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THE EFFICIENCY OF *ARABIDOPSIS THALIANA* (BRASSICACEAE) ROOT HAIRS IN PHOSPHORUS ACQUISITION[1]

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Arabidopsis thaliana root hairs grow longer and denser in response to low-phosphorus availability. In addition, plants with the root hair response acquire more phosphorus than mutants that have root hairs that do not respond to phosphorus limiting conditions. The purpose of this experiment was to determine the efficiency of root hairs in phosphorus acquisition at high- and low-phosphorus availability. Root hair growth, root growth, root respiration, plant phosphorus uptake, and plant phosphorus content of 3-wk-old wild-type *Arabidopsis* (WS) were compared to two root hair mutants (*rhd6* and *rhd2*) under high (54 mmol/m^3) and low (0.4 mmol/m^3) phosphorus availability. A cost-benefit analysis was constructed from the measurements to determine root hair efficiency. Under high-phosphorus availability, root hairs did not have an effect on any of the parameters measured. Under low-phosphorus availability, wild-type *Arabidopsis* had greater total root surface area, shoot biomass, phosphorus per root length, and specific phosphorus uptake. The cost-benefit analysis shows that under low phosphorus, wild-type roots acquire more phosphorus for every unit of carbon respired or unit of phosphorus invested into the roots than the mutants. We conclude that the response of root hairs to low-phosphorus availability is an efficient strategy for phosphorus acquisition.

Key words: *Arabidopsis thaliana*; Brassicaceae; cost-benefit analysis; phosphorus; root hairs.

Plant root hairs are subcellular extensions from the root epidermis that are hypothetically important in the acquisition of immobile resources such as phosphorus. Several lines of indirect evidence have indicated the benefit of root hairs in phosphorus acquisition. Depletion of ^{32}P around roots shows that increased root hair length increases the size of the depletion zone around the root (Lewis and Quirk, 1967; Bhat and Nye, 1973). Comparisons of species with different root hair lengths suggest that plants with longer root hairs have greater phosphorus uptake (Bhat and Nye, 1974; Anghinoni and Barber, 1980; Foehse and Jungk, 1983; Misra, Alston, and Dexter, 1988; Nielsen et al., 1994). Differences in root hair length and consequently phosphorus uptake exist among different genotypes of the same species (Green, Beard, and Oprisko, 1991). Furthermore, models that predict phosphorus uptake in roots underestimate phosphorus uptake when root hairs are not included (Itoh and Barber, 1983). In contrast to these comparisons, a root hair mutant in corn shows normal growth and development, which has raised questions on the benefit of root hairs (Wen and Schnable, 1994).

In *Arabidopsis thaliana,* low-phosphorus availability increases root hair length by increasing root hair growth rate and growth duration, which is accompanied by increased root hair density in these plants (Bates and Lynch, 1996). Comparative growth analysis of wild-type *Arabidopsis* with two root hair mutant genotypes (*rhd6* and *rhd2*) in a range of phosphorus availabilities shows that root hairs are important in increasing plant phosphorus content at low-phosphorus availabilities (Bates and Lynch, unpublished data; Schiefelbein and Somerville, 1990; Masucci and Schiefelbein, 1994).

Root hairs would be considered efficient in phosphorus acquisition if the cost-benefit ratio were lower for a plant with root hairs than without root hairs. In a cost-benefit analysis, a finite supply of plant carbon (or other plant resource) is conservatively allocated to a particular plant organ or plant function for the purpose of acquiring more carbon (or other plant resource). In a nutrient-limiting environment, carbon may not be limiting plant growth, but rather the nutrient in question may be the primary limitation for plant growth (Arnone and Korner, 1995). Therefore, nutrient-stressed plants may expend an excess of relatively unlimited carbon for the acquisition of the limiting nutrient. The exchange rate for carbon and a nutrient will be different in a nutrient-limiting and nonlimiting condition (Bloom, Chapin, and Mooney, 1985; Koide and Elliott, 1989). With the use of root hair mutant genotypes, plants with and without root hairs can be compared within a phosphorus treatment and the efficiency of root hairs in phosphorus acquisition can be assessed, assuming no other plieotropic effects.

Low-phosphorus *Arabidopsis* root hairs have greater tip growth rate, and roots of those plants have increased total root surface area which indicates that low-phosphorus plants may expend energy or invest plant resources for the purpose of acquiring limiting phosphorus. The cost of root hairs, whether it is carbon expended or resource invested in either high- or low-phosphorus availability, is not known. Accurate assessment of the costs and benefits associated with root hairs will indicate the absolute importance or efficiency of root hairs in phosphorus acquisition.

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One measurement of root cost is total belowground expenditure of carbon, which is used for root construction, root maintenance, and ion uptake (Eissenstat and Yanai, 1997). Root costs can also be measured as the amount of nutrient investment in belowground organs (Reekie and Bazzaz, 1987; Koide and Elliott, 1989). In the case of *Arabidopsis,* the cost of root hairs can be measured by comparing CO_2 evolution or phosphorus investment in wild type and root hair mutants grown in high- and low-phosphorus availability.

The overall objective of this research was to determine the efficiency of root hairs in phosphorus acquisition at high- and low-phosphorus availability. The first goal was to measure the benefit of root hairs in phosphorus acquisition through tissue phosphorus analysis and phosphorus uptake in wild type and two root hair mutants of *Arabidopsis* (Krannitz, Aarssen, and Lefebvre, 1991a, b). The second goal was to determine the effect of root hairs on root surface area and phosphorus acquisition. The third goal was to measure the cost of root hairs by CO_2 evolution in roots or by phosphorus allocation to roots. A cost-benefit approach was used to determine the efficiency of root hairs in phosphorus acquisition.

MATERIALS AND METHODS

Plant material—*Arabidopsis thaliana* L. (Heynh) wild type (WS), *rhd6,* and *rhd2* mutants were obtained from the Ohio State University *Arabidopsis* Biological Resource Center. The *rhd6* and *rhd2* mutant lines are from the WS ecotype with phenotypic defects in root hair density (*rhd6*) or root hair elongation (*rhd2*) (Schiefelbein and Somerville, 1990; Masucci and Schiefelbein, 1994).

Growth conditions—*Arabidopsis* genotypes (WS, *rhd6,* and *rhd2*) were grown under three different experimental conditions for two purposes. First, sterile plant culture and hydroponics provided growth media that facilitated the measurements of root respiration and phosphorus uptake kinetics, which are difficult to obtain in soil conditions. Second, sand-alumina media provided phosphorus regimes that could be compared to plant growth and phosphorus uptake in the other growth conditions.

Root respiration—*Arabidopsis thaliana* plants were grown in media consisting of half-strength nutrient salts (Johnson et al., 1957), myo-inositol (0.55 mmol/L), MES buffer (2.5 mmol/L), sucrose (29.2 mol/L), and Phytagel (0.2%) dissolved in Millipore-filtered water and adjusted to a pH of 5.7 with dilute potassium hydroxide. For media deficient in phosphorus, ammonium sulfate was substituted for ammonium phosphate. High-phosphorus and low-phosphorus media were autoclaved for 15 min at 121°C and then 2 mL was pipetted onto Number 1, 24 × 60 mm cover slips (Corning Glass Works, Elmira, New York, USA). Seeds were surface sterilized by washing successively in 70% ethanol for 30 s, 20% commercial bleach, and Triton X-100 for 5 min, and then rinsed three times in sterile water. Seeds were sown on the solidified media, one seed per cover slip, placed in a sterile petri dish, and sealed with parafilm. Seeds were incubated for 3 wk at a 45° angle in a plant culture room with constant light (40 mmol photons·m^{-2}·s^{-1} photosynthetically active radiation) and temperature (25°C).

After 3 wk of growth, plants on cover slips were lifted out of the sterile petri dishes and placed on a Nikon Diphot inverted microscope (Melville, New York, 200× magnification). Root hair length, root hair width, root hair density, and root radius were measured with Metamorph image analysis software (Universal Imaging Corp., West Chester, Pennsylvania, USA).

For respiration measurements, plants were removed from the Phytagel and placed in a nutrient-solution-filled cylindrical plexiglass chamber (1.5 mL total volume) and measured with a CO_2 microelectrode. The chamber was constructed by joining four 5 × 5 cm square sheets of plexiglass together with silicone sealant (Dow Corning, Midland, Michigan, USA). A hole with a diameter of 1 cm was drilled into three of the four sheets. Sheet number 1, without a hole, served as the bottom of the chamber. Sheet number 2 was glued to sheet 1, and a small stirbar was placed in the drilled hole of sheet number 2. Between sheets 2 and 3, one layer of window screen was glued to separate the stirbar from the rest of the chamber. In sheet number 4, the top sheet, a groove radiating from the chamber hole was made to accommodate the CO_2 microelectrode tip. The respiration chamber was filled with nutrient solution of similar composition to the plant culture media without Phytagel or sucrose. The nutrient solution was adjusted to a pH of 5.0 before being pipetted into the chamber, and fresh nutrient solution was used for each replicate of the experiment. The lower 75% of the chamber was placed in a circulating water bath with a temperature of 23°C. The chamber and water bath were then placed onto a stir plate. A MI-720 Micro-Carbon Dioxide Electrode (Microelectrodes Inc., Bedford, New Hampshire, USA) was calibrated with solutions of 1 and 2% CO_2. After calibration, the electrode was placed into the groove of sheet number 4 so that the electrode tip was submerged into the nutrient solution of the respiration chamber. Millivolt readings from the electrode were monitored on an Orion 710A pH/concentration meter (Microelectrodes, Inc.). Electrode readings of freshly stirring nutrient solution in an open chamber were monitored for 10 min to confirm proper electrode operation. A ring of stopcock grease was placed around the open rim of the chamber and around the neck of the electrodes. A 3-wk-old plant was put into the chamber with the root in the nutrient solution, the shoot on the outside of the grease ring, and the stem gently submerged in the grease. A cover slip was then carefully placed on top of the grease ring to eliminate all air bubbles from the chamber. Millivolt readings from the electrode were recorded at 5-min intervals for 45 min. After 45 min, the chamber was opened, shoots were saved for phosphorus analysis, and roots were stained for root length measurements.

Phosphorus uptake kinetics—*Arabidopsis* plants were hydroponically grown in 2-mL microcentrifuge tubes (Fisher Scientific, Pittsburgh, Pennsylvania, USA) under high-phosphorus or low-phosphorus availability. Nutrient solution composition was the same as in plant tissue culture without Phytagel. Sterilization and growth conditions were also the same as the plant culture procedure. A total of 300 seeds, one seed per microcentifuge tube, were suspended in the nutrient solution with polyester batting, and plants were grown for 3 wk in microcentrifuge racks placed in sterile clear plastic containers.

To study ^{32}P uptake, ten treatment solutions were made in plastic containers containing 100 mL liquid nutrient media each. The treatment solutions were identical in pH and nutrient contents to the no-phosphorus growth media but contained unlabeled phosphorus concentrations ranging from 0.1 to 100 mmol/m^3 P. Each of the ten treatment solutions was supplemented with ^{32}P (ICN Pharmaceuticals, Inc., Costa Mesa, California, USA). One-millilitre samples of the radioactive treatment solutions were then taken to determine specific activity of the treatment solution prior to the experiment. Four replicates of each genotype and phosphorus growth condition (3 genotypes × 2 P levels × 4 replicates = 24 plants) were placed in a wire rack, and the plant roots were lowered into one of the ten treatment solutions for 5 min. To expose the root systems to the treatment solutions, the bottom tips of the microcentrifuge tubes were cut off. After the 5-min treatment, the plants were transferred to 200 mL 0.5 mmol/L $CaSO_4$ wash for 15 min. This procedure was repeated for all ten treatment solutions for a total of 240 treated plants. After the wash, individual plants were transferred to 3 mL of ScintiVerse (Fisher Scientific, St. Louis, Missouri, USA) in hanging scintillation vials and analyzed by liquid scintillation spectroscopy (Packard Tri Carb 1500 Liquid Scintillation Analyzer, Packard Instru-

TABLE 1. Root characteristics of 3-wk-old WS, *rhd6,* and *rhd2* plants in tissue culture.

P level	Genotype	Root hair length (μm)	Root hair width (μm)	Root hair density (no./mm)	Root width (μm)
High P	WS	157.8 b	6.4 b	48.0 d	94.0 a
	rhd6	100.0 b	6.9 bc	14.2 b	100.0 a
	rhd2	0.0 a	0.0 a	0.0 a	100.0 a
Low P	WS	562.0 d	6.6 bc	65.2 e	94.0 a
	rhd6	448.0 c	7.0 c	23.6 c	101.2 a
	rhd2	0.0 a	0.0 a	0.0 a	100.0 a
Significance					
P level		**	ns	**	ns
Genotype		**	**	**	ns
P level × Genotype		**	ns	**	ns

Note: ns = not significant, ** = significant at the 1% level. Numbers with different letters differ significantly at the 5% level by Duncan's significant difference.

ments Co., Downers Grove, Illinois, USA). Of the remaining 60 plants, 48 were collected and used for root length and phosphorus analysis.

Sand-alumina—A solid-phase-buffered growth medium was used to grow the three *Arabidopsis* genotypes at high (54 mmol/m^3) and low (1.0 mmol/m^3) phosphorus. This system employs kinetic equilibria to provide a buffered phosphorus supply in a manner that mimics phosphorus availability in natural soil (Lynch et al., 1990). The seeds were sown on the solid phase sand-alumina phosphorus buffering media and drip irrigated with one tenth strength nutrient salts (Johnson et al., 1957) minus phosphorus. For zero-phosphorus nutrient solution, ammonium sulfate salt was substituted for ammonium phosphate and no additional source of phosphorus was used. Plants were automatically irrigated for 1 min each day from a common nutrient solution reservoir. Therefore, the source of each of the two phosphorus treatments came from the 1% (w/v) of alumina within each individual pot.

Root and shoot measurements—For each phosphorus × genotype treatment, shoots were separated from roots and placed in microcentrifuge tubes and dried at 60°C for 24 h. Roots were placed in microcentrifuge tubes filled with neutral red stain (0.016 g/L) to prepare them for scanning. After 1 h of staining, roots from each genotype and phosphorus treatment were spread out in a petri dish of deionized water with tweezers, digitally scanned (Deskscan II, Hewlett Packard Co., Palo Alto, California, USA), and root lengths were measured using Delta-T Scan software (Delta-T Devices LTD, Cambridge, UK). Each group of five root systems was then dried at 60°C for 24 h. Biomasses of dried shoots and roots were measured on a microbalance (Sartorius, Edgewood, New York, USA) and recorded. Roots and shoots were ashed in a muffle furnace (Thermolyne 48000, Debuque, Iowa, USA) at 495°C for 5 h and total phosphorus was measured using the Murphy and Riley reagents for phosphorus determination (Watanabe and Olsen, 1965).

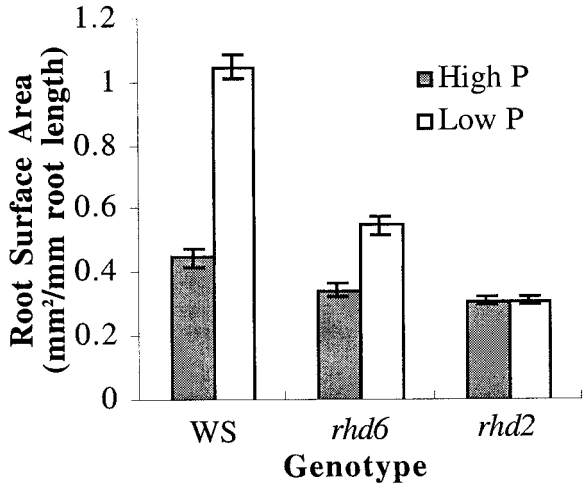

Fig. 1. Total root surface area per millimetre root of three *Arabidopsis* genotypes grown at two phosphorus availabilities. Low-phosphorus availability increased root surface area by increasing root hair length and density. $N = 10$. Bars indicate ± 1 SE.

Experimental design—Respiration, phosphorus uptake, and plant growth experiments were analyzed in a 3 × 2 factorial design with three *Arabidopsis* root hair genotypes and two phosphorus treatments. There were a total of five replicate plants for each factor combination, with the exception of the radioactive phosphorus uptake experiment, which had four replicates. A two-factor analysis of variance (ANOVA) was conducted, and data were checked for normality by plotting residuals vs. predicted values.

RESULTS

Nutrient solution vs. sand-alumina analysis—Three-week-old *Arabidopsis* plants grown in nutrient solution or sand-alumina media were compared for root morphology, plant biomass, and tissue phosphorus content. Root hair length and density were greater in WS plants than *rhd6* plants at both phosphorus treatments; in addition, low-phosphorus availability increased root hair length and density in both WS and *rhd6* genotypes (Table 1). In both high- and low-phosphorus availability, the *rhd2* genotype was devoid of elongated root hairs. Root hair width and root width were not significantly influenced by phosphorus availability or genotype.

Root and root hair data were used to calculate root surface area in a 1-mm segment of root length (Fig. 1). The *rhd2* surface area represents the surface area of the root cylinder without root hairs. The root surface area of WS and *rhd6* genotypes was greater than the *rhd2* mutant because of the addition of root hair surface area. Low-phosphorus availability had a greater effect than high-phosphorus availability on root surface area because of its effect on root hair length and density. Root surface area of WS high-phosphorus plants was similar to the root surface area of *rhd6* low-phosphorus plants.

Root length and shoot biomass were measured in 3-wk-old plants to compare overall plant growth in nutrient solution and sand-alumina media. High-phosphorus plants had greater root length and shoot biomass than low-phosphorus plants (Tables 2 and 3). However, among

TABLE 2. Root growth, shoot growth, and phosphorus status of 3-wk-old high-phosphorus-grown WS, *rhd6,* and *rhd2* plants.

Media	Genotype	Root length (mm/plant)	Shoot biomass (mg/plant)	Total plant P/root length (nmol P/mm root)	Total plant P/root surface area (nmol P/mm² root)
Nutrient solution	WS	582.0 b	3.43 ab	11.7 a	26.1 a
	rhd6	602.5 b	3.95 ab	11.7 a	33.6 a
	rhd2	541.5 b	2.83 a	13.5 a	42.9 a
Sand-alumina	WS	323.7 a	4.54 b	13.0 a	28.9 a
	rhd6	352.7 a	4.02 ab	10.5 a	30.2 a
	rhd2	336.7 a	4.14 ab	10.6 a	33.9 a
Significance					
Genotype		ns	ns	ns	ns
Media		**	*	ns	ns
Genotype × Media		ns	ns	ns	ns

Note: ns = not significant, * = significant at the 5% level, ** = significant at the 1% level. Numbers with different letters differ significantly at the 5% level by Duncan's significant difference.

high-phosphorus plants, there was no effect of genotype or growth media on any of the measured parameters. Among low-phosphorus plants, there was no effect of genotype or growth media on root length; however, there was both an effect of genotype and growth media on shoot biomass. Low-phosphorus WS plants grown in sand-alumina media had the greatest shoot biomass of all the low-phosphorus grown plants. This suggests that the response of *Arabidopsis* root hairs to low-phosphorus availability is most beneficial in a phosphorus-diffusion-limited situation.

Tissue phosphorus content was analyzed with respect to both root length and root surface area. As with root length and shoot biomass, there was no significant difference in the amount of total plant phosphorus per unit root length or root surface area in high-phosphorus grown plants (Table 2). Plants grown in low-phosphorus nutrient solution showed no significant difference in total plant phosphorus per unit root length. However, WS plants in low-phosphorus sand-alumina had significantly greater total plant phosphorus per unit root length than did the two root mutants (Table 3). This difference in total phosphorus per root length became insignificant when surface area was introduced into the calculation. Therefore, in a phosphorus-diffusion-limited situation, WS plants took up more phosphorus per unit root length than the two root hair mutants. This difference in phosphorus uptake could be accounted for by differences in root surface area caused by differential root hair growth in the WS genotype.

Root respiration and ^{32}P uptake—To understand the possible respiration cost associated with root hair maintenance, CO_2 given off by intact roots of the three genotypes was measured. In addition, the benefit of phosphorus acquisition by root hairs was measured in the three genotypes by kinetic analysis of ^{32}P uptake.

Low-phosphorus roots emitted approximately twice as much CO_2 per millimetre root length as did high-phosphorus roots (Fig. 2). Uptake kinetic analysis of ^{32}P showed that low-phosphorus plants took up more phosphorus per unit root length at each substrate (P) concentration than did high-phosphorus plants (Fig. 3). In addition, there was no significant difference between genotypes in phosphorus uptake per minute per root length. Low-phosphorus plants had greater maximum transport capacity (V_{max}) than high-phosphorus plants (0.0275 and 0.00416 nmol $\cdot$ min^{-1} $\cdot$ mm root^{-1}, respectively), which indicates that the phosphorus transport mechanism in the root hair mutants was not affected by the mutation. Low-phosphorus availability induced maximum transport capacity for phosphorus by plants.

Since the phosphorus uptake kinetic analysis was analyzed in nutrient solution and root hairs showed little effect on plant growth in nutrient solution, we compared the specific phosphorus uptake of ^{32}P with the specific

TABLE 3. Root growth, shoot growth, and phosphorus status of 3-wk-old low-phosphorus-grown WS, *rhd6,* and *rhd2* plants.

Media	Genotype	Root length (mm/plant)	Shoot biomass (mg/plant)	Total plant P/root length (nmol P/mm root)	Total plant P/root surface area (nmol P/mm² root)
Nutrient solution	WS	106.2 b	0.90 bc	0.8 a	0.8 a
	rhd6	72.8 a	0.78 b	1.4 a	2.5 ab
	rhd2	75.3 a	0.69 b	1.2 a	3.7 b
Sand-alumina	WS	88.4 ab	1.13 c	2.5 b	2.3 ab
	rhd6	77.4 ab	0.21 a	1.5 ab	2.7 ab
	rhd2	83.4 ab	0.10 a	0.9 a	2.9 ab
Significance					
Genotype		ns	**	ns	ns
Media		ns	**	ns	ns
Genotype × Media		ns	**	*	ns

Note: ns = not significant, * = significant at the 5% level, ** = significant at the 1% level. Numbers with different letters differ significantly at the 5% level by Duncan's significant difference.

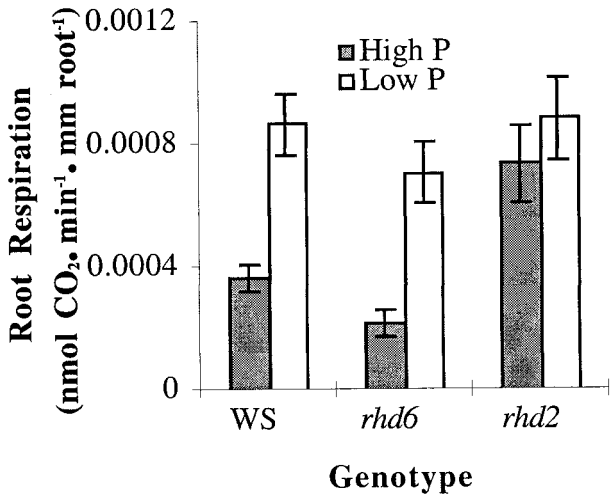

Fig. 2. Root respiration of three *Arabidopsis* genotypes grown at two phosphorus availabilities. Except for the *rhd2* mutant, low-phosphorus plants had greater root respiration than high-phosphorus plants. $N = 10$. Bars indicate ± 1 SE.

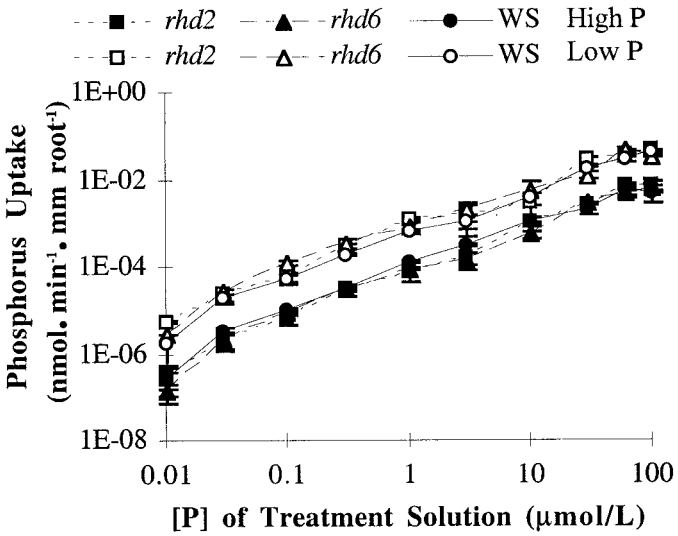

Fig. 3. Uptake of phosphorus by high- and low-phosphorus nutrient-solution-grown *Arabidopsis* plants at ten external phosphorus concentrations labeled with ^{32}P. Low-phosphorus plants took up more phosphorus per minute per millimetre root than did high-phosphorus plants, however there was no effect of genotype on phosphorus uptake. $N = 5$. Bars indicate ± 1 SE.

phosphorus uptake of sand-alumina grown plants. Phosphorus uptake rate of 3-wk-old plants was determined from a 6-wk growth analysis in sand-alumina media. Specific phosphorus uptake (in nmoles of phosphorus per minute per millimetre of root) was similar in all high-phosphorus grown plants (Table 4). However, at low phosphorus, sand-alumina plants had different specific phosphorus uptake with WS > *rhd6* > *rhd2* (Table 5). Therefore, root hairs increased the uptake of phosphorus at low-phosphorus availability in plants that were grown in sand-alumina media, but root hairs had no effect on phosphorus uptake in nutrient solution.

Cost-benefit analysis—To demonstrate the absolute importance of root hairs in phosphorus acquisition, we calculated the cost-benefit of root hairs in two ways. In the first case, ''cost'' was defined as CO_2 evolution per minute per root and ''benefit'' was defined as phosphorus uptake per minute per root. Root construction carbon costs were not included into the analysis because the root lengths were similar among genotypes within a phosphorus level. In all high-phosphorus plants and in low-phosphorus nutrient solution plants, WS plants had a cost-benefit ratio that was less efficient than *rhd6* plants (Table 4, 5). High-phosphorus *rhd2* plants had a poor cost-benefit ratio when compared to the other high-phosphorus plants because of its high respiration rate. Low-phosphorus WS plants grown in sand-alumina had a more favorable cost-benefit ratio than *rhd6* plants and much more favorable ratio than *rhd2* plants (Table 5).

In the second analysis, the ratio was inverted to a benefit-cost analysis or a profit-investment analysis. ''Benefit'' in this case was defined as phosphorus uptake per minute per root and ''cost'' was defined as total phosphorus invested in the root at the time of the uptake measurement. At high phosphorus, WS did not have a different benefit-cost ratio than the other plants (Table 4).

TABLE 4. Phosphorus uptake efficiency of WS, *rhd6*, and *rhd2* plants under high-phosphorus conditions.

Media	Genotype	Specific P uptake[1]	Cost-benefit[2]	Benefit-cost[3]
Nutrient solution	WS	0.001170 a	213.14 b	0.02830 a
	rhd6	0.001246 a	105.02 ab	0.01657 a
	rhd2	0.001033 a	420.97 c	0.01629 a
Sand-alumina	WS	0.001509 a	85.94 ab	0.02225 a
	rhd6	0.001533 a	44.62 a	0.01726 a
	rhd2	0.001315 a	234.10 b	0.01658 a
Significance				
Genotype		ns	**	ns
Media		ns	**	ns
Genotype × Media		ns	ns	ns

Note: Numbers with different letters differ significantly at the 5% level by Duncan's significant difference. ns = not significant, ** = significant at the 1% level.

[1] Units are nmol P · min^{-1} · mm $root^{-1}$.

[2] Units are nmol CO_2 · nmol P^{-1} · min^{-1} · $root^{-1}$.

[3] Units are nmol P · min^{-1} · $root^{-1}$ · nmol root P^{-1}.

TABLE 5. Phosphorus uptake efficiency of WS, *rhd6*, and *rhd2* plants under low-phosphorus conditions.

Media	Genotype	Specific P uptake[1]	Cost-benefit[2]	Benefit-cost[3]
Nutrient solution	WS	0.000217 bc	425.48 a	0.00136 a
	rhd6	0.000305 d	162.66 a	0.00162 ab
	rhd2	0.000319 d	249.28 a	0.00303 bc
Sand-alumina	WS	0.000263 cd	293.47 a	0.00362 c
	rhd6	0.000165 b	404.42 b	0.00213 abc
	rhd2	0.000086 a	1053.88 c	0.00113 a
Significance				
Genotype		ns	**	ns
Media		**	**	ns
Genotype × Media		**	**	**

Note: Numbers with different letters differ significantly at the 5% level by Duncan's significant difference. ns = not significant, ** = significant at the 1% level.

[1] Units are nmol P · min^{-1} · mm $root^{-1}$.

[2] Units are nmol CO_2 · nmol P^{-1} · min^{-1} · $root^{-1}$.

[3] Units are nmol P · min^{-1} · $root^{-1}$ · nmol root P^{-1}.

For the benefit-cost ratio at low phosphorus, WS > *rhd6* > *rhd2* in sand-alumina but WS < *rhd6* < *rhd2* in nutrient solution (Table 5). These data show that at low-phosphorus availability, root hairs are beneficial in sand-alumina but detrimental in nutrient solution.

DISCUSSION

Root hair mutants—Several studies compare the importance of root hairs in the acquisition of phosphorus, however these studies compare root hair growth of different plant species such as onion and wheat. The discovery of root hair mutants in *Arabidopsis* has made it possible to research the importance of root hairs in phosphorus acquisition of a single species.

We examined the growth of the root hair mutants under different phosphorus availabilities and growth conditions and measured root and root hair characteristics to identify mutational influences. The WS wild type responded similarly to low-phosphorus availability as the RLD wild type used in previous studies (Bates and Lynch, 1996). In this response, root hairs grew longer and more dense under low-phosphorus availability. The *rhd6* mutant is termed a root hair formation or density mutant, but it responded to low-phosphorus availability in a similar way to the wild type. Root hair length and density increased under low-phosphorus availability, but the mutation prevented root hair density from ever equaling that of the wild type under the same phosphorus regime. The *rhd2* mutant is termed a root hair elongation mutant, and the root hair phenotype did not change under low-phosphorus availability. The response of root hair growth to low-phosphorus availability was the same for nutrient solution and sand-alumina media. Root growth, as indicated by root length, was the same for all three genotypes within a phosphorus treatment.

The most obvious effect of differential root hair growth on root morphology was on root surface area per unit root length. The response of root hair length and density to low-phosphorus availability significantly increased root surface area in the WS and *rhd6* genotypes but not in the *rhd2* genotype, which is important because it differentiates roots of high, medium, and low surface area at two phosphorus levels.

Phosphorus uptake kinetics showed that the root hair mutation did not affect phosphorus transport at either high- or low-phosphorus availability. Although phosphorus transport was greater in low-phosphorus plants, the similarity in genotypes within a phosphorus level indicated that the root hair mutation was restricted to root hair growth and did not affect the phosphorus transport system.

Root respiration in the *rhd2* mutant indicated an abnormality that may be an effect of the mutation. For the other two genotypes, root respiration was greater for low-phosphorus plants than high-phosphorus plants and that WS respiration was greater than the respiration *rhd6*. This respiratory pattern in WS and *rhd6* may reflect the genomic effect of root surface area and the environmental effect of increased phosphorus transport. The *rhd2* mutant did not follow this pattern, but rather showed relatively high respiration for both high- and low-phosphorus plants. This may be a pleiotropic effect of the mutation or may be stress induced by decreased root hair elongation. Close examination of *rhd2* root hairs showed that many of the root hair papillae burst at the site of root hair elongation. This bursting of root hairs may induce increased respiration during wounding. The deviation of the *rhd2* respiration, especially in high-phosphorus plants, must be noted when examining the cost-benefit analysis.

Surface area and phosphorus nutrition—In high-phosphorus plants, genotype and growth media had little or no effect on root length, shoot biomass, tissue phosphorus content, whether based on root length or root surface area, and specific phosphorus uptake. This indicates that root hairs are not important in phosphorus acquisition at high-phosphorus availability.

In low-phosphorus plants, shoot biomass, tissue phosphorus content on a root length basis, and specific phosphorus uptake were significantly different in sand-alumina grown plants but not in nutrient-solution-grown plants. In low-phosphorus nutrient-solution-grown plants, phosphorus was available to the root surface even if root hairs were absent because of nutrient solution mixing. Therefore, shoot biomass and tissue phosphorus on a root length basis were equal. The sand-alumina media represents soil conditions where soil particles bind phospho-

rus, phosphorus is diffusion limited, and roots create depletion zones of phosphorus uptake. In low-phosphorus sand-alumina conditions, WS plants had larger shoots, had more plant phosphorus per unit root length, and took up more phosphorus per minute than the two root hair mutants. However, when root hairs were included into the analysis and plant phosphorus was calculated on a root surface area basis, the three genotypes were equal. These data show that root hairs are important for phosphorus acquisition under diffusion-limited low-phosphorus conditions because root hairs increase root surface area and extend the root surface into the soil environment.

Cost-benefit analysis—For the response of increased root hair elongation and density to be efficient, the cost-benefit ratio of the response must be lower than if the response never happened. For example, if longer root hairs help in phosphorus acquisition but respire most of the plant carbon or require considerable phosphorus investment, then longer root hairs may become less important or even detrimental to the plant.

We examined the cost-benefit of root hairs from the perspective of respiration and phosphorus investment. Using respiration as a cost, CO_2 is exchanged for acquired phosphorus. Using investment as a cost, phosphorus invested in the root system is exchanged for acquired phosphorus.

The cost-benefit analysis of low-phosphorus plants in sand-alumina supports root hair growth as a successful strategy. When using respiration as the cost, low-phosphorus sand-alumina plants respired less CO_2 for each unit of phosphorus gained than did the two root mutants. When using phosphorus investment as the cost, low-phosphorus sand-alumina plants gained more phosphorus for every unit of phosphorus invested in the root system. On the other hand, *rhd2* plants used more carbon and invested more phosphorus for every unit of phosphorus gained. The cost-benefit ratio of *rhd6* plants was intermediate to that of WS and *rhd2*.

In this study, it is not valid to compare high-phosphorus and low-phosphorus plants in the cost-benefit analysis for the efficiency of root hairs because phosphorus stress induces other stress responses in roots. It is more appropriate to compare two low-phosphorus plants where the root hair response has been blocked in one of the plants. From the surface area calculation, high-phosphorus WS and low-phosphorus *rhd6* roots had similar surface areas per unit root length. In addition, low-phosphorus WS and *rhd6* plants had similar root lengths. Therefore, from a root surface area perspective, low-phosphorus WS and *rhd6* plants represent low-phosphorus wild-type plants with and without the root hair growth response, respectively. Since low-phosphorus WS plants had a more favorable cost-benefit analysis than low-phosphorus *rhd6* plants, it is more cost effective for *Arabidopsis* plants to increase root surface area through root hairs under low phosphorus than not to increase root hair length and density.

We conclude that root hairs increase plant phosphorus acquisition under low-phosphorus soil conditions by increasing the root surface area. In addition, the strategy of stimulated root hair length and density under low-phosphorus availability is an efficient one for phosphorus acquisition.

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