probes Inc., Eugene, OR, USA) in its acetoxymethyl ester form (i.e. BCECF-AM, 5 μM). BCECF-AM loads preferentially into trichoblast files over atrichoblast files of the living roots (Bates 1998). Dye-loaded roots were viewed on a Nikon Diaphot inverted microscope (Nikon, Garden City, New Jersey, USA) with a dry Fluor X20 objective lens (Nikon). The excitation and emission wavelengths were set at 505 ± 8 and 535 ± 8 nm, respectively, obtained from a 75 W xenon arc lamp with scanning monochrometers (Photon Technology International, South Brunswick, New Jersey, USA), and separated by a 515 nm dichroic mirror. BCECF fluorescence was isolated through a 535 ± 25 nm bandpass filter (Nikon). A Hammamatsu Photonics C2400 intensifier (Hammamatsu, Japan, setting = 9·0) was used to enhance the intensity of the fluorescence emission. Fluorescence images of trichoblast files of low- and high-phosphorus roots were acquired with a Hammamatsu Photonics XC-77 charged-couple device (CCD) camera, and captured on the computer screen with software Metamorph (Universal Imaging Co., West Chester, PA, USA).

Image analysis

Metamorph was employed to measure the length of trichoblasts and cortical cells from non-fluorescent dye-loaded root images. Prior to determining cell length, roots were first boiled in 5% KOH for 20 min, then rinsed in deionized water and acidified in 1% HCl for 1 h. Roots were then boiled in Trypan Blue for another 1 h, then rinsed and destained with a solution consisting of the staining solution without the Trypan Blue. This procedure allows for a relatively good view of cell walls under the microscope. To measure cortical cell length, microscopic focus was carefully adjusted to show the cortical layer. To determine the areas of different cell types, image analysis was performed using PhotoFinish (version 1·02, ZSoftCorporation, Palo Alto, CA, USA) and Delta-T scan (version 2·0, Delta-T Devices Ltd, Cambridge, UK). PhotoFinish was used to separate images of individual cells by 'cutting' them out one by one from the cross-sectional image, and the images of the single cells were pasted and saved in individual files. Delta-T scan was then used to quantify the areas of cells of different cell types.

Data analysis

Statistical analyses of the data were conducted using SAS (Statistical Analysis Systems Institute 1982). Randomized-block analysis of variance (ANOVA) and Waller–Duncan K-ratio *t*-test were used to compare root hair density data of different treatments measured from each root segment over the time course. Completely randomized ANOVA was used when analysing data on root hair response to nutrient deficiencies, and on anatomical features (cell length of trichoblasts, cortical cell number, areas of individual trichoblasts, atrichoblasts, cortical cells, and areas of steles). Regression analysis was performed to study the effect of growth regulators on changes in root hair density. A *P*-value of 0·05 or less was considered to be statistically significant.

RESULTS

Phosphorus availability regulates root hair density in *Arabidopsis*

Root hair density was highly regulated by phosphorus availability, increasing significantly in roots exposed to low-phosphorus availability (Fig. 1). The root hairs produced during the first week of radicle growth were of equal density regardless of phosphorus availability, but by 9 d after planting, root hair density had declined under high phosphorus and increased under low phosphorus (Fig. 1). Within phosphorus levels, root hair density of the third, fourth, and fifth segments did not vary significantly after 9 d of plant growth. We therefore used the third segment, i.e. 3 mm from the root tip (solid columns in Fig. 1) for subsequent evaluations of root hair density.

Over a broad range of phosphorus concentrations, root hair density decreased logarithmically with increasing phosphorus availability in the media (Fig. 2). At 2000 mmol m^{-3} phosphorus, there was almost no root hair growth. The increase in root hair density as phosphorus concentration declined was not due to reduced radicle elongation rate, since the radicle elongation rate was reduced by only 28% in the absence of phosphorus compared with 1000 mmol m^{-3} phosphorus, whereas root hair density was increased more than five-fold (data not shown).

Specificity of root hair density response to phosphorus availability

Root hair density was uniquely sensitive to phosphorus deficiency as opposed to that of other nutrients (Fig. 3). Phosphorus deficiency caused a five times increase in root

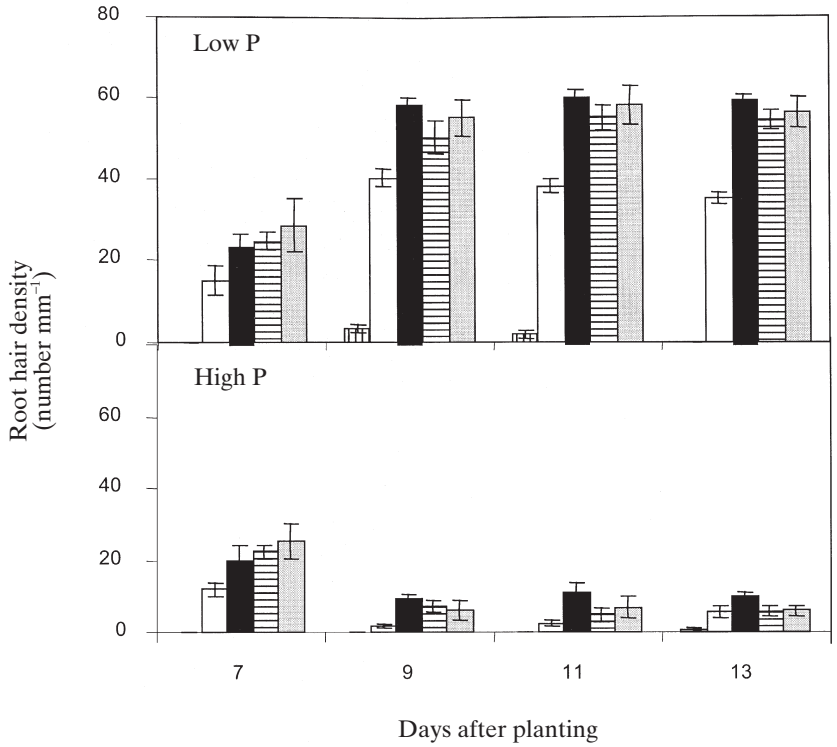

Figure 1. Root hair density of wild-type *Arabidopsis* plants through time. Plants were grown in either low phosphorus (1 mmol m^{-3}) or high phosphorus (1000 mmol m^{-3}) media, and root hair density was measured over time on five 1 mm root segments starting from the root tip. Within time points, the five bars from left to right represent 0–1, 1–2, 2–3, 3–4, and 4–5 mm root segments from the tip. Each bar represents the mean of six plants, and error bars represent SE. ANOVA indicates a significant effect of phosphorus treatment on root hair densities starting from day 9 (F = 164·03, P = 0·0001).

hair density in comparison with control (1000 mmol m^{-3} P). Deficiencies of Fe and Zn increased root hair density by 64 and 71%. Deficiencies of Mn increased root hair density by 2·2 times. Calcium, Mg, B, and Cu deficiencies had no significant effect on root hair density, whereas deficiencies in S, K, or N caused a reduction in root hair density.

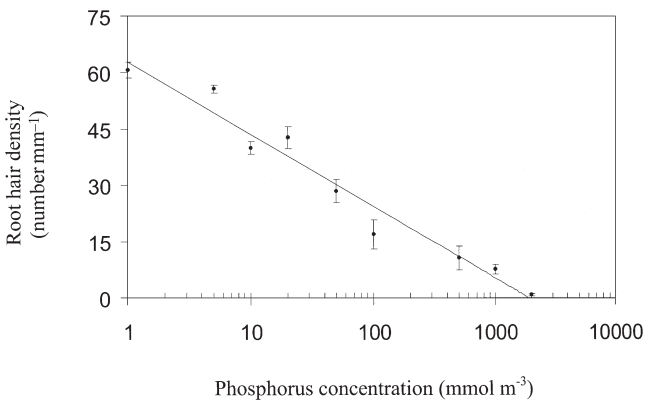

Figure 2. Phosphorus availability regulates root hair density in *Arabidopsis*. Root hair density decreased linearly as phosphorus availability increased logarithmically in the growth media. The x-axis is on a log scale and the solid line is a regression line (Density = –8·31 ln(concentration) + 62·73, R^2 = 0·961). Each data point represents the mean of six plants, and error bars represent SE.

Involvement of auxin and ethylene in the root hair density response to phosphorus availability

There was a significant effect of auxin (IAA) on root hair density of plants grown in either high- or low-phosphorus media, and of an auxin transport inhibitor (CMPA) on hair density of plants grown in low-phosphorus media. Root hair density in plants grown in high-phosphorus media increased more than three-fold in response to exogenously applied IAA, whereas the root hair density of low-phosphorus plants increased only slightly. CMPA induced a 15% decrease in root hair density in low-phosphorus roots (Fig. 4).

ACC increased root hair density in plants grown in high-phosphorus media, but had no effect on plants grown in low-phosphorus media. AOA treatment less than 1 mmol m^{-3} increased root hair density in plants grown under high phosphorus, but had no significant effect on root hair density of plants grown under low phosphorus (Fig. 4).

Root hair density as related to anatomical alterations in roots

Plants grown in low- or high-phosphorus media had approximately equal trichoblast length (Table 1), but trichoblast length under Mn deficiency was 124·25 ± 1·6 μm, a reduction of about 46%**.** Cross-sections showed that roots grown in low-phosphorus media were about 9% larger in diameter than roots from high-phosphorus media (Fig. 5, Table 1). Low phosphorus significantly changed root

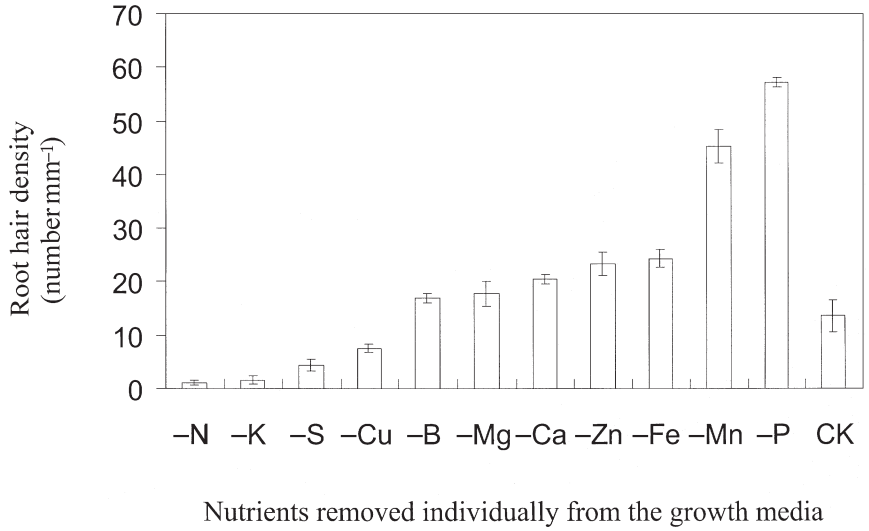

Figure 3. *Arabidopsis* root hair density responds to nutrient removal from the growth media. Root hair density was most sensitive to phosphorus deficiency as opposed to that of other nutrients. Each bar represents the mean of six plants, and error bars represent SE. *P*-values from ANOVA for each of the nutrients are: –N: 0·0029 ($F = 17·92$); –K: 0·004 ($F = 15·45$); –S: 0·018 ($F = 8·71$); –Cu: 0·085 ($F = 3·86$); –B: 0·3 ($F = 1·22$); –Mg: 0·3 ($F = 1·24$); –Ca: 0·05 ($F = 5·15$); –Zn: 0·029 ($F = 7·00$); –Fe: 0·014 ($F = 9·95$); –Mn: 0·0001 ($F = 54·87$); –P: 0·0001 ($F = 164·03$).

anatomy, causing a 31% decrease in the cross-sectional area of individual trichoblasts, a 40% decrease in the cross-sectional area of individual atrichoblasts, without affecting the cross-sectional area of individual cortical cells (Fig. 6). Low phosphorus increased the average number of trichoblast files from 8·3 to 12 (Table 1). As the number of cortical cells in low-phosphorus roots was greater, there were more epidermal cells located over the junction of cortical cells, where trichoblasts normally occur (Fig. 5, Table 1). Fluorescent imaging of living roots confirmed that low-phosphorus roots developed more trichoblast files under phosphorus stress (Fig. 7).

Low phosphorus increased the total cross-sectional area of the roots by 18%, the stele by 47%, and the cortex by 33% (Figs 5 & 8). Epidermal tissue (trichoblasts and atrichoblasts) occupied a smaller fraction of the root cross-sectional area under low phosphorus (Fig. 8).

DISCUSSION

Arabidopsis plants grown in low-phosphorus media produced five times greater root hair density than those in high-phosphorus media. Root hair densities did not differ between phosphorus treatments at 7 d after germination, but after that, root hair density increased on low-phosphorus roots and declined on high-phosphorus roots, leading to a large and persistent difference in density (Fig. 1). Although roots elongated 1·6 times faster under high phosphorus than low phosphorus, the rate of root hair production was 3·5 times greater under low phosphorus (data not shown). The effect of phosphorus on root hair density did not appear until 9 d after germination. The increasing response to external phosphorus availability could represent a developmental sensitization to cues received by the root epidermal cells from its environment and neighbouring cells.

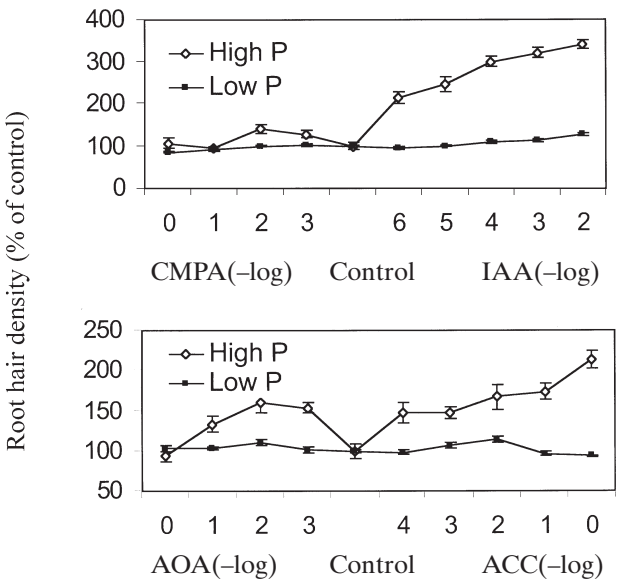

Figure 4. *Arabidopsis* root hair density as affected by the interaction of phosphorus availability and growth regulators, or their inhibitors. Plants were grown in either low (1 mmol m^{-3}) or high-phosphorus (1000 mmol m^{-3}) media, to which new media containing IAA, CMPA, ACC, or AOA was added. Root hair densities were measured 3 d later. The concentration of growth regulators/inhibitors is on a negative log scale. No growth regulators or inhibitors were added to the media of control plants, which had average root hair densities of 60 for low-phosphorus roots and 15 for high-phosphorus roots. Each data point represents the mean of six plants, and error bars represent SE (error bars not shown where their size is smaller than that of the symbols). Regression analysis shows that under high phosphorus, for IAA: $R^2 = 0·74$, $P < 0·0001$; for CMPA: $R^2 = 0·23$, $P < 0·03$; for ACC: $R^2 = 0·45$, $P < 0·0002$; and for AOA: $R^2 = 0·55$, $P < 0·0002$. Under low phosphorus: for IAA: $R^2 = 0·69$, $P < 0·0001$; for CMPA: $R^2 = 0·62$, $P < 0·0001$; for ACC: $R^2 = 0·07$, $P < 0·20$; and for AOA: $R^2 = 0·0014$, $P < 0·88$.

Table 1. Calculated and observed root hair density of low- and high-phosphorus roots. Theoretic root hair density is calculated from 1 mm/trichoblast length × number of trichoblast files × (1 root hair/trichoblast), and percentage of trichoblasts not elongated is calculated by [(theoretic root hair density – observed root hair density)/theoretic root hair density] × 100%

	High P	Low P	*F*-value	*P*-value
Length of trichoblasts (μm)	181·6 (3·68)	171·3 (4·03)	3·58	0·08
Length of cortical cells (μm)	170·3 (2·99)	150·3 (1·86)	32·23	0·0001
Number of trichoblasts per cross-section	8·3 (0·51)	12 (1·26)	43·21	0·0001
Theoretic root hair density (number of root hairs/1 mm root segment)	46	70		
Observed root hair density (number of root hairs/1 mm root segment)	11 (2·4)	63 (3·1)	164·03	0·0001
Percentage of trichoblasts not elongated	76%	10%		
Root diameter (μm)	123·23 (0·56)	133·90 (0·17)	5·36	0·042

Measured values are means of six plants, followed by standard error in parentheses.

Image analysis showed that the cross-sectional areas of individual trichoblasts and atrichoblasts, as well as the total areas occupied by these components differed in low-phosphorus and high-phosphorus roots (Figs 6 & 8). Since there are more cortical cells under low phosphorus, there are more sites where trichoblasts may develop, thereby permitting production of more root hairs, which would facilitate phosphorus acquisition. It would be interesting to know the cause of these anatomical changes associated with increased root hair density under low phosphorus. It was found that low-phosphorus availability increased ethylene production in bean roots (Borch *et al*. 1999). Auxin was previously shown to mimic the elongation response of root hairs to low phosphorus (Bates & Lynch 1996). There is reason to believe that ethylene and auxin are involved in regulating root hair densities in response to phosphorus (Lynch & Brown 1997). IAA increased both root hair density (Fig. 4) and root hair elongation (Bates & Lynch 1996) up to three-fold under high phosphorus but only slightly under low phosphorus, when density and length are already high. The IAA transport inhibitor CMPA, which would be expected to have the opposite effects to IAA, did produce a dramatic decrease in root hair elongation (Bates & Lynch 1996) at low phosphorus, but only slightly (15%) decreased root hair density. Under high phosphorus, most CMPA concentrations increased root hair density (Fig. 4) but had little effect on length (Bates & Lynch 1996). The CMPA results are inconclusive, as the response was small and showed a complex dose–response relationship. Although CMPA is an auxin transport inhibitor, it is difficult to know what its effects might be on auxin concentration along the root axis. The IAA results are consistent with a role for auxin in root hair density and elongation.

One way in which auxin might act could be to increase ethylene production. Ethylene is known to increase root hair length and density and is often produced in response to high auxin concentrations (Abeles, Morgan & Saltveit 1992). Comparison of the effects of IAA and ACC on root hair density (Fig. 4) reveals a similar pattern of strong stimulation only in roots grown in high phosphorus. These compounds may not have affected root hair density of low-phosphorus plants because the response was already saturated in those plants. The absolute values for root hair density at the highest IAA or ACC concentration were still lower than control (no IAA or ACC) values for low-phosphorus plants. In high-phosphorus plants, IAA and ACC may have increased root hair density via a mechanism similar to or distinct from the mechanism utilized in the low-phosphorus situation.

If ethylene is important for mediating the low-phosphorus response, then AOA should have reduced root hair density. However, it had very little effect (Fig. 4). This suggests that low phosphorus does not act via ethylene, and that ACC (or IAA, via ethylene production) could increase root hair density at high phosphorus via a separate mechanism. We speculate that phosphorus stress may trigger some

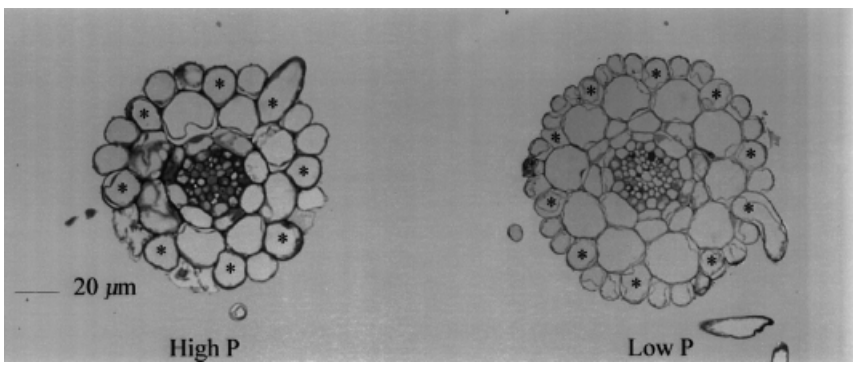

Figure 5. Cross-section images of low- and high-phosphorus roots from the apical third mm segment (magnification: 400×). Asterisks indicate trichoblasts.

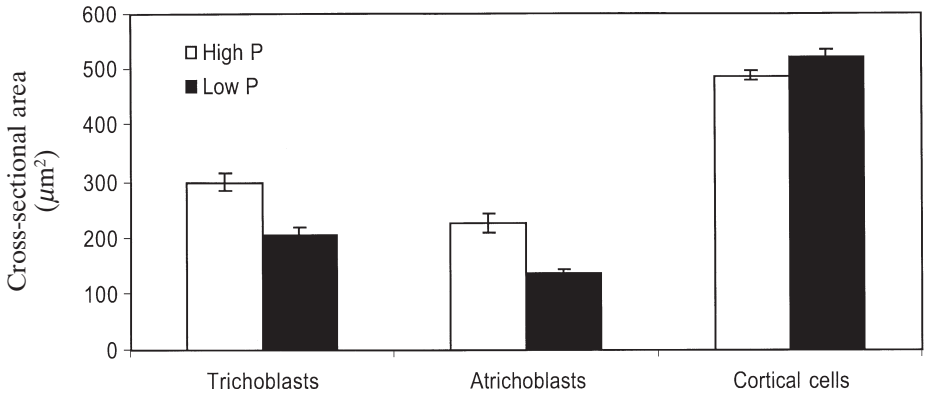

Figure 6. Cross-sectional areas of the individual cells of different tissue types in *Arabidopsis* roots grown in high and low phosphorus. Each bar represents the mean area of 60 cells, 10 from each of six plants, and error bars represent SE. ANOVA indicates significant differences in areas of trichoblasts ($F = 20{\cdot}56$, $P = 0{\cdot}0007$) and atrichoblasts ($F = 23{\cdot}88$, $P = 0{\cdot}0064$), and no difference in areas of cortical cells ($F = 2{\cdot}74$, $P = 0{\cdot}12$), between phosphorus treatments.

other signal transduction pathway that in turn causes anatomical changes in the ontogeny of root hairs.

Root hair density decreased logarithmically with increasing phosphorus availability in the media (Fig. 2). Deficiencies in other nutrients either had little effect or caused very poor root hair growth and even early death, as in the case of nitrogen deficiency. Only manganese (Mn) deficiency substantially increased root hair density (Fig. 3). However, trichoblast length decreased by 46% under Mn deficiency, suggesting that the primary effect of Mn deficiency on root hair density occurred via reduced root elongation.

Several possible mechanisms may account for the increase in root hair density under low phosphorus. First, increased density may arise from a decrease in trichoblast length, which may cause root hairs to stack more tightly and appear more dense. Secondly, since trichoblasts form in cell files in *Arabidopsis*, it is possible that more trichoblast files may be initiated. Thirdly, the occurrence of trichoblasts with multiple root hairs (i.e. ectopic hairs) may contribute to increased root hair density. Fourth, atrichoblasts may be recruited into trichoblasts during development under phosphorus stress. Finally, it is possible that the initiation of new trichoblast files, the occurrence of ectopic root hairs, and the recruitment of atrichoblasts into trichoblasts may occur in combination.

Measurements of the lengths of trichoblasts from which root hairs arise indicate that trichoblast lengths were approximately equal in high- and low-phosphorus plants (Table 1), suggesting other causes for increased root hair density in low-phosphorus plants. Root cross-sections and images of fluorescent dye loaded roots show that there are more trichoblast files under phosphorus stress (Fig. 5 and Fig. 7). No ectopic hairs were observed under high phosphorus, but under low phosphorus we would not easily have seen them because root hairs were so dense. Ectopic root hair development could have contributed to the increase in root hair density.

Root hair densities of low- and high-phosphorus roots

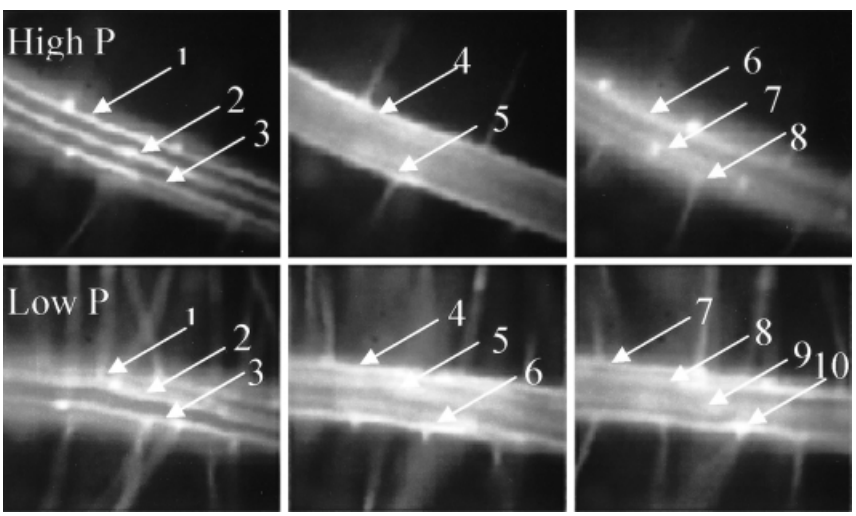

Figure 7. Fluorescence images of high-phosphorus and low-phosphorus roots loaded with 5 μM BCECF-AM showing root hair cell files. Root hair cell files load more quickly and more intensely than non-root hair cell files. By capturing a series of longitudinal 'z'-sections, a three-dimensional image stack can be created that allows the counting of cell files. The three images in the top/bottom panel represent three visually distinct planes from high-phosphorus and low-phosphorus roots and show that low-phosphorus roots have more root hair cell files than high-phosphorus roots.

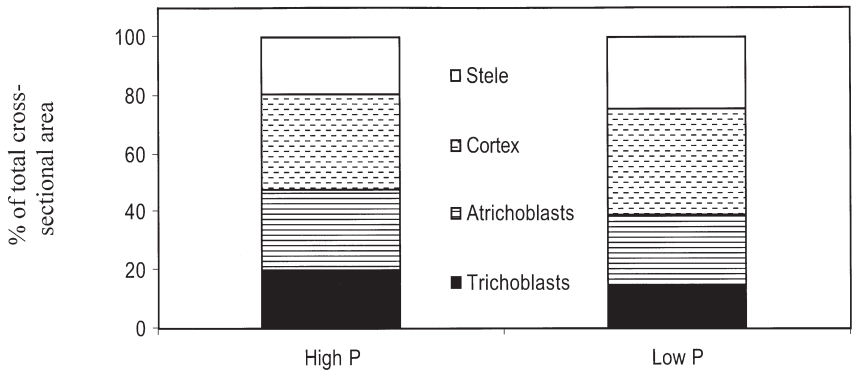

Figure 8. Relative distribution of the total areas of different tissue types in high- and low-phosphorus roots. Values are calculated from means of six roots. ANOVA indicates significant differences in the areas occupied by cortex ($F = 51{\cdot}14$, $P < 0{\cdot}0001$) and stele ($F = 14{\cdot}82$, $P < 0{\cdot}0001$) between high- and low-phosphorus treatments. For atrichoblasts, $F = 0{\cdot}09$, $P = 0{\cdot}7732$; and for trichoblasts, $F = 3{\cdot}38$, $P = 0{\cdot}09$.

calculated from trichoblast length and the number of trichoblast files should be 70 and 46 per mm root length, respectively (Table 1). However, the observed root hair densities were 63 and 11 per mm root length for low- and high-phosphorus roots, respectively, suggesting that not all of the trichoblasts developed root hairs under either low- or high-phosphorus conditions. About 10% of the trichoblasts did not form root hairs under low phosphorus, whereas 76% of the trichoblasts did not form root hairs under high phosphorus (Table 1). Therefore, the initiation of new trichoblast files in low phosphorus and the failure of most trichoblasts to form root hairs in high phosphorus both contributed to the increase in root hair density under low phosphorus.

It is interesting that there were some trichoblasts that did not form root hairs regardless of external phosphorus availability, and that additional trichoblast files developed under low-phosphorus conditions to produce increased root hair density, even though roots have the potential of having root hair density as high as 46 per mm root length even under high phosphorus (Table 1). One advantage associated with the development of additional trichoblast files rather than development of root hairs on 100% of trichoblasts may be that individual root hairs within trichoblast files would not be too close together, minimizing competition for nutrients.

The development of additional trichoblast files resulted from the increased number of cortical cells in plants grown under low phosphorus (Fig. 5). This increased the number of epidermal cell files located at the radial (anticlinal) walls of the underlying cortical cells, positions associated with trichoblast formation (Dolan 1996). This change in anatomy must have been determined during differentiation of these cells in the meristem. Previous work showed that the number of cortical cells was stable (at eight cells in radial section) along the length of the root and in the mutants *rdh6* (impaired root hair production, rescued by IAA or ACC), *axr2* (auxin, ethylene, and ABA insensitive, fewer root hairs), and *ctr1* (constitutive ethylene response, ectopic root hairs) (Dolan *et al*. 1994; Masucci & Schiefelbein 1994; Masucci & Schiefelbein 1996). In addition, treatments with ACC and AVG did not change the number of cortical cells (Masucci & Schiefelbein 1996). This indicates that low phosphorus has a specific effect on meristem differentiation and cell patterning that is distinct and independent of the known effects of ethylene, auxin, and described root hair mutants.

Low-phosphorus availability has been shown to increase root hair elongation and density (Bates & Lynch 1996; this paper). The increased surface area resulting from longer and more dense root hairs may be important in the acquisition of immobile phosphorus from the environment. Although high root hair density may lead to some competition for nutrients among root hairs, as root hairs become longer, they would be less likely to have overlapping zones of depletion. Therefore, increasing the density of root hairs may serve to allow the root to exploit a larger radius of influence around the root. Although the mechanics of response to phosphorus may be different between root hair elongation (a localized cell-level response) and density (a developmental response), together they represent a coordinated strategy to exploit phosphorus in the root environment. Plants may have to invest more energy to produce more and longer root hairs. However, a cost–benefit analysis from a recent study shows that wild-type *Arabidopsis* plant roots are able to acquire more phosphorus for every unit of carbon consumed under low-phosphorus availability (Bates & Lynch, 2000b). Therefore, root hair response in length and density appears to be an efficient strategy employed by plants to deal with low-phosphorus availability.

These results show that root hair density is highly regulated by phosphorus availability. Considering the role of root hairs in acquiring diffusion limited nutrients such as phosphorus in the soil, the regulation by phosphorus of root growth in terms of changes in root hair density and root hair length is of particular significance to the mineral nutrition of plants. The fact that only phosphorus appears to actively regulate root hair density through anatomical alterations (the effect of Mn being a secondary result of reduced root extension), suggests that phosphorus has unique importance in the evolution of root form and function, perhaps due to its prevalence as a constraint to plant growth (Lynch & Deikman 1998).

ACKNOWLEDGMENTS

The authors thank Rosemary Walsh and Michelle Peiffer for assistance with microscopic work, Steve Halperin for

help with plant culture and florescence imaging, Jennifer Tomscha, Dr Terrence Bates and Dr Gerardo Rubio for helpful discussions and suggestions. The authors would also like to thank Dr Terrence Bates for fluorescence images.

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Received 18 September 2000; received in revised form 20 December 2000; accepted for publication 20 December 2000