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Characterization of a Phosphate-Accumulator Mutant of *Arabidopsis thaliana*

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We have characterized a novel mutation of *Arabidopsis thaliana* at a locus designated *pho2*. *pho2* mutants accumulated up to 3-fold more total P in leaves, mostly as inorganic phosphate (Pi), than wild-type seedlings. In addition, we isolated a mutant (locus designated *pho1–2*, an allelle of *pho1–1* described by Y. Poirier, S. Thoma, C. Somerville, J. Schiefelbein [1991] Plant Physiol 97: 1087–1093) with low Pi concentrations in leaves. When grown under high transpiration conditions, leaves of *pho2* seedlings became severely P intoxicated, whereas shoots of *pho1–2* mutants were P deficient and wild-type seedlings were normal. A *pho1/pho2* double mutant resulting from a cross between the single mutants was identified in the F_2 generation and shown to have a *pho1* phenotype. Prior to the development of P toxicity symptoms, P was the only mineral nutrient whose concentration was greater in *pho2* mutants than wild-type seedlings. Compared to wild-type, *pho2* mutants had greater Pi concentrations in stems, siliques, and seeds, but roots of *pho2* mutants had similar or lower Pi concentrations than either *pho1* mutants or wild-type seedlings. We suggest that the *pho2* mutation affects a function normally involved in regulating the concentration of Pi in shoots of *Arabidopsis*.

Much is known of the molecular mechanisms used by microorganisms to regulate their uptake of Pi. When starved of Pi, *Escherichia coli* and other microorganisms activate a Pi-scavenging system known as the *pho* regulon (Torriani-Gorini, 1987). The *pho* regulon consists of genes whose expression are co-regulated by the P status of the organism. These genes are activated when Pi becomes limiting and encode proteins that include phosphatases, a high-affinity Pi transporter, and other proteins involved in the transport and assimilation of Pi.

In plants, by contrast, little is known of the molecular mechanisms that regulate P nutrition. There is circumstantial evidence to show that plants possess an analogous system to the *pho* regulon (Goldstein et al., 1989). Pi transport into roots or cells in the culture of many species is enhanced severalfold by P deficiency and there is evidence that in some species the synthesis of a high-affinity Pi transporter is induced (Lefebvre and Glass, 1982; McPharlin and Bieleski, 1989; Lee et al., 1990; Mimura et al., 1990; Bieleski and Läuchli, 1992; Furihata et al., 1992). When P deficient, many species increase their acid-phosphatase activity in various plant parts (see Bieleski, 1973), as well as secreting acid phosphatases from roots or cells in culture (Ueki and Sato, 1971; Goldstein et al., 1988; Duff et al., 1991). A possible function of secreted acid phosphatases is to increase the availability of Pi by cleaving off phosphate groups esterified to organic compounds. In addition, several species excrete organic acids when P deficient to enable the dissolution of poorly soluble Pi-containing compounds, such as Ca-phosphates (Gardner et al., 1983; Lipton et al., 1987; Hoffland et al., 1992). These observations suggest that plants respond to P deficiency by activating specific mechanisms in an effort to increase P availability and uptake. Some of these responses to low P status may result from activation of specific genes, whereas others may be metabolically regulated.

As with other plant processes, analysis of mutants provides a way of dissecting the mechanisms involved in the P nutrition of plants. Poirier et al. (1991) described *pho1*, a mutation of *Arabidopsis thaliana*, which causes a defect in translocating Pi from roots to shoots. In this paper we describe a novel mutation of *Arabidopsis* at a locus designated *pho2*, which causes seedlings to accumulate excessive Pi concentrations in their leaves.

MATERIALS AND METHODS

Isolation of Mutants

Mutants were generated in the Col ecotype of *Arabidopsis* using ethyl methylsulfonate as the mutagen on about 50,000 seeds. An M_2 population was screened for mineral nutrition mutants using x-ray fluorescence spectrometry as described previously (Delhaize et al., 1993). Briefly, single leaves were pressed onto paper discs and analyzed for a range of elements using x-ray fluorescence spectrometry. Putative mutants with abnormal count rates for P were identified and grown to produce seeds. Only seedlings that showed an inherited abnormality were selected for further study. Mutants were backcrossed at least three times to wild-type plants before being used in physiological experiments.

Concentrations of Elements in Plant Material

The concentrations of Mg, P, Cl, S, K, and Ca were determined by x-ray fluorescence spectrometry (Norrish

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Abbreviations: CAPS, cleaved amplified polymorphic sequences; Col, Columbia ecotype; Ler, Landsberg *erecta* ecotype.

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and Hutton, 1977). For Mn, Fe, and Zn, samples were dry ashed at 520°C, taken up in 10 mL of 2.5% nitric acid, and analyzed by atomic absorption spectroscopy. Inorganic P was determined using acid extraction of fresh plant material by a method modified from Irving and Bouma (1984). One to five leaves were collected and weighed, and approximately 40 μL of 5 M H_2SO_4 was added per 20 mg of fresh sample. The samples were ground in a hand-held device and then 3 mL of water were added to the mixture. The resulting solution was filtered (Whatman No. 4 filter paper) and a subsample, ranging from 20 μL to 1.5 mL, depending on the Pi concentration, was analyzed for Pi. The subsample was made up to 1.5 mL with water, added to 0.5 mL of Malachite green reagent (Irving and McLaughlin, 1990), and then mixed vigorously. After at least 30 min the A_{650} of the solution was measured. Standards in the range of 125 to 500 ng of P as KH_2PO_4 were used. Roots and seedlings grown immersed in nutrient solution were washed in distilled water for 5 min, blotted dry, and then weighed before being analyzed for Pi as described above.

Growth of Seedlings

Seeds to be grown on soil were planted on a potting mixture of moderate fertility, pH 6.3, and high in composted organic matter in freely draining 15-cm-diameter pots. Seeds to be grown on sterile agar were surface sterilized by soaking for 20 min in a 1:1 mixture of ethanol:5% H_2O_2 and then planted in test tubes (165 mm height × 28 mm diameter) that contained 15 mL of agar and were subsequently plugged with sterile polystyrene wool. The agar was prepared by autoclaving 0.75% (w/v) agar (Bacto-Agar; Difco Laboratories, Detroit, MI) in a solution that contained 29 mM Suc, 2.5 mM KNO_3, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 1 mM KH_2PO_4, 25 μM $FeCl_3$, 25 μM Na_2EDTA, 88 μM H_3BO_3, 16 μM $MnCl_2$, 2.8 μM $ZnCl_2$, and 1.6 μM $CuCl_2$ (all at a final pH of 6.0). Analysis of the agar without added nutrients showed that a 0.75% (w/v) gel contained 30 μM P as contaminant. When the Pi concentration of the agar was varied, the change in K ions was not adjusted with KCl as was done for hydroponic culture experiments (see below).

Seedlings grown in hydroponic culture were germinated and grown on plugs of rockwool that were dipped into aerated nutrient solution (2.5 L of one-quarter strength of that used for agar-grown seedlings with both Suc and agar omitted). The containers used for hydroponic culture experiments were painted black and covered with aluminum foil to prevent algae from growing in the solutions. In experiments in which the Pi concentration of the nutrient solution was reduced to less than 250 μM, KCl was added to maintain the concentration of K ions. In these experiments the Pi concentrations of the nutrient solutions were monitored daily and replenished if they decreased by 10% or more. In a variation of the hydroponic culture method, seedlings were grown under sterile conditions fully immersed in nutrient solution of the same composition as agar-grown seedlings except that the agar was omitted. Four milligrams of seeds (approximately 200 seeds) were surface sterilized as described above and washed five times with 1-mL aliquots of water before being placed in a 150-mL flask that contained 20 mL of nutrient solution. The flask was plugged with polystyrene wool and incubated on an orbital shaker (110 rpm) at 23°C. After 10 d of growth, the nutrient solution was replaced with 40 mL of fresh nutrient solution. Continuous lighting was provided with fluorescent tubes (100–150 μmol photons m^{-2} s^{-1}) for seedlings grown on agar or by hydroponic culture, and the temperature was maintained at 23 C°.

Genetic Crosses

The P mutants were crossed to lines that carried phenotypic markers for the various chromosomes (lines were obtained from the Arabidopsis Biological Resource Center, Ohio State University, and included the following markers: *ap1*, *gl2*, *ch1*, *cp2*, *as1*, *hy2*, *gl1*, *bp1*, *ap2*, *yi1*, and *ttg1*). The P mutants were used as the female parents, and in an initial screen, 40 seedlings that showed the chromosomal markers in the F_2 generation were assayed for leaf Pi concentrations. To facilitate Pi assays, unweighed leaf plugs of 5.5 mm diameter were taken with a cork borer, and the resulting extracts from these leaf samples were not filtered prior to the Pi assay described above. After the initial screen showed that *pho2* was linked to *as1* on chromosome 2, a total of 133 seedlings with the *as1* phenotype were identified in the F_2 generation and their leaf Pi concentration was determined. To determine linkage to CAPS markers (Konieczny and Ausubel, 1993), the P mutants (Col genetic background) were crossed to wild-type seedlings of the Ler genetic background. The leaf Pi concentrations of individual seedlings in the F_2 generation were determined, and DNA from seedlings that showed the *pho2* phenotype was amplified using the CAPS primers as described by Konieczny and Ausubel (1993). To determine whether the three *pho2* mutants identified complemented one another genetically, the mutant used as the female parent also carried the *gl-2* mutation to confirm in the F_1 generation that the cross was successful. A similar method was used when the *pho1–1* mutant carrying the *gl-1* mutation was crossed to the *pho1–2* mutant. The progeny of these crosses was grown on soil, and leaf Pi concentrations were determined and compared to the parental genotypes and wild-type seedlings.

RESULTS

Isolation and Phenotype of Mutants

Identification of the mutants in the Col genetic background using x-ray fluoresence spectrometry of single leaves pressed onto filter paper has previously been described (Delhaize et al., 1993). Initially, seedlings were assessed visually (two independent M_2 populations of approximately 50,000 each) and those that showed phenotypes with symptoms consistent with a nutrient toxicity or deficiency (Reuter and Robinson, 1986) were then analyzed by x-ray fluorescence spectrometry (approximately 2000 seedlings). Three seedlings that gave higher than normal count rates for P and one seedling with a low count rate for P were identified. We confirmed that the traits were in-

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herited, and crosses between the P-accumulator mutants showed that they were unable to genetically complement one another. Two of the mutants were isolated from independent populations, and the loci were named *pho2–1* and *pho2–2*, to denote that they are alleles. From the number of seedlings identified in this study we were unable to determine the frequency of mutation, since it later became apparent that visible symptoms of P toxicity on *pho2* mutants were dependent on the transpiration rate. Because the seedlings were grown in a greenhouse where the transpiration rate was not controlled, not all mutants would have been identified. Only *pho2–1* was characterized in further detail.

The mutation that caused the low P content in leaves was designated *pho1–2* because it was found to be an allele of *pho1–1*, a mutant previously described by Poirier et al. (1991). When grown under high transpiration conditions on soil, the margins of *pho2* leaves became necrotic, symptoms that are typical of P toxicity, and under extreme conditions, the whole leaf senesced. Wild-type seedlings were unaffected when grown under the same conditions, and *pho1–2* seedlings were dark green with purple petioles, symptoms typical of P deficiency. Analysis of Pi concentrations in leaves confirmed that the *pho2* mutants consistently accumulated more Pi than wild-type seedlings. In leaves of seedlings grown on agar with all nutrients supplied, *pho2* mutants had 2.28 ± 0.08 mg Pi g^{-1} fresh weight (mean ± SE, n = 35), wild-type seedlings had 0.66 ± 0.02 mg Pi g^{-1} fresh weight (n = 101), and the *pho1–2* mutant had 0.05 ± 0.01 mg Pi g^{-1} fresh weight (n = 23). *pho2* mutants flowered at about the same time as did wild-type seedlings (*pho2* seedlings bolted 35.1 ± 2.2 d [n = 24] versus 35.7 ± 3.7 d [n = 24] for wild-type seedlings) but were generally smaller (approximately 50% fresh weight of wild type after 33 d growth on soil).

Genetic Analysis of Mutants

Both the *pho2* and *pho1–2* mutations were recessive and segregated in F_2 populations as single loci (Table I). The *pho2* and *pho1–2* mutants genetically complemented one another, since the F_1 resulting from a cross between these mutants all had the phenotype of wild-type seedlings. The *pho2* locus was linked to *as1* on chromosome 2 (of 133 *as1* mutants identified in the F_2 generation of a *pho2* by *as1* cross, none showed a *pho2* phenotype based on Pi assays). Linkage to chromosome 2 was confirmed with the CAPS marker *m429* (31 of 32 *pho2* mutants identified in the F_2 generation of a Ler by *pho2* cross were homozygous for the Col allele; one seedling was heterozygous). *pho1* mapped to chromosome 3 and showed linkage to the CAPS marker *GAPC* (from 26 *pho1* mutants identified in an F_2 population resulting from a cross between a *pho1* mutant and a Ler seedling, 19 were homozygous for the Col allele and 7 were heterozygous Col/Ler).

To identify a *pho1/pho2* double mutant, an F_2 population resulting from a cross between a *pho2* mutant and a *pho1* mutant was analyzed. Seedlings in this population segregated with phenotypes based on the Pi content of leaves with ratios that had the best fit for 9:4:3, wild type:*pho1*:*pho2* (Table I). However, there is a relatively low probability that sampling error accounts for the discrepancy between the predicted values and the actual values. Analysis of larger numbers of seedlings may have resolved this discrepancy, but the data suggested that a *pho1/pho2* double mutant has a *pho1* phenotype and this was subsequently confirmed by genetic analysis. To identify a double mutant (i.e. *pho1pho1/pho2pho2*) in the F_2 population among other seedlings with a *pho1* phenotype, i.e. those that were either heterozygous for *PHO2* (i.e. *pho1pho1/pho2PHO2*) or homozygous for *PHO2* (i.e. *pho1pho1/PHO2PHO2*), *pho1* seedlings were used as the female parents when crossed to a *pho2* mutant. Analysis of the F_1 population identified a *pho1* parent that produced *pho2* progeny only (n = 24) and, therefore, must have been a double mutant, since the other possible genotypes would have resulted in seedlings either being all wild-type or segregating 1:1 for *pho2* and wild type.

Elemental Composition of *pho2* and Wild-Type Seedlings

Leaves of soil-grown seedlings were analyzed for their mineral nutrient composition to determine whether the *pho2* mutation specifically affected P nutrition. After 23 d of growth, *pho2* mutants did not show visible symptoms of P toxicity and yet had twice the total P of wild-type seedlings (Table II; Fig. 1A). At the second harvest (30 d) *pho2* seedlings showed mild symptoms of P toxicity (marginal necrosis of leaves), and by the final harvest (35 d) the symptoms were severe (some leaves totally necrotic). Total P concentrations in *pho2* mutants increased at each subsequent harvest (Fig. 1A), consistent with the observed in-

Table I. *Segregation ratios of progeny resulting from genetic crosses among pho1–2, pho2, and wild-type (WT) seedlings*

Genotypes Crossed and Generation Screened	Phenotype of Progeny[a]			Ratio Tested	χ^2	P[b]
	Wild type	*pho2*	*pho1–2*			
WT × *pho2* F_2	101	35		3 WT:1 *pho2*	0.04	0.7–0.8
WT × *pho1–2* F_2	50		13	3 WT:1 *pho1–2*	0.75	0.3–0.5
pho2 × *pho1–2* F_2	62	16	38	9 WT:3 *pho2* :4 *pho1–2*	4.57	0.1–0.2
				10 WT:3 *pho2* :3 *pho1–2*	14.67	<0.01
				9 WT:4 *pho2* :3 *pho1–2*	17.61	<0.01

[a] Seedlings with Pi concentrations greater than 0.2 but less than 1.4 mg g^{-1} fresh weight were classed as wild type, those with more than 1.4 mg Pi g^{-1} fresh weight were classed as *pho2*, and those with less than 0.2 mg Pi g^{-1} fresh weight were classed as *pho1–2*. [b] Probabilities of departure from the stated ratios are due to sampling error.

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Table II. *Concentrations of various mineral nutrients in leaves of wild type and pho2 mutants grown on soil for 23 d*

Leaf Type	Mg	P	S	Cl	K	Ca	Mn	Fe	Zn
			% dry wt[a]				μg g^{-1} dry wt[a]		
Wild type	0.52 ± 0.05	0.68 ± 0.04	0.78 ± 0.09	0.08 ± 0.01	4.21 ± 0.48	3.19 ± 0.23	46 ± 3	98 ± 4	135 ± 4
pho2	0.51 ± 0.05	1.45 ± 0.08	0.86 ± 0.08	0.10 ± 0.01	4.03 ± 0.24	3.28 ± 0.14	46 ± 5	131 ± 60	132 ± 4

[a] Mean concentrations in leaves taken from three replicate pots ± SD.

crease in severity of P toxicity symptoms. Pi concentrations showed larger percentage differences between genotypes than total P concentrations but followed a similar trend with each harvest (Fig. 1B). If we assume that the shoot dry weight is approximately 10% of the fresh weight, most of the excess P in *pho2* mutants could be accounted for by the higher Pi concentrations. *pho2* mutants also had greater total P (Fig. 2) and Pi (data not shown) concentrations in stems, siliques, and seeds than wild-type seedlings.

None of the other elements analyzed at the first harvest had concentrations that were significantly different from wild-type seedlings (Table II). Although Ca concentrations in leaves of *pho2* mutants were similar to wild-type seedlings at the first harvest, *pho2* mutants had greater concentrations at the second and third harvests (Fig. 3). However, these differences were much less pronounced than the differences in total P concentrations (Fig. 1A). None of the other macronutrients analyzed differed markedly between wild type and *pho2* mutants at the later harvests (data not shown).

Effect of Pi Supply and Transpiration Rate on Pi Accumulation in Leaves and Roots

The Pi concentration supplied to seedlings had little effect on leaf Pi concentrations of either *pho2* mutants or wild-type seedlings grown in hydroponic culture (Fig. 4). Over a range encompassing a 50-fold change in Pi concentrations supplied, *pho2* mutants maintained greater Pi concentrations in their leaves than did wild-type seedlings. This was also evident in agar-grown seedlings in which the Pi concentration of leaves of both wild type and *pho2* mutants remained stable over Pi supplies ranging from 0.2 to 10 mM (data not shown). Although *pho1* mutants usually had about one-tenth the Pi concentration in leaves compared to wild-type seedlings when grown on agar medium, a high concentration of Pi supplied overcame this block in Pi transport to shoots (0.05 ± 0.01 mg g^{-1} fresh weight Pi at 0.2 mM, 1 mM, and 5 mM Pi supply; 0.42 ± 0.05 mg g^{-1} fresh weight at 10 mM Pi supply). This observation is similar to that described by Poirier et al. (1991).

The severity of P toxicity in the *pho2* mutant appeared to be related to the transpiration rate. Seedlings grown on soil in a glasshouse during periods when cool, overcast days predominated (low transpiration conditions) showed either no sign of P toxicity or only mild symptoms. The same seedlings developed severe toxicity symptoms when they were subsequently grown under artificial lighting and were cooled by a fan to cause high transpiration conditions (data not shown). *pho2* mutants grown in hydroponic culture under lights and air cooled with a fan also showed severe P toxicity symptoms and accumulated leaf Pi con-

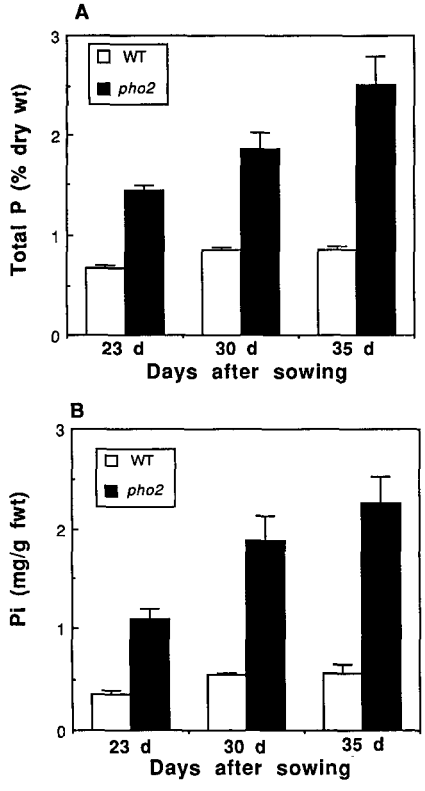

Figure 1. Total P concentration (A) and Pi concentrations (B) in leaves of wild type (WT; empty bars) and *pho2* mutants (filled bars) grown on soil for 23, 30, and 35 d. Error bars denote the SE of the mean from seedlings of three replicate pots. fwt, Fresh weight.

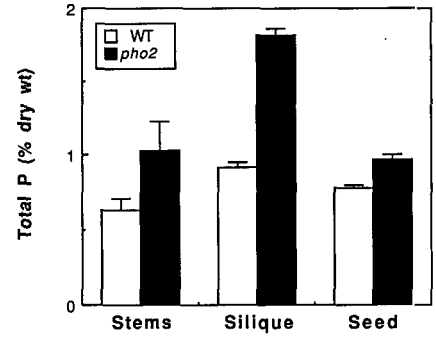

Figure 2. Total P concentrations in stems, green siliques, and mature seeds of wild type (WT; empty bars) and *pho2* mutants (filled bars) grown on soil for 35 d. Error bars denote the SE of the mean from seedlings of three replicate pots. wt, Weight.

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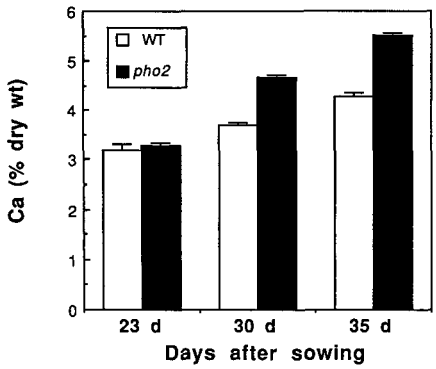

Figure 3. Total Ca concentrations in leaves of wild type (WT; empty bars) and *pho2* mutants (filled bars) grown on soil for 23, 30, and 35 d. Error bars denote the SE of the mean from seedlings of three replicate pots. wt, Weight.

centrations in excess of 2 mg g^{-1} fresh weight (Fig. 5A). Under the same conditions wild-type seedlings grew vigorously and had approximately 0.6 mg Pi g^{-1} fresh weight in leaves, whereas leaves of *pho1* mutants were P deficient and had less than 0.1 mg Pi g^{-1} fresh weight. In contrast to the high Pi concentrations in shoots of *pho2* mutants, the concentration in the roots was similar or slightly less than in wild-type seedlings (Fig. 5A). *pho1* mutants had the greatest Pi concentrations in roots of the three genotypes grown in hydroponic culture (Fig. 5A).

To determine whether a high transpiration rate was necessary to obtain the *pho2* phenotype, seedlings were grown fully immersed in nutrient solution to stop all transpiration. If a high transpiration rate was necessary for the high Pi phenotype, then in conditions in which no transpiration was occurring we would expect the *pho2* mutant to have similar Pi concentrations in its leaves to wild-type seedlings. Figure 5B shows that *pho2* seedlings still accumulated more Pi in shoots compared to wild-type seedlings when grown fully immersed. Although the difference was not as great as that obtained for seedlings grown under high transpiration conditions, this result indicates that the *pho2* phenotype is not entirely dependent on a high transpiration rate. *pho1* mutants grown immersed in nutrient solution had greater Pi concentrations in shoots than seedlings

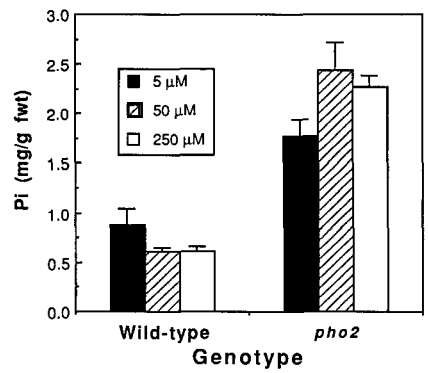

Figure 4. Concentrations of Pi in leaves of wild-type and *pho2* mutants grown with different Pi supplies. Seedlings were grown in hydroponic culture for 23 d. Error bars denote the SE of the mean of four seedlings. fwt, Fresh weight.

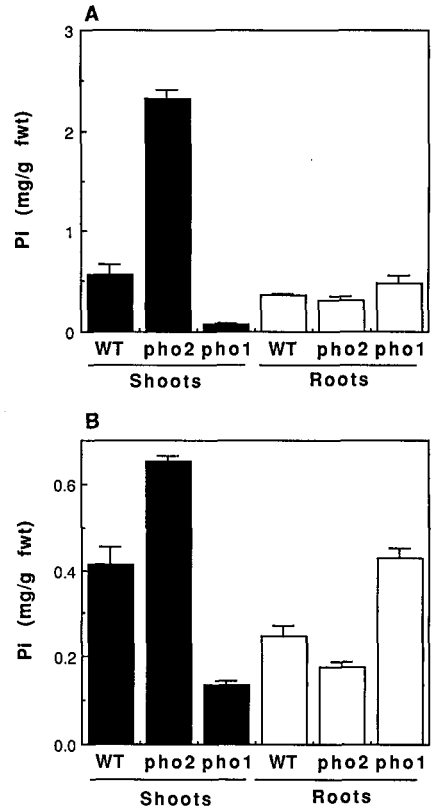

Figure 5. Concentrations of Pi in shoots (filled bars) and roots (empty bars) of wild-type seedlings (WT), *pho2* mutants, and *pho1* mutants grown in hydroponic culture for 21 d (A) or grown immersed in nutrient solution for 13 d (B). Error bars denote the SE of the mean of either four replicate seedlings (A) or seedlings from three replicate flasks (B). fwt, Fresh weight.

grown by hydroponic culture (cf. with Fig. 5A) but still had considerably lower Pi concentrations than either wild-type or *pho2* seedlings. By contrast, root Pi concentrations showed an inverse relationship to leaf Pi concentrations as observed for seedlings grown by hydroponic culture: *pho2* mutants had the lowest root Pi concentration, whereas *pho1* mutants had the greatest root Pi concentration (Fig. 5).

Mobility of Pi in *pho2* Mutants

P is considered to be a mobile element in plants and moves freely in xylem and phloem (Bieleski, 1973). It is possible that *pho2* mutants had a defect in retranslocating Pi from leaves, possibly because of an inability to load Pi from leaves into the phloem, and this may explain the high concentrations of Pi in leaves. The continual accumulation of Pi into shoots may be a consequence of an inability to remobilize Pi back to roots to switch off the uptake of Pi. To test the ability of *pho2* mutants to remobilize Pi, seedlings were grown under normal conditions to ensure that leaves became loaded with Pi and then were transferred to nutrient solution that lacked Pi. As P deficiency developed, Pi was retranslocated from old leaves of both wild-type seedlings and *pho2* mutants (Table III), indicating that the defect in *pho2* mutants did not appear to be due to an inability to

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load Pi into the phloem of such leaves. Necrotic regions on leaves of *pho2* mutants were avoided for the Pi assays since these regions retained high Pi concentrations and did not retranslocate the Pi. With prolonged growth in nutrient solution that lacked Pi, leaves of *pho2* mutants that had shown P toxicity symptoms when grown in the presence of Pi became severely P deficient and showed symptoms of P deficiency (data not shown).

DISCUSSION

Plants normally regulate the concentration of Pi in their leaves within certain limits. For example, wild-type *Arabidopsis* seedlings in this study accumulated approximately the same concentration of Pi in leaves over a wide range of Pi concentrations supplied (Fig. 4). By contrast, the *pho2* mutant accumulated 2- to 5-fold greater Pi in shoots compared to wild-type seedlings under the same range of Pi supplies. The concentrations of P accumulated in leaves of *pho2* mutants are in the range that have been determined to be high or toxic for many plant species (Reuter and Robinson, 1986). We propose that the *pho2* mutation deregulates the control that is normally exerted on the amount of Pi accumulated into shoots of *Arabidopsis*. The specificity of the mutation in increasing P concentrations of leaves (Table II) supports the hypothesis that the wild-type gene is primarily involved in regulating Pi within plants and not mineral nutrients in general. The *pho1* phenotype of the *pho1/pho2* double mutant is consistent with the hypothesis that *pho1* results in inefficient loading of Pi into the xylem (Poirier et al., 1991), whereas *pho2* results in defective regulation of the amount of Pi accumulated into shoots. If Pi is being inefficiently loaded into the xylem, then the double mutant is unable to show the *pho2* phenotype. The *pho1* mutants that were grown immersed in nutrient solution containing 1 mM Pi did not accumulate Pi in shoots to the concentrations present in wild-type shoots (Fig. 5B). This suggests either that transport of Pi directly from the nutrient solution into shoots is relatively inefficient or that *pho1* affects some function of Pi transport in shoots in addition to the defective loading of Pi into xylem of roots.

In some respects the excessive accumulation of Pi into shoots of *pho2* seedlings is similar to that observed in Zn-deficient seedlings of several species (Cakmak and Marschner, 1986; Marschner and Cakmak, 1986; Webb and Loneragan, 1988). Marschner and Cakmak (1986) suggested that leaves of Zn-deficient plants were deregulated for Pi uptake because leaves were unable to retranslocate Pi back to roots via the phloem, which under normal conditions would switch off Pi uptake by roots. They postulated that Zn plays a critical role in providing the signal to recycle Pi from shoots. It was possible that *pho2* mutants were Zn deficient because of a defect in Zn uptake, which resulted in the observed deregulated Pi accumulation into shoots. However, Zn concentrations in leaves of *pho2* mutants were similar to those of wild-type seedlings (Table II) and well within the range considered adequate for maximal growth of many plant species (Reuter and Robinson, 1986). Furthermore, *pho2* mutants were able to retranslocate Pi out of old leaves when seedlings were deprived of a Pi supply, indicating that Pi was still able to move freely through the phloem under these conditions. However, the retanslocated Pi in *pho2* mutants may have remained in the shoot, e.g. if it was taken up by young leaves, and experiments using radiolabeled Pi are required to determine whether any of the retranslocated Pi, finds its way to roots.

Coincident with the appearance of P toxicity symptoms, Ca concentrations in leaves of *pho2* mutants were greater than in wild-type seedlings. It is possible that Ca was acting as a counterion for Pi, since there was no other macronutrient cation whose concentration increased with the increased Pi concentrations. However, at the first harvest shown in Figure 3, Ca concentrations in *pho2* mutants were the same as in wild-type seedlings, and it was only at later harvests that Ca concentrations increased above wild-type concentrations. It is probable that at the first harvest the increased Pi accumulation was compensated for by decreased synthesis of organic acid anions within the plant to maintain electroneutrality. Changes in the synthesis of organic acid anions within plants to maintain electroneutrality is a well-documented process (Kennedy, 1986).

Several authors have suggested that uptake rates of mineral nutrients in plants are regulated by their internal concentrations through negative feedback mechanisms (Glass, 1983; Clarkson and Lüttge, 1991). Uptake of a specific nutrient is enhanced when its concentration is low in the plant and repressed when internal concentrations increase. This model suggests that shoots indirectly control nutrient uptake by roots by recycling nutrients through the phloem back to roots when nutrients are in quantities greater than requirements for plant growth. Under most conditions Pi is taken up by roots through an active transport mechanism and the Pi concentration can be in the order of 10,000-fold greater inside root cells compared to the external solution (Bieleski, 1973). The processes involved in unloading Pi from xylem vessels to the apoplast and then uptake by leaf cells are not fully understood, but it is likely that Pi uptake into leaf cells also requires active transport (Mimura et al., 1990). The excessive accumulation of Pi in *pho2* shoots might be due to a Pi transporter, either in root cells or in shoot cells, that does not respond to high Pi concentrations in shoots and continues to transport Pi beyond require-

Table III. *Pi concentrations in old leaves after transfer of seedlings to nutrient solutions that lacked Pi*

Treatment	Days of Growth	
	23	27
	mg Pi g^{-1} fresh wt in old leaves[a]	
Wild type (control)[b]	0.62 ± 0.04	0.83 ± 0.04
Wild type (± Pi)[c]	0.61 ± 0.05	0.16 ± 0.03
pho2 (control)[b]	2.44 ± 0.29	2.76 ± 0.12
pho2 (± Pi)[c]	2.27 ± 0.11	0.52 ± 0.09

[a] Old leaves are defined as fully expanded leaves present before transfer of seedlings to new solutions. [b] Seedlings were grown with a 250 μM Pi supply during the course of the experiment. [c] Seedlings were grown with a 250 μM Pi supply during the initial 23 d of growth and then transferred to nutrient solutions that lacked Pi for an additional 4 d.

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ments. The defect in *pho2* mutants may be in a gene encoding Pi transport or in a gene encoding a protein that senses the Pi concentration and in turn regulates the activity of a Pi transporter.

The molecules involved in regulating Pi uptake by roots and shoots are not known. An increased capacity for Pi influx across the plasmalema of root cells when plants become P deficient precedes any detectable decrease in cytoplasmic Pi concentration, suggesting either that a decline in cytoplasmic Pi concentration is not the trigger inducing this increased transport capacity (Lee et al., 1990) or that only small changes in cytoplasmic Pi concentrations are required to elicit the response. There is evidence that a sensory function also acts at the tonoplast. In roots or shoots of several species it was shown that the vacuole acts as a store for Pi when it is in excess of requirements, whereas the cytosolic Pi concentration is kept relatively constant under conditions ranging from deficient to excessive Pi supply (Lauer et al., 1989; Lee et al., 1990; Mimura et al., 1990). The concentration of Pi in vacuoles responds to the P status of the plant and is diverted to the cytoplasm under conditions of P deficiency. The signals that cause Pi transport across the tonoplast to switch from uptake into the vacuole (generally by passive transport) to efflux into the cytoplasm (requiring active transport) are also unknown. Further analysis of the *pho2* mutants and other yet-to-be identified *Arabidopsis* mutants in P nutrition will serve to clarify the roles of genes involved in regulating the uptake and homeostasis of Pi in plant cells.

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