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A Mutant of *Arabidopsis* Deficient in Xylem Loading of Phosphate[1]

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ABSTRACT

A mutant of *Arabidopsis thaliana* deficient in the accumulation of inorganic phosphate has been isolated by screening directly for plants with altered quantities of total leaf phosphate. The mutant plants accumulate approximately 5% as much inorganic phosphate, and 24 to 44% as much total phosphate, as wild-type plants in aerial portions of the plant. Growth of the mutant is reduced, relative to wild type, and it exhibits other symptoms normally associated with phosphate deficiency. The phosphate deficiency is caused by a single nuclear recessive mutation at a locus designated *pho1*. The rate of phosphate uptake into the roots was similar between mutant and wild-type plants over a wide range of external phosphate concentrations. In contrast, when plants were grown in media containing 200 micromolar phosphate or less, phosphate transfer to the shoots of the mutant was reduced to 3 to 10% of the wild-type levels. The defect in phosphate transfer to the shoots could be overcome by providing higher levels of phosphate. Transfer of sulfate to the shoots was essentially normal in the mutant, indicating that the *pho1* lesion was not a general defect in anion transport. Movement of phosphate through the xylem of the shoots was not impaired. The results suggest that the mutant is deficient in activity of a protein required to load phosphate into the xylem.

The mechanisms that regulate fluxes of phosphate between various tissues of higher plants are not well characterized. Potential points of control are the level of root Pi uptake, transfer of the absorbed Pi to the shoot, and compartmentalization into different subcellular organelles. There appear to be two major points of regulation for ion transport across the roots: the initial uptake across the plasma membrane into the symplast of the epidermal and cortical cells and the subsequent release into the xylem (7, 8, 12). An increase in the capacity of the roots for phosphate uptake occurs when the roots are exposed to a limited supply of phosphate. Changes in the number of phosphate transporters or in their affinity for phosphate have been postulated to explain these changes in uptake kinetics (3, 7, 11). Under conditions of limited phosphate availability, the capacity to transfer the absorbed phosphate to the shoots is also increased, suggesting an increase in phosphate release into the xylem (3, 7, 11). Compartmentalization of Pi appears to be important at the cellular level, with the vacuole acting as a storage site for excess Pi that can be released under conditions of cytosolic Pi deficiency (3, 12). Thus, understanding the mechanisms that regulate Pi acquisition is complicated by both the existence of several potential sites of control and the presence of adaptive responses.

As one approach to understanding how Pi levels are regulated, a genetic study was initiated to define loci that play a role in this process. The goal was to identify plants from a mutagenized population of *Arabidopsis thaliana* that display quantitative differences in leaf total phosphate content when compared with wild-type plants. We describe here the properties of a novel mutant that displayed a leaf Pi content that is only approximately 5% of the wild-type level. Measurements of the rate of root Pi uptake and transfer to the shoots, under various external phosphate concentrations and physiological conditions, indicate that the mutant is most likely deficient in activity of a protein required to load phosphate into the xylem.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The mutant line PL9 was isolated from an M_2 population derived from the Columbia wild type of *Arabidopsis thaliana* (L.) Heynh. following mutagenesis with ethyl methanesulfonate by previously described methods (9). Before its use in physiological experiments, the mutant was backcrossed to the Columbia wild type four times. Unless otherwise indicated, all plants were grown at 22°C under continuous fluorescent illumination (100–150 μmol m^{-2} s^{-1} in the 400- to 700-nm range) on a potting mixture of fine sphagnum:perlite: vermiculite (1:1:1) irrigated with a mineral solution containing 2.5 mM Pi (9).

Phosphate Measurements

Total phosphate in plant samples was determined by the method of Ames (1). To screen for mutants, uniform-sized leaf discs (0.7 cm diameter) from the center of mature leaves of approximately 18-d-old plants were placed individually in 16- × 100-mm glass test tubes, ashed over a Bunsen burner flame in the presence of 200 μL of 10% $Mg(NO_3)_2$, treated

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with 300 μL of 0.5 N HCl at 65°C for 30 min, and assayed spectrophotometrically for Pi content with an ammonium molybdate/ascorbic acid reagent in a final volume of 1 mL, as described by Ames (1). For most purposes, discs from two different leaves of the same plant were analyzed.

In experiments in which only cellular Pi was measured, the plant tissues were placed in 1% acetic acid followed by repeated freezing and thawing, and Pi released in the solution was assayed by the method of Ames (1).

For seed phosphate analysis, mature dry seeds were suspended in 0.4 M HCl, boiled for 5 min, and homogenized by sonication. The homogenate was dried, and total phosphate was determined as described above.

Fractionation and quantification of phosphate-containing compounds was performed essentially as described by Chapin and Bieleski (4) using freeze-dried material from the aerial portion of plants at the initiation of bolting.

Measurements of Germination, Growth, and Seed Production

Germination tests were conducted by sowing seed on the potting mixture described above and counting the number of seedlings after 7 d. For growth measurements, the fresh weight of the entire aerial portion of individual plants ($n = 8$) was determined at 2-d intervals. Seed yield was determined from plants grown to maturity (>60 d) at low density (20 cm^2/plant).

Elemental Analysis

Seeds from the wild-type and mutant lines were surface-sterilized and germinated on the surface of agar-solidified medium containing mineral elements, as previously described (17). After 8 d, plants were transferred to the potting mixture and grown as described above. The entire aerial portion of mutant and wild-type plants was harvested at the bolting stage (16–18 d after potting) and dried at 120°C overnight. The mineral content of plant tissues was determined by plasma emission spectroscopy, except for nitrogen which was determined by the Kjeldahl method.

Short-Term Sulfate and Pi Uptake Experiments

The basic nutrient medium used when growing plants in agar contained 2.5 mM KNO_3, 100 μM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 25 μM Fe-EDTA, 14 μM $MnCl_2$, 0.5 μM $CuSO_4$, 1 μM $ZnSO_4$, 70 μM H_3BO_3, 0.2 μM $NaMoO_4$, and 10 μM NaCl (final pH 5.7). A variable amount of Pi was provided in the media as KH_2PO_4. In [^{35}S]sulfate uptake experiments, low sulfate concentration was achieved by using an experimental medium with a final concentration of 6.5 μM $MgSO_4$ and 1 mM $MgCl_2$.

Wild-type and mutant plants were grown in 100- × 25-mm Petri dishes, under sterile conditions, on a 300-μm mesh nylon screen placed on the surface of an agar-solidified medium containing mineral nutrients, 1% (w/v) sucrose, and 0.7% (w/v) agar. Under these conditions, the roots of the seedlings grew through the openings of the nylon screen into the agar medium. Plants were grown for 12 to 14 d at 23°C in continuous fluorescent illumination (50 μmol m^{-2} s^{-1}). The day before the labeling experiment, the Petri dish covers were slightly opened to permit the plants to adjust to the ambient RH. At the start of an experiment, the plants were gently pulled out of the agar by lifting the nylon screens. The roots were washed and placed in the same experimental media used for the labeling experiment but without the radioisotopes. This was done by placing each nylon screen on the rim of a small vial containing the experimental medium, such that the roots were immersed in solution but the shoots were in air. After 1 h, the plants were transferred to 5.5 mL of an aerated experimental medium containing 1 μCi/mL of [^{32}P]Pi or 2 μCi/mL of [^{35}S]sulfate. Care was taken to avoid contact between the shoots and the labeled solution. Plants were illuminated with fluorescent light (50 μmol m^{-2} s^{-1}), and agitation was provided throughout the uptake experiment. Aeration of the medium during uptake was not necessary because of the relatively short labeling period, as well as the large ratio of medium to root volume. At the end of the labeling period (1 h), plants were rinsed with 20 mL of unlabeled experimental medium. Fresh weights of shoots and roots were recorded, and tissues were dried overnight at room temperature. The dried tissues were then placed overnight in scintillation cocktail (Safety-Solve, RPI Corp., Mount Prospect, IL), and the radioactivity incorporated was measured by scintillation spectrometry. The uptake of [^{32}P]Pi and [^{35}S]sulfate to the shoot and root was linear for at least 2 h. The rates of root Pi and sulfate uptake were measured as the amount of element taken into the whole plant, per gram fresh weight of roots and per hour of labeling. Shoot transfer is defined as the fraction of the absorbed element that was located in the shoots at the end of the labeling experiment (1 h).

In one experiment, plants were transferred to a sucrose-free nutrient medium 3 d before measuring Pi uptake and transfer as described above.

Short-Term Uptake into the Hypocotyl

To measure uptake of [^{32}P]Pi into the hypocotyl, plants having long hypocotyls were raised by placing 3-d-old seedlings in the dark for 48 h, followed by 8 d under normal conditions of illumination. Plants were partially submerged in medium, and the roots were cut off at the base of the hypocotyl. The cut end of the hypocotyl was placed in unlabeled experimental medium for 30 min. Phosphate uptake was measured by transferring the stems to 5.5 mL of mineral solution containing 8 μM [^{32}P]Pi (125 μCi/μmol) for 15 to 60 min. After the plants were labeled, they were washed for 2 min in unlabeled medium and dried, and the radioactivity incorporated was measured by scintillation spectrometry as described before. Uptake was linear for at least 1 h.

Growth of Mutant and Wild-Type Plants at High External Pi

Plants were grown for 7 d on an agar-solidified medium containing 200, 1000, or 5000 μM Pi and then transferred to hydroponic culture in aerated sucrose-free mineral solution having the same Pi concentration. Plants were grown for an

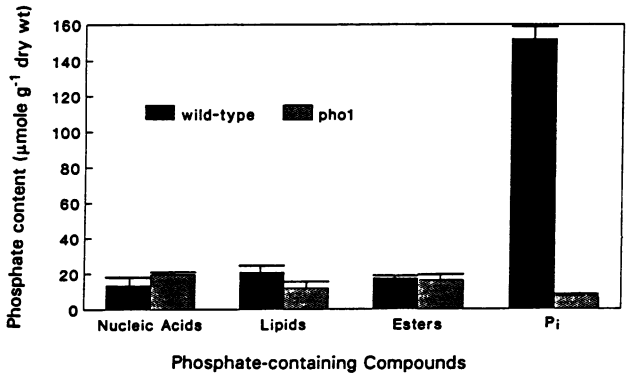

Figure 1. Content of various phosphate-containing compounds in wild-type and *pho1* mutant plants. Plant were grown under continuous illumination and harvested at the bolting stage. Columns, means of three measurements; bars, SE.

additional 12 d under continuous fluorescent illumination (50 μmol m^{-2} s^{-1}). At the end of the growth period, the fresh weights of the roots and shoots were measured.

RESULTS

Mutant Isolation and Analysis of Phosphate Content

Approximately 2200 randomly chosen *A. thaliana* M_2 plants were screened for alteration in leaf total phosphate content. One plant was identified that displayed a substantial reduction in total leaf phosphate compared with wild-type plants. This M_2 plant gave rise to a mutant line designated PL9. Further experiments on this line were performed on plants derived from four rounds of backcrossing to wild type.

Total phosphate was measured for the entire above-ground portion of mutant and wild-type plants at 10 and 18 d postgermination. Wild-type plants contained an essentially constant amount of total phosphate with values of 27.9 ± 1.6 (mean ± SE, n = 8) and 28.4 ± 0.7 μmol g^{-1} fresh weight for 10- and 18-d-old plants, respectively. In contrast, mutant plants had a total phosphate content of 12.3 ± 0.7 μmol g^{-1} fresh weight at 10 d, and 6.8 ± 0.4 μmol g^{-1} fresh weight at 18 d. Thus, total phosphate in the aerial portion of mutant plants was reduced to 44 and 24% of wild-type plants for 10- and 18-d-old plants, respectively. Total phosphate was also measured in mature dry seeds from mutant and wild-type plants. The mutant seeds contained significantly less phosphate than wild type with values of 44 ± 6 *versus* 218 ± 20 μmol g^{-1} dry weight (n = 4), respectively.

To determine how the total phosphate present in leaf tissue from the mutant was distributed among the various phosphate compounds, the phosphate-containing compounds were fractionated into Pi, phosphate esters, phospholipids, and nucleic acids. The only significant difference between mutant and wild type was in the amount of phosphate in the Pi fraction (Fig. 1). The Pi present in these two plant lines differed by a factor of approximately 21 (152 ± 16 *versus* 7.1 ± 0.5 μmol g^{-1} dry wt [n = 3] in the wild type and mutant, respectively).

Genetic Characterization

The genetic basis for the phosphate-deficient phenotype was determined by crossing the mutant to the wild type and measuring the total phosphate content of the F_1 and F_2 progeny. The phosphate content of leaf discs from F_1 plants (33.1 ± 1.1 μmol g^{-1} fresh weight, n = 7) did not differ significantly from that of wild-type leaf discs. However, F_2 plants fell into two distinct groups. The leaf discs from one group displayed low phosphate content typical of the PL9 line (11.7 ± 1.0 μmol g^{-1} fresh weight, n = 14), whereas the other group contained phosphate levels characteristic of wild type (36.7 ± 0.9 μmol g^{-1} fresh weight, n = 38). Each of the plants in the low-phosphate group also displayed the altered growth characteristics that are present in the mutant line (see below), whereas none of the plants with a normal phosphate content displayed these abnormalities. The ratio of normal to phosphate-deficient plants in the F_2 population (38:14) fits the 3:1 segregation ratio (χ^2 = 0.103, P > 0.5), indicating that the phosphate deficiency is caused by a single nuclear recessive mutation at a locus designated *pho1*.

Measurement of Growth

The growth of the mutant, measured as the increase of the fresh weight of the aerial portion of the plant, was significantly impaired by the *pho1* mutation (Fig. 2). In addition to reduced growth, the mutant plants displayed several other visual characteristics generally associated with phosphate deficiency (13, 16). The plants possessed thinner stalks, smaller leaves, and very few secondary inflorescences. Flowering was delayed by approximately 6 d in the mutant. Furthermore, the leaves and stems of mutant plants accumulated elevated levels of anthocyanins. The mutant plants were fertile but produced only 5% as many seeds as wild type. The seeds produced were smaller in size and successfully germinated at a frequency of only 55% compared with 95% for wild-type seeds.

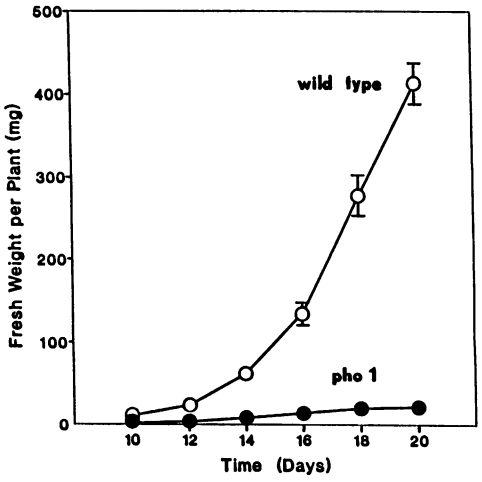

Figure 2. Growth of wild-type and mutant plants. Points, means of eight measurements of fresh weight of the entire aerial portion of the plant. Where no error bar (SE) is shown, the SE is less than the size of the symbol.

Elemental Analysis

To determine whether the mutation at the *pho1* locus affects accumulation of other mineral ions, the concentrations of various elements in leaf tissues of mutant and wild-type lines were compared. Elements analyzed were Al, B, Ca, Cu, Fe, Mg, Mn, N, P, K, and Zn. All elements tested were present in approximately equal amounts in the two lines, except for the expected fourfold difference in phosphorus (1.6 ± 0.2%, w/w [n = 3] *versus* 0.4 ± 0.05% [n = 4] for wild type and mutant, respectively) and a small but statistically significant difference in potassium (7.2 ± 1.2 *versus* 4.8 ± 0.9% for wild type and mutant, respectively).

Root Uptake and Transfer of Pi and Sulfate

The capacity of the roots to absorb and transfer Pi and sulfate to the shoots was compared for the *pho1* mutant and wild-type plants. The results of short-term uptake experiments with plants grown at low external Pi and sulfate concentration (8 μM Pi and sulfate) are shown in Table I. Root sulfate uptake was not significantly different between the wild type and mutant. Similarly, Pi was absorbed at the same rate by the roots of wild-type and mutant plants. In contrast, the fraction of Pi absorbed by the root system that was transferred to the shoots was greatly reduced in the mutant. Whereas wild-type roots transferred 35% of the absorbed Pi to the shoots, the mutant transferred only 0.9%. Transfer of the absorbed sulfate to the shoots was only slightly lower (50% reduction) in the mutant compared with the wild type. These results indicate that the *pho1* mutation does not affect the root Pi uptake *per se* but, rather, impairs the transfer of the absorbed Pi to the shoots.

Uptake and transfer of Pi were also measured in plants transferred for 3 d before labeling on sucrose-free medium containing 8 μM Pi. As for plants grown continuously on sucrose-containing medium, transfer of Pi to the shoots was greatly reduced in the mutant (27 ± 2 *versus* 0.7 ± 0.2%, n = 3, for wild type and mutant, respectively). However, in contrast to the results of Table I, roots of the mutant grown in sucrose-free medium showed a significant decrease in Pi uptake (1369 ± 88 *versus* 396 ± 24 nmol g^{-1} h^{-1} for wild type and mutant, respectively). These results indicate a significant effect of the availability of sucrose on the ability of the mutant to absorb Pi.

Table II. *Uptake and Transfer of Pi in Plants Grown at Various External Pi Concentrations*

Plants were grown for 12 to 14 d on an agar-solidified medium containing 1% sucrose. Labeling was done at the same Pi concentration used for growth. Values represent the means of six measurements.

Plant Line	Pi in Medium	Root Uptake	Transfer to Shoot
	μM	nmol g^{-1} h^{-1}	%
Phol	0.32	142 ± 19	1.5 ± 0.1
Wild type	0.32	93 ± 8	21 ± 3
Phol	1.6	513 ± 46	2.3 ± 0.4
Wild type	1.6	460 ± 36	34 ± 2
Phol	8	1807 ± 48	1.8 ± 0.3
Wild type	8	1845 ± 176	49 ± 2
Phol	40	2101 ± 299	4.0 ± 0.9
Wild type	40	3839 ± 222	42 ± 1
Phol	200	693 ± 108	24 ± 5
Wild type	200	1814 ± 97	39 ± 1
Phol	1000	681 ± 100	47 ± 8
Wild type	1000	859 ± 83	30 ± 2

Uptake of Pi into the Hypocotyl

A reduction in Pi transfer to the shoots could, in principle, be due to a block at a step before or after loading of Pi into the xylem. To examine the possibility that movement of Pi through the xylem is impaired in the *pho1* mutant, uptake of Pi into hypocotyls of plants from which the roots were excised was measured. Phosphate uptake was 3.5 ± 0.3 nmol g^{-1} h^{-1} (n = 12) for wild type and 4.7 ± 0.3 nmol g^{-1} h^{-1} for the *pho1* mutant. The movement of Pi in the xylem of the shoot tissue is, therefore, not impaired in the *pho1* mutant. The very low rate of Pi uptake into the hypocotyl reflects the fact that mass flow of water through the xylem is the major driving force for Pi transport under these conditions. This illustrates the large contribution of the root system to the active uptake of Pi.

Uptake and Transfer of Pi in Plants Grown under Different External Pi Concentrations

To analyze whether the defect in Pi transfer in the *pho1* mutant is dependent upon the external Pi concentration, root Pi uptake and transfer were measured in plants grown on a wide range of Pi concentrations (Table II). When the plants were grown on medium containing between 0.32 and 40 μM

Table I. *Root Uptake and Transfer to the Shoot of Phosphate and Sulfate*

Experiments were done on 12 to 14-d-old plants grown on an agar-solidified medium containing 1% sucrose, 8 μM phosphate, and 8 μM sulfate. Values represent the means of 8 to 11 measurements.

Plant Line	Sulfate		Phosphate	
	Root uptake	Transfer to shoot	Root uptake	Transfer to shoot
	nmol g^{-1} h^{-1}	%	nmol g^{-1} h^{-1}	%
Phol	367 ± 34	12 ± 2	1559 ± 144	0.9 ± 0.1
Wild type	291 ± 25	25 ± 2	1593 ± 130	35 ± 3

Pi, transfer of Pi to the shoots of the *pho1* mutant was 4 to 10% of that of wild type. At 200 and 1000 μM Pi, transfer of Pi to the shoots of the mutant was 65 and 150% of that of wild type, respectively. By contrast, the rates of root Pi uptake were very similar between the mutant and wild type at all Pi concentrations, except for a 2.5-fold difference observed at 200 μM Pi, which is probably artifactual. These results indicate that the defect in Pi transfer to the shoot can be overcome by an increase in the external Pi concentration.

Effect of High External Pi Concentration on Plant Growth and Pi Content

Short-term uptake experiments indicated that the *pho1* mutant grown at 1000 μM Pi was able to transfer Pi to the shoots as efficiently as wild type (Table II). This suggested that the effect of the *pho1* mutation on growth might be overcome by increasing the level of extracellular Pi available to the plant. Wild-type and *pho1* plants were grown for 17 d on 200, 1000, and 5000 μM Pi. Fresh weights of the roots and shoots were measured at the end of the growth period, as well as the amount of Pi and total phosphate (Table III). The final fresh weight of the shoots of mutant plants was 19 to 29% of wild type for all levels of extracellular Pi used. In the shoots of the mutant grown in 200 and 1000 μM Pi, both Pi and total phosphate were lower than in the wild type. However, when the mutant and wild type were grown at 5000 μM Pi, shoot Pi and total phosphate levels were similar. Thus, even though an appropriate supply of Pi to the shoots was achieved, there was no restoration of the growth rate of the mutant to wild-type level.

Comparison of the Pi content of the shoots and roots of the *pho1* mutant grown in various Pi concentrations provided further evidence for a block in Pi transport between the roots and shoots. For all treatments, wild-type shoots contained an equal or slightly higher level of total phosphate and Pi than the roots. In contrast, *pho1* mutant plants grown at 200 and 1000 μM Pi had a higher content of both Pi and total phosphate in their roots compared with shoots. This difference is most pronounced at 200 μM Pi, where tissue Pi content is 22-fold higher in the roots than the shoots. This difference in Pi content between shoots and roots is abolished by growth in the highest external Pi concentration (5000 μM). The apparent discrepancies between Pi levels in mutant plants grown mainly on sucrose-free solution and values for Pi transfer to the shoots obtained for plants grown on sucrose (compare values at 200 and 1000 μM Pi in Tables II and III) may reflect a synergistic effect occurring between photosynthesis and Pi transport.

DISCUSSION

By using a simple method for directly estimating the quantity of phosphate in leaf tissue, we isolated an *Arabidopsis* mutant that accumulates <5% of the amount of Pi present in shoots of mature wild-type plants. Despite the dramatic reduction in Pi content, this mutant is viable and fertile, although growth and seed yield are severely depressed. The phosphate content of the seeds is also significantly reduced, which probably contributes to their lower germination frequency. Genetic analysis has shown that the phosphate deficiency is caused by a single nuclear recessive mutation at a locus we have designated *pho1*.

The small amount of Pi relative to organic phosphate compounds in the *pho1* mutant is consistent with current views concerning phosphate distribution in leaf tissue under conditions of low phosphate availability. Pi levels are generally found to be more sensitive to phosphate availability than are the levels of organic phosphate compounds (3).

When an adequate supply of Pi is available to the leaf tissue, approximately 80% of the Pi is normally located in the vacuole, with the remaining 20% located in the cytosol, chloroplasts, and other organelles (3). Under conditions of Pi starvation, the proportion of Pi in the vacuole decreases to

Table III. *Effect of High External Pi Concentration on Plant Growth and Phosphate Content*

Plants were grown for 7 d on an agar-solidified medium containing 1% sucrose and between 200 and 5000 μM Pi. This was followed by 12 d of growth in an aerated sucrose-free mineral solution containing the same Pi concentration. Values represent the mean of five to six measurements.

Plant Line	Pi in Medium	Tissue	Fresh Weight	Pi in Tissue	Total Phosphate in Tissue
	μM		mg	μmol g^{-1} fresh wt	μmol g^{-1} fresh wt
Phol	200	Shoot	6.4 ± 0.6	0.6 ± 0.3	11.4 ± 0.8
		Root	3.8 ± 0.4	12 ± 2	34.2 ± 0.8
Wild type	200	Shoot	33 ± 2	19 ± 2	27 ± 1
		Root	11.6 ± 0.8	10.0 ± 0.4	26 ± 2
Phol	1000	Shoot	6.4 ± 0.8	5.1 ± 1.5	15 ± 3
		Root	3.2 ± 0.4	15.3 ± 0.6	39 ± 2
Wild type	1000	Shoot	22 ± 4	10.3 ± 0.7	29 ± 3
		Root	7 ± 1	10.1 ± 0.4	30 ± 2
Phol	5000	Shoot	7.6 ± 0.8	29 ± 10	43 ± 11
		Root	4.6 ± 0.9	24 ± 4	38 ± 5
Wild type	5000	Shoot	26 ± 4	24 ± 7	37 ± 9
		Root	10 ± 1	14 ± 1	31 ± 1

50% or less (3, 12). Because the *pho1* mutant has <5% of wild-type levels of Pi, it seems likely that very little, if any, Pi is present in the vacuole, and any available Pi is probably used to maintain essential metabolic processes.

Short-term uptake experiments have shown that, for plants growing at low external Pi concentrations (<200 μM), transfer of Pi to the shoot is reduced by 90 to 98% in the *pho1* mutant compared with the wild type (Tables I and II). These values are in agreement with a 95% reduction in the amount of leaf Pi of mutant plants grown in soil (Fig. 2), in which the average Pi concentration is between 0.2 and 10 μM (2). At higher external Pi concentrations (≥1000 μM), this defect can be overcome, and wild-type levels of Pi in the shoots of the mutant can be achieved (Tables II and III). In contrast, at all external Pi concentrations used, there was no consistent difference between the wild type and the mutant in the rate of Pi uptake into roots. The defect in ion transfer to the shoot was specific for Pi (Table I). The small decrease in sulfate transfer observed in the *pho1* mutant could be caused by a number of factors unrelated to a defect in ion transport, such as lower transpiration due to a smaller leaf surface area or a lower requirement for sulfate due to decreased metabolic demand.

The results from the elemental analysis further indicated that the effect of the *pho1* mutation is largely Pi specific. However, elemental analysis indicated that potassium content was also reduced by 30% in the mutant. Because potassium plays a predominant role in maintaining the cation-anion balance in plant cells, this decrease may be a direct response to the decreased amount of Pi anion.

The defect in Pi transfer to the shoots was observed in plants grown in the presence or absence of sucrose in the growth medium. In plants grown on sucrose-free medium, a significant decrease in the rate of root Pi uptake was observed. Previous studies have shown that limited availability of Pi can decrease the photosynthetic capacity of the chloroplast (18) and increase the conversion of photosynthate to starch (15). These two factors are likely to reduce the export of photosynthate to the roots, thereby contributing to the decrease in Pi uptake and transfer in the *pho1* mutant. In this respect, the synergistic interaction between photosynthesis and Pi uptake is likely to be a major reason for the reduced growth rate of the *pho1* mutant grown in soil, where the plant is dependent on photosynthesis for carbon assimilation.

According to the two-pump and the chemiosmotic models (10, 14), loading of ions into the xylem is an active process. These models are supported by evidence for electrogenic ion pumps at both the root epidermal surface and the stele (5, 6). There is also good evidence that the xylem parenchymal cells are actively involved in ion loading into the xylem (3, 8, 14). Two major potential points of regulation of ion transport are the initial uptake across the plasmalemma of the epidermal and cortical cells and the final release into the xylem (7, 8, 12). In the *pho1* mutant, the specific deficiency in transfer of Pi from the roots into the shoots indicates that loading of Pi into the xylem is impaired rather than the initial uptake into the epidermal cells. Thus, a defect in a Pi-specific transporter in the xylem parenchymal cells is an attractive model to explain the phenotype of the *pho1* mutant.

The defect in Pi transfer to the shoots of the mutant can be overcome at high external Pi concentrations. This may indicate that at a high Pi concentration, leakage of ions into the xylem or mass movement through the apoplast may compensate for a defect in active xylem loading (14). Alternatively, a second (low affinity) transporter may be active at higher Pi levels and be responsible for Pi loading into the xylem under these conditions. This hypothesis would be consistent with the multiple carrier model developed initially to explain the kinetics of ion uptake in fungi and plant roots growing under a large range of ion concentrations (3). Finally, it is also possible that the *pho1* mutation may result in a transporter with a higher K_m for Pi, resulting in a deficiency in translocation to the shoots at a low Pi concentration.

An unexplained property of the mutant is that, when an appropriate level of Pi was obtained in the leaves of the *pho1* mutant by provision of a high external Pi concentration, growth was still impaired (Table III). One possible explanation is that the *pho1* mutation affects a component of a Pi transporter that is expressed in cell types other than the xylem parenchymal cells and that cannot be supplemented with high levels of exogenous Pi. Another possibility is that a defect in phosphate loading into the xylem might lead to a nonphysiological buildup of Pi in a cell type and/or subcellular compartment of the root cells growing at high external Pi concentrations. Alternatively, in spite of having been backcrossed four times to the wild type, the PL9 line used in this study may have a second mutation at a site closely linked to the *pho1* locus, which leads to reduced growth despite adequate Pi transport. Additional genetic studies will be required to evaluate this possibility.

The use of laboratory generated mutants for the study of mineral nutrition has not been extensively exploited. Recently, another mutant of *Arabidopsis* deficient in Pi uptake was reported (19). Although limited information is available, this mutant appears to be different from the *pho1* mutant because influx of Pi into the roots was shown to be impaired. In addition to providing insights into the mechanism of ion transport, the isolation and characterization of the *pho1* mutation and similar mutations in *Arabidopsis* are expected to facilitate the isolation and characterization of the genes involved in plant mineral nutrition.

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