WRKY75 Transcription Factor Is a Modulator of Phosphate Acquisition and Root Development in Arabidopsis

Ballachanda N. Devaiah, Athikkattuvalasu S. Karthikeyan, and Kashchandra G. Raghothama*
Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, Indiana 47907–1165

Phosphate (Pi) deficiency limits plant growth and development, resulting in adaptive stress responses. Among the molecular determinants of Pi stress responses, transcription factors play a critical role in regulating adaptive mechanisms. WRKY75 is one of several transcription factors induced during Pi deprivation. In this study, we evaluated the role of the WRKY75 transcription factor in regulating Pi starvation responses in Arabidopsis (Arabidopsis thaliana). WRKY75 was found to be nuclear localized and induced differentially in the plant during Pi deficiency. Suppression of WRKY75 expression through RNAi silencing resulted in early accumulation of anthocyanin, indicating that the RNAi plants were more susceptible to Pi stress. Further analysis revealed that the expression of several genes involved in Pi starvation responses, including phosphatases, Mt4/TPS1-like genes, and high-affinity Pi transporters, was decreased when WRKY75 was suppressed. Consequently, Pi uptake of the mutant plant was also decreased during Pi starvation. In addition, when WRKY75 expression was suppressed, lateral root length and number, as well as root hair number, were significantly increased. However, changes in the root architecture were obvious under both Pi-sufficient and Pi-deficient conditions. This indicates that the regulatory effect of WRKY75 on root architecture could be independent of the Pi status of the plant. Together, these results suggest that WRKY75 is a modulator of Pi starvation responses as well as root development. WRKY75 is the first member of the WRKY transcription factor family reported to be involved in regulating a nutrient starvation response and root development.

Phosphate (Pi) is one among the least-available macronutrients required by plants. It is a constituent of key molecules such as ATP, nucleic acids, and phospholipids. It plays a crucial role in energy transfer, metabolic regulation, and protein activation (Marschner, 1995). Although abundant phosphorus (P) is present in many soils, very little of it is present in ionic forms that are available to plants. Consequently, plants have evolved several adaptive mechanisms by way of altered morphology, physiology, and biochemical processes that help them cope with Pi deficiency (Bucher et al., 2001; Raghothama and Karthikeyan, 2005). These biochemical and physiological adaptations, such as elevated phosphatase activity (Lipton et al., 1987) and secretion of organic acids (Marschner, 1995), help augment the availability of both endogenous and exogenous Pi. In addition, developmental responses involving changes in root growth and architecture enhance the exploration of soil Pi resources (López-Bucio et al., 2003).

Over the past decade, many genes, including Pi transporters, phosphatases, RNases, and others of unknown function that help plants adapt to Pi stress, have been characterized. It is also becoming clear that the acquisition, allocation, and metabolism of Pi are highly regulated processes that directly affect plant performance (Franco-Zorilla et al., 2004). Microarray analysis of 22,810 Arabidopsis (Arabidopsis thaliana) genes revealed a coordinated induction and suppression of 612 and 254 Pi-responsive genes, respectively (Misson et al., 2005). While details of the adaptive mechanisms in response to Pi starvation are emerging, the molecular determinants that regulate these adaptive processes have yet to be clearly elucidated. Among the known regulators, miRNA mir399, representing a class of noncoding small RNAs that generally function as posttranscriptional negative regulators, has been implicated in regulating Pi homeostasis (Bari et al., 2006; Chiou et al., 2006). SIZ1, a SUMO E3 ligase, is also known to control Pi homeostasis at the posttranslational level through sumoylation (Miura et al., 2005). At the transcriptional level, the spatiotemporal regulation of transcription factors responsive to Pi starvation has been described by microarray analysis in rice (Oryza sativa) and Arabidopsis (Wasaki et al., 2003; Wu et al., 2003; Misson et al., 2005). A potential transcription factor candidate, Phi-2, coding for a bZIP transcription factor in tobacco (Nicotiana tabacum), was reported to be induced during Pi starvation earlier (Sano and Nagata, 2002). Despite these developments, to date only two transcription factors involved in regulating Pi starvation responses in higher plants have been examined in detail. The Myb transcription factors...
factor PHR1 was first reported to play a regulatory role in Pi starvation responses in Arabidopsis (Rubio et al., 2001). More recently, OsPTF1, a bHLH transcription factor, has been shown to provide tolerance to Pi starvation in rice (Yi et al., 2005). However, PHR1 expression is not responsive to Pi starvation and it controls only a small subset of Pi starvation responses, whereas OsPTF1 does not regulate any known high-affinity Pi transporter. A transcriptional regulatory model for the Pi starvation responses in plants has been proposed on the basis of microarray analysis (Hammond et al., 2004). Thus, the regulation of the global Pi starvation responses by transcription factors is an interesting area of study that needs further attention.

Previously, we analyzed the expression patterns of Pi stress-responsive genes, including transcription factors, in Arabidopsis through microarray analysis (Misson et al., 2005). Among the putative candidates, WRKY75, a transcription factor, was strongly induced during Pi deprivation. Members of the plant-specific WRKY transcription factor family are known to be involved in regulating many plant processes, including biotic and abiotic stresses. An analysis of the expression profiles of all the members of the WRKY gene family suggested that the expression of WRKY75 is also induced during pathogen infection (Dong et al., 2003). Although most WRKY proteins studied thus far have been implicated in regulating biotic stress responses, several of them play a role in the regulation of abiotic stresses. In barley (Hordeum vulgare), a transcription factor belonging to the WRKY family, SUSIBA2 (a sugar-responsive element binding factor), was shown to be involved in sugar signaling (Sun et al., 2003). Interestingly, sugar signaling has been implicated in the regulation of Pi starvation responses and root architecture in Arabidopsis (Müller et al., 2005; Karthikeyan et al., 2007). Another WRKY gene in barley, Hv-WRKY38, is involved in regulating cold and drought stress response (Mare et al., 2004). In Arabidopsis, microarray studies have suggested a role for WRKY transcription factors in drought, cold, or high salinity stress (Seki et al., 2002). Several WRKY transcription factors, such as WRKY4, 6, 11, and 53, of Arabidopsis have also been shown to play a role in leaf senescence (Eulgem et al., 2000; Robatzek and Somssich, 2001; Miao et al., 2004). The involvement of WRKY75 during senescence has also been suggested based on a microarray analysis (Guo et al., 2004) as well as unpublished data (Y. Guo and S. Gan). This is particularly interesting since leaf senescence and anthocyanin accumulation are often observed during nutrient stress (Buchanan-Wollaston et al., 2003).

In this study, the function of WRKY75 is investigated and its characterization as a regulator of Pi stress responses is reported. We demonstrate that WRKY75 is responsive to Pi stress and is nuclear localized. RNAi suppression of WRKY75 resulted in impaired Pi starvation responses. In contrast, the same mutant shows increased root growth independent of the Pi status of the plant. These results indicate that WRKY75 is a positive regulator of Pi starvation responses besides having an impact on root development. To our knowledge, this is the first report showing a WRKY transcription factor involved in regulation of nutrient starvation responses in plants.

RESULTS WRKY75 Responds to Pi Starvation and Is Nuclear Localized

The relative transcript abundance of WRKY75 in mature plants as well as young seedlings grown under Pi-sufficient (P+) or Pi-deficient (P−) conditions was evaluated (Fig. 1A). Although a basal level of WRKY75 transcripts was observed under Pi-sufficient conditions, during Pi deprivation there was a distinct but differential increase in the transcript’s abundance in different parts of the plants. This highlights the spatial regulation of WRKY75 by Pi. Upon transferring Pi-deprived plants into medium with sufficient Pi, a decrease in the elevated transcript levels was observed within 3 h (Supplemental Fig. S1). These results suggest that WRKY75 is among the early induced genes that respond to altered Pi status of the plant.

It is known that nutrient starvation responses could be regulated by controlling the subcellular localization of a transcription factor (Beck and Hall, 1999). Therefore, to identify the subcellular localization of WRKY75, its coding region was fused with the 3’ end of an ENHANCED GREEN FLUORESCENT PROTEIN (EGFP) reporter gene and expressed constitutively under the control of a strong cauliflower mosaic virus (CaMV) 35S promoter. The EGFP gene alone under the control of the CaMV 35S promoter served as a control. Transgenic Arabidopsis plants expressing the control EGFP gene and the chimeric WRKY::EGFP gene were analyzed under both Pi-sufficient and Pi-deficient conditions. In the control transgenic plant, GFP fluorescence was uniformly distributed all over the cell, whereas, in the plants with the WRKY75-GFP protein, fluorescence was localized in the nucleus (Fig. 1B). The WRKY75-GFP fusion protein was localized to the nucleus irrespective of the Pi regimen under which the plants were grown. Further, WRKY transcription factors are defined by the presence of a highly conserved WRKY domain and a novel zinc finger motif in their amino acid sequence. An examination of the amino acid sequence of WRKY75 revealed the presence of a single WRKY domain as well as the characteristic C2H2 zinc finger motif located at the C-terminal end (Fig. 1C). Together, these data suggest that WRKY75 is a Pi starvation-responsive transcription factor localized in the nucleus.

Suppression of WRKY75 Expression through RNAi-Mediated Silencing

To characterize the role of WRKY75 during Pi starvation, RNAi mutant plants harboring inverted repeats
of a WRKY75 fragment under the control of a CaMV 35S promoter were generated. Silencing of WRKY75 in the RNAi plants was confirmed by semiquantitative reverse transcription (RT)-PCR analysis of the wild-type control and two independent RNAi lines grown under different Pi regimens (Fig. 2). Amplifying the first-strand cDNA through 25 cycles of PCR indicated the absence of any WRKY75 transcripts in the RNAi plants, suggesting the silencing of the gene. However, when 2 μL of the product from this PCR was used as the template for a second PCR with an additional 25 cycles, faint DNA bands were observed in the lanes representing the RNAi samples. Strong DNA bands were also observed in lanes representing the wild-type plants grown under Pi-sufficient conditions after the second set of PCR cycles (data not shown). The presence of WRKY75 transcripts in the wild-type plants during Pi-sufficient conditions was consistent with the results of northern analysis (Fig. 1A). On the other hand, the presence of a few copies of the WRKY75 transcripts in the RNAi plants suggests that the expression of WRKY75 is drastically reduced, but not completely silenced, in the RNAi lines. A representative RNAi line showing almost complete suppression of WRKY75 was selected from among 10 lines for subsequent characterization. In addition, a SALK T-DNA insertion mutant for WRKY75 (kindly provided by Dr. Z. Chen, Department of Botany and Plant Pathology, Purdue University) was used as an internal control for most of the experiments and showed similar results as the RNAi line (data not shown).

Suppression of WRKY75 Leads to Early Accumulation of Anthocyanin during Pi Deprivation

One of the most striking symptoms of Pi starvation in plants is the accumulation of anthocyanin (Marschner, 1995). When WRKY75 RNAi and the wild-type plants were grown in petri dishes on medium without Pi, it was observed that WRKY75 RNAi plants turned purple by the third day of Pi starvation, whereas the wild-type plants were still green (Fig. 3A). Comparative analysis of the anthocyanin content in 10-d-old WRKY75 RNAi and the wild-type seedlings was done after growing them for 3 d on medium with (1 mM) or without Pi (Fig. 3B). The results indicated that the anthocyanin content in the RNAi seedlings was approximately 5-fold higher than in the wild-type seedlings after 3 d of Pi deprivation. The anthocyanin content in the RNAi seedlings grown under Pi-sufficient conditions did not vary significantly in comparison to the wild type. The wild-type plants accumulated similar levels of anthocyanin after three more days of Pi deprivation (data not shown). The data indicate that the suppression of WRKY75 accelerates the process of anthocyanin accumulation during Pi starvation. Further, it was observed that the accelerated accumulation of anthocyanin in RNAi seedlings did not occur during nitrogen,
Potassium, or iron deficiency, indicating that it was specific to Pi starvation (data not shown). This suggested that some of the adaptive mechanisms to Pi stress could be impaired by the suppression of WRKY75 in the plant.

Pi Starvation-Induced Genes Are Attenuated upon Suppression of WRKY75

Global gene expression analysis using microarray techniques has shown an array of spatiotemporally regulated Pi-responsive genes involved in Pi mobilization and acquisition from the soil and its remobilization within the plant (Misson et al., 2005). Therefore, to elucidate the molecular components involved in triggering the early Pi stress response in WRKY75 RNAi plants, expression of some of the well-characterized Pi-responsive genes involved in maintaining Pi homeostasis was evaluated in both the wild-type and WRKY75 RNAi plants grown with Pi and without Pi on the third day of Pi starvation. Values are mean ± st (n = 5), and different letters above the bars indicate that the means are statistically different (P < 0.05).

[See online article for color version of this figure.]

Figure 3. Anthocyanin accumulation in WRKY75 RNAi plants during Pi deprivation. Seven-day-old seedlings of wild-type and WRKY75 RNAi plants grown on half-strength MS medium were transferred into MS medium in petri dishes with Pi (1 mM) or without Pi (P−). Images and anthocyanin content were recorded on the third day of treatment. A, The seedlings grown in P− conditions were scanned at 600 dpi, showing the early accumulation of anthocyanin in the hypocotyl and cotyledonary leaves of the WRKY75 RNAi plants as compared to the wild type (WT). The samples are representative of 15 seedlings of each genotype. B, Anthocyanin content was determined in wild-type and WRKY75 RNAi plants grown with Pi and without Pi on the third day of Pi starvation. Values are mean ± se (n = 5), and different letters above the bars indicate that the means are statistically different (P < 0.05).

Pi Starvation-Induced Genes Are Attenuated upon Suppression of WRKY75

To find if defective mobilization of Pi from the media was the cause of early Pi stress symptoms in the mutant, the expression of AtPS2-1, AtPS2-2, and AtPS2-3, encoding three members of a phosphatase family specifically induced by Pi stress (K.G. Raghothama, unpublished data), which are orthologs of the LePS2 gene family from tomato (Solanum lycopersicum; Baldwin et al., 2001), was evaluated. In addition, the expression of AtACP5, encoding an acid phosphatase involved in Pi stress responses (Del Pozo et al., 1999), was also evaluated. There was a complete suppression of AtPS2-1 expression, significant reduction in the transcript levels of AtPS2-2, and no change in the AtPS2-3 and AtACP5 transcripts in the mutant as compared to the wild-type plants. These results showed that suppression of WRKY75 had differential effects on the genes involved in Pi mobilization. Recent studies on at4, a loss-of-function mutant, revealed the role of At4, a member of the Mt4/TPSI1 gene family, in Pi distribution between roots and shoots (Shin et al., 2006). We
Reduced Pi Uptake by the WRKY75 RNAi Mutant during Pi Deprivation

Pi uptake experiments were carried out to further decipher the effect of an impaired Pi starvation response caused by the suppression of WRKY75. The wild-type plants and WRKY75 RNAi mutants were grown under Pi-sufficient or Pi-deficient conditions. Ten-day-old seedlings were transferred into a Pi uptake solution containing 50 µM Pi supplemented with 33P, a radiotracer. Pi uptake over a 2-h period was measured (Fig. 5). There was no significant difference in Pi uptake of the wild-type and WRKY75 RNAi plants grown under Pi-sufficient conditions (Fig. 5A). In contrast, WRKY75 RNAi plants showed a substantial reduction in Pi uptake relative to the wild type when the plants were grown under Pi-deficient conditions (Fig. 5B). The suppression of WRKY75 thus appears to have an effect on Pi uptake rate during Pi deprivation, but not during normal conditions, when the Pi starvation response mechanism is not active. This result was consistent with the reduced expression of several genes involved in Pi homeostasis observed in the WRKY75 mutant (Fig. 4). Together, these data suggest that WRKY75 functions as a positive regulator of the Pi starvation response mechanism, possibly through the control of various genes involved in the process. In an in planta labeling experiment, 10-d-old wild-type and WRKY75 RNAi seedlings were allowed to absorb 33P in the uptake solution for 4 h and the pattern of distribution of the radiotracer was monitored after 10 d (Supplemental Fig. S2). The results indicated no difference in the pattern of movement of 33P within the WRKY75 mutant as compared to the wild-type plants. This suggests that the acquisition of Pi, but not its internal movement, is affected by the suppression of WRKY75.

Suppression of WRKY75 Affects the Development of Lateral Roots and Root Hair

Modulation of the root system architecture (RSA) is one of the adaptive responses to Pi starvation in Arabidopsis (López-Bucio et al., 2003). Since the suppression of WRKY75 caused the early accumulation of anthocyanin, reduced expression of some of the Pi-responsive genes, and reduced 33P uptake (Figs. 3–5), it was logical to investigate its effect on the RSA as well. We therefore examined the RSA of the wild type and the WRKY75 RNAi mutant grown in vertically oriented agar plates under Pi-sufficient and Pi-deficient conditions for 7 d (Fig. 6). The Pi deprivation resulted in a significant reduction of primary root growth in both the wild type and the mutant (Fig. 6B). This result was in agreement with an earlier study showing that Pi deficiency induced progressive loss of meristematic cells in the primary root, thereby causing determinate growth (Sánchez-Calderón et al., 2005). However, the effect of WRKY75 suppression in the mutant was not evident on the primary root growth both under Pi-sufficient and Pi-deficient conditions and was comparable to that of the wild-type plants (Fig. 6B). This result indicated that localized Pi deficiency-induced inhibition of primary root growth (Ticconi et al., 2004) is not influenced by the suppression of WRKY75. A recent study on the analysis of the pldz2 mutant suggested the role of Pi deficiency-induced PLDZ2 in the maintenance of RSA (Cruz-Ramirez et al., 2006). We therefore compared the expression of PLDZ2 in the wild-type and the mutant grown under Pi-sufficient and Pi-deficient conditions (Fig. 6C). No difference was observed in the levels of PLDZ2 transcripts in Pi-deficient mutants as compared to the wild-type plants, thus substantiating the data on primary root growth. On the other hand, there was a significant increase (P < 0.05) in the number (Fig. 6D) and total length (Fig. 6E) of the lateral roots of the mutant under both Pi-sufficient and Pi-deficient conditions as compared to the wild-type plants. This suggested that the effect of WRKY75 suppression on the RSA may be independent of the Pi status of the plant. A similar accentuated effect of WRKY75 suppression was also evident on the number of root hairs in the region spanning 5 mm from the root tip of the mutant grown under Pi-sufficient condition (Fig. 6, F and G). However, the root hair length was comparable to the wild type (Fig. 6F). Together, the results suggest that WRKY75 plays an important role in root development by negatively regulating lateral root and root hair growth in a process that might be independent of the Pi status of the plant.

Silencing WRKY75 Influences Accumulation of Total P and Reduces Phosphatase Activity

An increase in the total root surface of a plant improves its ability to explore a greater soil volume and...
thus acquire more P (López-Bucio et al., 2003). To further analyze the effect of increased root surface area in the WRKY75 RNAi mutants, total P concentration was estimated in the leaves and the roots of 4-week-old wild-type and mutant plants grown hydroponically in media with (P+) or without (P−) P (Fig. 7). A significant increase (P < 0.05) in the total P concentration was observed in the leaves and the roots of the WRKY75 RNAi plants as compared to the wild-type plants under both P-sufficient and P-deficient conditions (Fig. 7). However, relative to plants grown with P, the increase in total P concentration under P-deficient conditions was significantly lower. These results were in contrast to the Pi uptake measurements where lower Pi uptake in the mutant seedlings was observed (Fig. 5B). To resolve this discrepancy, we estimated the total P concentration of the wild-type and the WRKY75 RNAi mutant seedlings grown in agar plates on media with or without P for 7 d (Supplemental Fig. S3). The age of these seedlings is closer to those used for Pi uptake analysis. The Pi concentrations in the leaves and roots of the WRKY75 RNAi and wild-type seedlings grown under P-sufficient conditions did not show any significant differences. In seedlings grown under P-deficient conditions, there was no significant difference in the Pi concentration of the roots, whereas the leaves of the mutant seedling had significantly lower (P < 0.05) Pi content relative to the wild-type leaves (Supplemental Fig. S3). This result is in agreement with the results from Pi uptake measurements (Fig. 5) and the accelerated accumulation of anthocyanin observed in young mutant seedlings during Pi deprivation (Fig. 3). Together, these results suggest that, despite an impaired Pi uptake mechanism, the increased root surface area of the WRKY75 RNAi plants may help the mutants accumulate more P over a period of time.

An increase in the production of extracellular and intracellular phosphatases is a distinct and universal response of higher plants to Pi starvation. Consequently, phosphatase activity has been used as a potential marker of the Pi status of plants (Ascencio, 1994). A comparative analysis of the total acid phosphatase activity in the leaves and the roots of wild-type and WRKY75 RNAi plants grown hydroponically under P-sufficient or P-deficient conditions was performed (Fig. 8). Phosphatase activity was significantly reduced (P < 0.05) in the leaves of the mutant plants grown under both P-sufficient and P-deficient conditions as compared to the wild-type plants. However, no significant variation was observed in the roots of the WRKY75 RNAi plants relative to the wild-type plants under both P regimens.

**DISCUSSION**

Proteins belonging to the WRKY superfamily of transcription factors are known to regulate a multiplicity of biotic and abiotic stress responses. Members of the plant-specific WRKY transcription factor family have been implicated in the regulation of genes involved in pathogen-induced stress, as well as drought, cold, and salinity stresses (Seki et al., 2002; Dong et al., 2003). They are also believed to play an important role during senescence (Miao et al., 2004). Their regulatory effect is primarily through their binding with conserved W box elements on the promoters of specific genes (Ulker and Somssich, 2004). We report here the functional characterization of WRKY75, a member of

### Table 1. In silico analysis of the cis-regulatory regions of Pi starvation-responsive genes for predicted WRKY-binding W boxes (ttgacc/t)

| Gene   | No. of W Box Promoter Motifs | Gene Function during Pi Starvation | Reference                                      |
|--------|------------------------------|-----------------------------------|------------------------------------------------|
| At4    | 2                            | –                                 | Internal Pi allocation                        | Shin et al. (2006) |
| AtIPS1 | –                            | 1                                 | Cytokinin signaling                          | Martin et al. (2000) |
| Phr1:1 | 1                            | 4                                 | Pi transporter                               | Muchhal et al. (1996) |
| Phr1:3 | 1                            | 3                                 | Pi transporter                               | Okumura et al. (1998) |
| Phr1:4 | 1                            | 3                                 | Pi transporter                               | Muchhal et al. (1996) |
| Phr1:5 | –                            | 1                                 | Pi transporter                               | Okumura et al. (1998) |
| Phr1:7 | –                            | 3                                 | Pi transporter                               | Mudge et al. (2002) |
| Phr1:8 | –                            | 1                                 | Pi transporter                               | Mudge et al. (2002) |
| Phr1:9 | –                            | 2                                 | Pi transporter                               | Mudge et al. (2002) |
| AIPS2-1| 3                            | 3                                 | Protein phosphatase                          | K.G. Raghothama (unpublished data) |
| AIPS2-2| –                            | 1                                 | Protein phosphatase                          | K.G. Raghothama (unpublished data) |
| AIPS2-3| –                            | –                                 | Protein phosphatase                          | K.G. Raghothama (unpublished data) |
| PIR1   | –                            | –                                 | Transcription factor                         | Rubio et al. (2001) |
| ACP5   | –                            | –                                 | Type 5 acid Phosphatase                       | Del Pozo et al. (1999) |
| PAP11  | 1                            | –                                 | Purple acid phosphatase                      | Li et al. (2002) |
| PDD2:1 | –                            | –                                 | Phosphate recycling                         | Cruz-Ramirez et al. (2006) |
| RNS1   | 1                            | 1                                 | Ribonuclease RNS1                            | Bariola et al. (1994) |
| SQD1   | 1                            | –                                 | Sulfolipid biosynthesis gene SQD1            | Essigmann et al. (1998) |
| SQD2   | 1                            | 2                                 | Sulfolipid biosynthesis gene SQD2            | Yu et al. (2002) |
| VSP2   | 3                            | 1                                 | Vegetative storage protein                   | Utsugi et al. (1998) |
This family. This study shows that WRKY75 is likely to be involved in modulating Pi stress responses and root development. The involvement of WRKY75 in the modification of the root system in addition to regulating Pi starvation responses highlights the intricate cross talk between these biological processes.

**WRKY75 Has a Role in Regulating Pi Starvation Responses**

The response of higher plants to Pi starvation is a highly regulated process involving the transcriptional activation of numerous genes that help in establishing an adaptive mechanism (Franco-Zorilla et al., 2004). However, information about the transcription factors involved in this complex process is limited. In this study we demonstrate that WRKY75, encoding a WRKY family transcription factor, is induced differentially in different parts of the plant during Pi stress, suggesting its involvement in regulating Pi starvation responses. WRKY75 was also found to be localized to the nucleus irrespective of the Pi status of the plant. This pattern of localization is similar to that of PHR1, a Myb transcription factor from Arabidopsis that has been shown to be involved in regulating several Pi starvation responses (Rubio et al., 2001). Since the expression of PHR1 is not induced upon Pi starvation and it is localized to the nucleus independent of the Pi status of the plant, it was suggested that PHR1 is regulated posttranslationally. In support of this idea, it was recently found that PHR1 is a sumoylation target of SIZ1, a SUMO E3 ligase, and that sumoylation is one of the control mechanisms of Pi deficiency responses (Miura et al., 2005). However, considering that the expression of WRKY75 is strongly induced upon Pi starvation (Fig. 1A) and is rapidly suppressed by Pi resupply (Supplemental Fig. S1), as well as its possible involvement in regulating root growth independent of the Pi status of the plant, sumoylation may not be involved in the regulation of WRKY75.

Gene silencing is one of the effective ways to analyze the function of a gene. We used this approach to generate RNAi lines that showed significant reduction in the expression of the WRKY75 transcripts (Fig. 2). It was observed that the mutant demonstrated accelerated Pi starvation symptoms, as seen by the rapid accumulation of anthocyanin during Pi deprivation (Fig. 3A). Anthocyanin accumulation is a common response to several nutrient stresses. However, the accelerated accumulation of anthocyanin in the WRKY75 RNAi seedlings appears to be a specific response to Pi deprivation and was not observed when they were deprived of other nutrients, such as nitrogen, potassium, or iron. An investigation of the probable cause showed that the expression of a broad spectrum of genes, encoding different components of the Pi starvation response mechanism, was reduced (Fig. 4). These genes included phosphatases such as AtPS2-1 and AtPS2-2 (K.G. Raghothama, unpublished data), high-affinity transporters Pht1;1 and Pht1;4 (Muchhal et al., 1996), and components of the Pi signaling and allocation mechanisms (AtIPS1 and At4, respectively; Martín et al., 2000; Shin et al., 2006). This suggests that WRKY75 could be implicated in the regulation of genes involved in the acquisition of Pi during Pi starvation. Interestingly, the suppression of WRKY75 resulted in the reduced expression of several genes involved in Pi starvation responses but had no negative effect on the anthocyanin accumulation during Pi starvation. This was surprising considering that several genes in the anthocyanin biosynthetic pathway are induced during Pi deprivation (Misson et al., 2005). However, the constitutive accumulation of anthocyanin in the plants upon Pi deprivation, when WRKY75 transcripts are induced in wild-type plants but remain suppressed in WRKY75 RNAi plants, suggests that WRKY75 has a

Figure 5. Decreased Pi uptake in WRKY75 RNAi plants during Pi starvation. Wild-type plants (black circles) and WRKY75 RNAi plants (white squares) were grown on 0.5× MS medium for 7 d and then transferred as groups of 10 seedlings into Pi-sufficient or Pi-deficient medium for 3 d. The Pi uptake of these 10-d-old seedlings was monitored over a 2-h period. A, Pi uptake in plants from Pi-sufficient conditions. B, Pi uptake in plants from Pi-deficient conditions. Error bars represent SE (n = 3).
minor, if any, role in regulating anthocyanin biosynthesis. The accelerated accumulation of anthocyanin in WRKY75 RNAi plants during Pi stress could also be a response to the significant decrease in the leaf Pi concentration (Supplemental Fig. S3) due to the reduced Pi uptake in these plants (Fig. 5B). Thus, although the results presented here reinforce the notion that anthocyanin accumulation is a primary response to Pi starvation, more detailed studies are required to understand the role of WRKY75 in this process.

WRKY transcription factors are known to regulate specific genes by binding to the conserved TTGAC/TW boxes on their promoter regions (Ulker and Somssich, 2004). A differentially reduced transcript level of Pi stress-responsive genes involved in maintaining Pi homeostasis was observed in the WRKY75 RNAi plants (Fig. 4). The differential level of suppression appeared to be correlated to a combination of the number and type of W boxes predicted in the promoter regions of Pi-responsive genes by in silico analysis (Table I). Genes that were completely suppressed in the WRKY75 RNAi plants, such as AtPS2-1 and At4, had multiple predicted W boxes on their promoters, with at least two of them having the TTGACC motif. On the other hand, genes that were not affected by the suppression of WRKY75 (AtACP5, AtPS2-3, and PLDZ2) had no predicted W box motifs on their promoters. Genes that were partially suppressed, such as AtPS2-2 and AtIPS1, had only one predicted W box with the TTGACT motif. Partially suppressed genes with multiple predicted W boxes, Phl1/1 and Phl1/4, had more W boxes with the TTGACT motif, which could potentially lead to competition with the apparently more active TTGACC motif. Future studies with specific
mutations in these conserved WRKY-binding sequences will provide confirmatory data on the role of these target sequences in gene activation during Pi stress. Our studies suggested that WRKY75 could be working as a positive regulator of several genes involved in the global Pi starvation response. These results are interesting considering that PHR1 and OsPTF1 (Rubio et al., 2001; Yi et al., 2005), the two transcription factors implicated in the regulation of Pi stress responses, did not appear to be regulating genes encoding high-affinity Pi transporters or phosphatases. Suppression of WRKY75 expression also led to decreased phosphatase activity (Fig. 8) in the leaves of the plants. This further confirms the role of WRKY75 in activating multiple responses associated with Pi starvation. In addition, the expression of AtPS2-1 and AtPS2-2, phosphatases responsive to Pi stress (K.G. Raghothama, unpublished data), was attenuated by the suppression of WRKY75 expression. These results further support the notion that WRKY75 is a positive regulator of Pi starvation responses. Considering the complex cross talk that occurs during plant stresses, it is often possible that genes induced by a specific stress response could also respond to other stresses as a secondary effect. For example, many of the genes involved in Pi starvation responses, such as RNases, ATPases, and purple acid phosphatases, are induced during senescence and vice versa (Buchanan-Wollaston et al., 2003). Similarly, LePS2, a Pi stress-responsive gene, is also induced during pathogen infection (Stenzel et al., 2003). However, the reduction in the expression of Pi stress-responsive genes in WRKY75 RNAi mutants appears to be rather specific to Pi stress as indicated by impaired Pi uptake during Pi deprivation (Fig. 5B).

The lack of any change in the Pi remobilization pattern of the mutant as compared to the wild-type plants (Supplemental Fig. S2) suggests that WRKY75 may not be directly involved in the internal Pi allocation and remobilization. Thus, WRKY75 can be implicated in the regulation of genes involved in the acquisition of P during Pi starvation. Noticeably, when 4-week-old plants were analyzed, there was a significant increase in the total P concentration in all parts of the WRKY75 RNAi mutants (Fig. 7) despite reduced Pi uptake in these mutants during Pi starvation. Although other possibilities could be considered, this increase could be attributed to the significant (≈75%) increase in the root surface area caused by the suppression of WRKY75 (Fig. 6). In addition, the age of the plants and rapid diffusion of Pi when the plants were grown in a hydroponic system may have contributed to the increased Pi concentration in the WRKY75 RNAi plants. This notion is supported by the fact that total P concentration in young seedlings of WRKY75 RNAi plants grown on agar media does not vary significantly in most parts relative to the wild-type seedlings (Supplemental Fig. S3). The presence of a hypothetical intracellular Pi-sensing mechanism that mediates the induction of Pi starvation-inducible (PSI) genes at the cellular level has been proposed (Abel et al., 2002). Therefore, WRKY75 could be influencing the Pi status of the plant directly by activating PSI genes during Pi deprivation while indirectly regulating it through the accumulation of Pi by increasing root surface area. Although the details of the actual regulatory mechanism are yet to be dissected, it is clear that WRKY75 acts as a positive regulator of Pi acquisition during Pi stress conditions.

Figure 7. Increased total P concentration in WRKY75 RNAi plants. Pi concentration in leaves (white bars) and roots (black bars) of wild-type and WRKY75 RNAi plants grown hydroponically with Pi (P+: 250 μM) and without Pi (P−) for 7 d. Error bars indicate se (n = 4), and different letters above the bars represent means that are statistically different (P < 0.05).

Figure 8. Decreased phosphatase activity in WRKY75 RNAi plants. Total acid phosphatase activity in leaves (white bars) and roots (black bars) of wild-type and WRKY75 RNAi plants grown hydroponically with Pi (P+: 250 μM) and without Pi (P−) for 7 d. Error bars indicate se (n = 4), and different letters above the bars represent means that are statistically different (P < 0.05).
WRKY75 Negatively Regulates Lateral Root and Root Hair Growth

The root architecture in Arabidopsis comprises three components, namely, the primary root, lateral root, and root hairs. The cell division at the primary root meristem enables indeterminate growth by adding new cells to the root. Lateral root formation increases the capacity of the root system to explore the rhizosphere. On the other hand, root hair formation increases the total surface area of primary and lateral roots. Changes in these components can influence root architecture and the ability of plants to grow in soils where nutrients are limiting. Typically, Pi deprivation results in proliferation of roots in the plants (López-Bucio et al., 2003). In this study, we show that WRKY75 negatively regulates lateral root and root hair development. In plants where WRKY75 expression was suppressed, a significant increase in lateral root length and number, as well as root hair number, was observed independent of the Pi status of the plant (Fig. 6). The suppression of WRKY75 resulted in increased root growth but attenuated expression of PSI genes. This supports the hypothesis that the effect of WRKY75 on the RSA is independent of its effect on PSI genes. Thus far, the phytohormone auxin is believed to be the critical factor influencing the components of the root architecture. Two distinct auxin transport mechanisms have been described in roots: acropetal transport from the shoot system to the root and basipetal transport from the root tip to the root base (Rashotte et al., 2000; Bhalerao et al., 2002). In Arabidopsis, acropetal movement of IAA has been implicated in the control of lateral root development (Reed et al., 1998). Auxin and the ethylene precursor ACC are known to regulate root hair formation in response to external stimuli such as nutrient stress (Masucci and Schiefelbein, 1994). It has also been reported that genetic and chemical reductions in protein phosphatase activity alter auxin transport and, thereby, lateral root growth (Rashotte et al., 2001). Therefore, a hypothetical model could be considered wherein WRKY75 transcriptionally regulates phosphatases, resulting in altered auxin transport and changes in lateral root growth. Future studies on WRKY75 could explore this hypothetical model to understand the mechanism by which WRKY75 regulates root architecture. In contrast to these results, the primary root development and expression of PDL2, a phospholipase gene known to be involved in root development (Cruz-Ramírez et al., 2006), were unaffected by the suppression of WRKY75 (Fig. 6, B and C). The localized Pi deficiency-induced inhibition of primary root growth was also unaffected in the WRKY75 RNAi plants. This could be attributed to the ontogenically distinct entity of the embryonic primary root from that of the postembryonically developed lateral roots. Evidences have been provided earlier showing that primary root growth is influenced by localized Pi deprivation (Linkohr et al., 2002; Ticconi et al., 2004), suggesting that lateral roots could be regulated systemically. Therefore, WRKY75 could be acting as a Pi-independent component of the systemic regulatory mechanism of the RSA. The presence of low levels of WRKY75 transcripts detected during Pi-sufficient condition (Fig. 1A) supports the notion of a biological function for WRKY75 even in the absence of Pi stress. These results suggest that WRKY75 may be negatively regulating lateral root and root hair development independent of the Pi status of the plant.

It has been suggested that two major transcriptional programs, one early and general while the other later and specific, are involved in response to Pi starvation (Hammond et al., 2004). The Pi-responsive transcription factor PHR1 (Rubio et al., 2001) is believed to be part of the second, more specific response to Pi starvation. The transcriptional program induced during the early stages of Pi stress is thought to involve genes with the characteristics of general stress response factors. A macroarray study involving mineral nutrition-related genes showed that many of the genes induced early by Pi stress are also activated by potassium and iron starvation (Wang et al., 2002). We found that WRKY75 is also induced during nitrogen, potassium, and iron starvation, but not by other abiotic stresses tested, such as cold or salt stress (data not shown). Interestingly, although WRKY75 is induced early during Pi stress, its expression persists. In this context, the importance of WRKY75 in maintaining the Pi homeostasis of the plant cannot be overemphasized. The control of root architecture by WRKY75 indirectly affects the Pi status of the plant during Pi-sufficient or Pi-deficient conditions as indicated by the increase in Pi accumulation upon its suppression (Fig. 7). On the other hand, its induction during Pi starvation conditions results in the coinduction of several genes that help the plant adapt to Pi stress.

In summary, this study shows that WRKY75 is a negative regulator of root development while it also acts as a positive regulator of Pi stress responses. This strengthens our understanding of transcriptional regulation during Pi starvation responses in higher plants and provides a base for future work in this area. WRKY75 could potentially be a useful tool in understanding multiple stress responses as it plays an important role in regulating root architecture, which is critical for many other nutrient stresses besides P.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Arabidopsis (Arabidopsis thaliana) ecotype Columbia was used in this study. To confirm observations under varied conditions, as well as to meet the specific requirements of individual experiments, one of the following growth conditions was used.

Hydroponic Culture

Seeds were germinated in Premier ProMix PGX peat mix (Premier Horticulture). Plants were grown under greenhouse conditions under a 16-h-light/8-h-dark cycle at 1,000 μmol m⁻² s⁻¹ photosynthetically active radiation.
Plant Transformation

Two different gene constructs were used to transform Arabidopsis plants by use of the floral-dip method (Clough and Bent, 1998). To generate the WRKY75 RNAi construct, a 223-bp fragment of the WRKY75 coding sequence—unique to WRKY75—was amplified using the primers 5′GGACTAAGCTTCCATGGCCAGAGCTGCATCAAGG3′ and 5′CCGC-GATCCGGCGCGCCATCTCCATATGC3′. The amplified fragment was initially digested with NcoI and Ascl and cloned in sense orientation into the binary double-stranded RNA vector pGSA1131 immediately after the CaMV 35S promoter to generate an intermediary vector. The same WRKY75 fragment was then digested with SpeI and BamHI and cloned in antisense orientation next to the GUS intron spacer region of the intermediary vector. The vector was sequenced to confirm the authenticity and correct orientation of the two cloned inserts. The pGSA1131 binary vector confers Basta resistance in planta. The second construct was used to generate a GFP::WRKY75 translational fusion. The full-length WRKY75 cDNA was amplified with the primers 5′GCCGATCCCTAGAAGGACGAGTGG3′ and 5′GGACTAAGCTTCCATGGCCAGAGCTGCATCAAGG3′. The amplified fragment was digested with HindIII and BamHI and cloned into the binary pEGAD expression vector with the EGFP as an N-terminal translational fusion.

Visualization of GFP

Wide-field fluorescence imaging was done using a NIKON E800 compound microscope equipped with a SPOT RT-slider digital camera (Diagnostic Instruments) interfaced to a computer. GFP excitation was done with standard FITC filters. Images of the roots were taken through FITC filters under the 20× objective. To confirm the nuclear localization of WRKY75, roots as well as mesophyll protoplasts from Arabidopsis were stained with 4′,6-diamino-phenylindole (DAPI). They were fixed in phosphate buffered saline, pH 7.2, containing 4% (w/v) paraformaldehyde, 50 mM EDTA, and 100 mM NaCl for 1 h and then washed three times over 40 min with DAPI stain solution (100 mM phosphate buffered saline, 50 mM EDTA, and 1 μg ml−1 DAPI). The nuclear-specific dye DAPI colocalized with GFP fluorescence in the cells (data not shown).

Liquid Culture

This method was used to generate material for all gene expression analyses. Surface-sterilized seeds were dispensed into conical flasks containing one-half-strength MS medium without agar. The seedlings were grown under a 16-h-light/8-h-dark cycle at 22°C with 75 μmol m−2 s−1 PAR. The plates were inclined at a 65° angle to allow the roots to grow along the agar surface.

Physiological Measurements

Anthocyanin Estimation

Plant material was raised through petri dish culture as described earlier. About 100 mg of frozen ground tissue from each treatment and line was used for the quantification of anthocyanins as described by Lange et al. (1971). The optical density was measured at A650 and A532. Subtraction of 0.24 A650 compensated for the small overlap in A650 by the chlorophylls. The concentration was determined by using the corrected absorbance and the molar extinction coefficient (ε) of 38,000 L mol−1 cm−1 for anthocyanin.

Quantification of Total Pi

Plant material was generated hydroponically. Total Pi concentration was quantified using a modification of the U.S. Environmental Protection Agency Method 363.2. About 50 mg of fresh sample was taken in a preweighed vial and oven dried. After recording their dry weight, the samples were flamed to ash and dissolved in 100 μl of concentrated HCl. Ten microliters of this solution was diluted in 790 μl of water. To a reaction containing 800 μl of diluted sample, 200 μl of assay solution (4.8 mM NH4MoO4,2.5 mM H2SO4, and 35 mM ascorbic acid) was added and incubated at 45°C for 20 min. Total Pi content was measured at A660 and expressed as total Pi/milligram tissue dry weight.

Quantification of Total Acid Phosphatase Activity

Plant material was generated hydroponically. Total acid phosphatase was measured as described earlier using the pNPP hydrolysis assay (Richardson et al., 2001). Samples were extracted from about 30 mg of finely ground frozen tissue. The enzyme activity was measured at A405. Total protein was estimated separately using Bradford’s reagent and the total acid phosphatase activity expressed as milliliters/milligram protein.

Pi Uptake Assay

Wild-type and WRKY75 RNAi seedlings were grown as described earlier in Pi-sufficient or Pi-deficient medium for 3 d. Groups of 10 seedlings were

DNA Gel-Blot Analysis and RT-PCR

Total RNA was extracted from plant samples using the TRizol reagent (Invitrogen). Ten micrograms of total RNA was electrophoretically separated in a denaturing formaldehyde agarose gel and blotted onto nylon membranes. The nylon membranes were hybridized overnight with 32P-labeled DNA probes at 42°C.

For RT-PCR analysis, two micrograms of DNAse-treated (RQ1 DNAse; Promega) total RNA was used as a template for first-strand cDNA synthesis with Superscript II (Invitrogen) and an elogid(T) primer. The EF1α gene was amplified with specific primers to calculate equal quantities of first-strand cDNA per reaction. The following gene-specific primers were used to detect WRKY75 cDNA: 5′ ATGACGGAATGACTAAGG3′ and 5′GAAGAAGGACTAGATTCC3′. Ten-microliter reactions were set up for each sample and amplified through 25 cycles. Eight microliters of the product was run on an agarose gel and the results were documented. The remaining 2 μl of the product was used as a template for a second 25-cycle PCR.

Measurements of Roots and Root Hairs

Seedlings were grown on petri dishes under Pi-sufficient or Pi-deficient conditions as described earlier. After 7 d of treatment, the primary root tips were photographed in a Nikon stereo microscope with a SPOT RT digital camera attached to a computer. Root tip images were captured under the 0.5× objective. The total number of root hairs in the region 5 mm from the root tip as well as the length of the root hairs in this region were measured using the ImageJ program (Abramoff et al., 2004). Data were recorded from five individual plants from each line per treatment. For measurement of primary root length, lateral root number, and lateral root length, the roots from 20 individual plants of each line per treatment were spread out carefully with a fine brush, scanned at 600 dpi, and different root traits were evaluated using the ImageJ program.

Role of WRKY75 in Phosphate Stress Response

Plant Physiol. Vol. 143, 2007 1799

Copyright © 2007 American Society of Plant Biologists. All rights reserved.
used as one biological sample. The roots of the seedlings were incubated in a pretreatment solution (5 mM MES and 0.1 mM CaCl₂, pH 5.7) for 20 min before moving them into 2 mL of uptake solution (5 mM MES, 0.1 mM CaCl₂, and 1 mM KH₂PO₄, pH 5.7) containing [³²P]orthophosphate (0.15 μCi/mL). Samples were moved into ice-cold desorption solution (5 mM MES, 0.1 mM CaCl₂, and 1 mM KH₂PO₄, pH 5.7) at the end of 1 and 2 h, respectively. After two washes with fresh desorption solution for 45 min, the samples were blotted dry, placed in preweighed scintillation vials, oven dried overnight at 65°C, and their dry weight recorded. Four milliliters of scintillation cocktail was added into each vial, and radioactivity was measured with a scintillation counter (Beckman Coulter).

For in planta labeling experiments, wild-type plants and WRKY75 RNAi plants were grown for 10 d in Pi-sufficient (1 mM) and Pi-deficient conditions. Roots of these seedlings were incubated for 4 h in the [³²P]orthophosphate uptake solution and subsequently desorbed for 2 h as described above. The seedlings were moved to one-half-strength Hoagland solution and grown for 10 d to allow emergence of new leaves. The nutrient solution was replaced every day. The apical meristem and pairs of leaves were harvested from the plants after 10 d and the radioactivity was measured.

Statistical Analysis
Statistical significance of difference between mean values was determined using Student’s t test. Different letters on the error bars of histograms were used to indicate means that were statistically different at P ≤ 0.05.

In Silico Analysis of Promoter Sequences
W box elements on the promoter regions of Pi stress-responsive genes were collated from the Arabidopsis cis-regulatory element database (AtcisDB) maintained by the Arabidopsis Gene Regulatory Information Server, Ohio State University (http://Arabidopsis.med.ohio-state.edu).

Supplemental Data
The following materials are available in the online version of this article.

Supplemental Figure S1. Decrease in WRKY75 transcripts as Pi-deprived plants are replenished with Pi.

Supplemental Figure S2. Comparative distribution pattern of Pi in WRKY75 RNAi plants.

Supplemental Figure S3. Total Pi concentration in WRKY75 RNAi and wild-type seedlings.

ACKNOWLEDGMENTS
We thank Ajay Jain, Mike Poling, and Madhuvanthi Ramaiah for their valuable help in preparing and editing this manuscript. We also thank the Arabidopsis Resource Center at The Ohio State University for providing the vectors used in this study and Dr. Zhixiang Chen, Department of Botany and Plant Pathology, Purdue University, for the homozygous SALK mutant line of WRKY75.

Received November 30, 2006; accepted February 20, 2007; published February 23, 2007.

LITERATURE CITED
Abel S, Ticcioni AC, Delatorre CA (2002) Phosphate sensing in higher plants. Physiol Plant 115: 1–8
Abramoff MD, Magelhaes PJ, Ram SJ (2004) Image processing with ImageJ. Biophotonics International 11: 36–42
Ascencio J (1994) Acid phosphatase as a diagnostic tool. Commun Soil Sci Plant Anal 25: 1553–1564
Baldwin JC, Karthikeyan AS, Raghothama KG (2001) LEPS2, a phosphorus starvation-induced novel acid phosphatase from tomato. Plant Physiol 125: 728–737
Bari R, Pant BD, Stitt M, Scheible W-R (2006) PHO2, microRNA399, and PHR1 define a phosphate-signaling pathway in plants. Plant Physiol 141: 988–999
Bariola PA, Howard CJ, Taylor CB, Verburg MT, Jaglan VD, Green PJ (1994) The Arabidopsis ribonuclease gene RNS1 is tightly controlled in response to phosphate limitation. Plant J 6: 673–685
Beck T, Hall MN (1999) The TOR signaling pathway controls nuclear localization of nutrient-regulated transcription factors. Nature 402: 689–692
Bhalerao RP, Eklöf J, Ljung K, Marchant A, Bennett M, Sandberg G (2002) Shoot derived auxin is essential for early lateral root emergence in Arabidopsis seedlings. Plant J 29: 325–332
Buchanan-Wollaston V, Earl S, Harrison E, Mathes J, Navabpour S, Page T, Pink D (2003) The molecular analysis of leaf senescence—a genomics approach. Plant Biotechnol J 1: 3–22
Bucher M, Rausch C, Daram P (2001) Molecular and biochemical mechanisms of phosphorus uptake into plants. J Plant Nutr Soil Sci 164: 209–217
Chiou T-J, Aung K, Lin S-I, Wu C-C, Chiang S-F, Su C-L (2006) Regulation of phosphate homeostasis by microRNA in Arabidopsis. Plant Cell 18: 412–421
Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J 16: 735–743
Cruz-Ramirez A, Oropeza-Aburto A, Razo-Hernández F, Ramirez-Chavez E, Herrera-Estrella L (2006) Phospholipase D2Z plays an important role in extraplastidic galactolipid biosynthesis and phosphate recycling in Arabidopsis roots. Proc Natl Acad Sci USA 103: 6765–6770
Del Pozo JC, Alloba I, Rubio V, Levy A, de la Pena A, Aragoncillo C, Del Pozo J, Eslava S, Gómez A, Rodriguez-Ravina A (1999) A type 5 acid phosphatase gene from Arabidopsis thaliana is induced by phosphate starvation and by some other types of phosphate mobilizing/oxidative stress conditions. Plant J 19: 579–589
Dong J, Chen C, Chen Z (2003) Expression profiles of the Arabidopsis WRKY gene superfamily during plant defense response. Plant Mol Biol 51: 21–37
Essigmann B, Guler S, Narang RA, Linke D, Benning C (1998) Phosphate availability affects the thylakoid lipid composition and the expression of SQDT1, a gene required for sulfolipid biosynthesis in Arabidopsis thaliana. Proc Natl Acad Sci USA 95: 1950–1955
Eulgem T, Rushton PJ, Robatzek S, Somssich IE (2000) The WRKY superfamily of plant transcription factors. Trends Plant Sci 5: 199–206
Franco-Zorrilla JM, Gonzalez E, Lustros R, Linhares F, Levy A, Paz-Ares J (2004) The transcriptional control of plant responses to phosphate limitation. J Exp Bot 55: 285–293
Guo Y, Cai Z, Gan S (2004) Transcriptome of Arabidopsis leaf senescence. Plant Cell Environ 27: 521–549
Hammond JP, Bradleyn MR, White PJ (2004) Genetic responses to phosphorus deficiency. Ann Bot (Lond) 94: 323–332
Karthikeyan AS, Varadarajan DK, Jain A, Held MA, Carpita NC, Raghothama KG (2007) Phosphate starvation responses are mediated by sugar signaling in Arabidopsis. Plant Cell 20: 907–918
Karthikeyan AS, Varadarajan DK, Mukharia UT, D’Urzo MP, Damz B, Raghothama KG (2002) Regulated expression of Arabidopsis phosphate transporters. Plant Physiol 130: 221–233
Kim KC, Fan B, Chen Z (2006) Pathogen-induced Arabidopsis WRKY7 is a transcriptional repressor and enhances plant susceptibility to Pseudomonas syringae. Plant Physiol 142: 1180–1192
Lange H, Shropshire W Jr, Mohr H (1971) An analysis of phytochrome-mediated anthocyanin synthesis. Plant Physiol 47: 649–655
Li D, Zha H, Liu K, Liu X, Leggewie G, Udvardi M, Wang D (2002) Purple acid phosphatases of Arabidopsis thaliana. Comparative analysis and differential regulation by phosphate deprivation. J Biol Chem 277: 27772–27781
Linkohr BL, Williamson LC, Fitter AH, Leyser HMO (2002) Nitrate and phosphate availability and distribution have different effects on root system architecture of Arabidopsis. Plant J 29: 751–760
Lipton DS, Lanchar RW, Blevins DG (1987) Citrate, malate and succinate concentration in exudates from P-sufficient and P-stressed Medicago sativa L. seedlings. Plant Physiol 85: 315–317
López-Bucio J, Cruz-Ramirez A, Herrera-Estrella L (2003) The role nutrient availability in regulating root architecture. Curr Opin Plant Biol 6: 280–287
Mare C, Mazzucotelli E, Crosati A, Francia E, Stanca AM, Cattivelli L (2004) Hv-WRKY38: a new transcription factor involved in cold- and drought-response in barley. Plant Mol Biol 55: 399–416
Role of WRKY75 in Phosphate Stress Response

Marschner H (1995) Mineral Nutrition of Higher Plants. Academic Press, London

Martin AC, del Pozo JC, Iglesias J, Rubio V, Solano R, de La Peña A, Leyva A, Paz-Ares J (2000) Influence of cytokinins on the expression of phosphate starvation responsive genes in Arabidopsis. Plant J 24: 1–11

Masucci JD, Schiefelbein JW (1994) The rhd8 mutation of Arabidopsis thaliana alters root-hair initiation through an auxin- and ethylene-associated process. Plant Physiol 106: 1335–1346

Miao Y, Lau T, Zimmermann P, Zentgraf U (2004) Targets of the WRKY53 transcription factor and its role during leaf senescence in Arabidopsis. Plant Mol Biol 58: 853–867

Misson J, Raghothama KG, Jain A, Jouhet J, Block MA, Bligny R, Orte P, Creff A, Somerville S, Rolland N, et al (2005) A genome-wide transcriptional analysis using Arabidopsis thaliana Affymetrix gene chips determined plant responses to phosphate deprivation. Proc Natl Acad Sci USA 102: 11934–11939

Miura K, Russ A, Shankhoo A, Yokoi S, Mitsukawa N, Shirano Y, Shibata D (2000) Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J 31: 279–292

Shin H, Shin H-S, Chen R, Harrison MJ (2006) Loss of At4 function impacts phosphate distribution between the roots and the shoots during phosphate starvation. Plant J 45: 712–726

Marschner H, Masucci JD, Schiefelbein JW (2001) A conserved MYB transcription factor involved in phosphate starvation genes in Arabidopsis. Plant Physiol 128: 1369–1378

Wang YH, Garvin DE, Kochian LV (2002) Rapid induction of regulatory and transporter genes in response to phosphorus, potassium, and iron deficiencies in tomato roots. Evidence for cross talk and root/rhizosphere-mediated signals. Plant Physiol 130: 1361–1370

Wasaki J, Ponseti R, Kuroda S, Shinano T, Yazaki J, Fujii F, Shimbo K, Yamamoto K, Sakata K, Sasaki T (2003) Transcriptomic analysis of metabolic changes by phosphorus stress in rice plant roots. Plant Cell Environ 26: 1513–1523

Wu P, Ma L, Hou X, Wang M, Wu Y, Liu F, Deng XW (2003) Phosphate starvation triggers distinct alterations of genome expression in Arabidopsis roots and leaves. Plant Physiol 132: 1260–1271

Yu K, Wu Z, Zhou J, Du L, Guo L, Wu W, Yu P (2005) OsPTF1, a novel transcription factor involved in tolerance to phosphate starvation in rice. Plant Physiol 138: 2087–2096

Yu D, Chen C, Chen Z (2001) Evidence for an important role of WRKY DNA binding proteins in the regulation of NPR1 gene expression. Plant Cell 13: 1527–1540

Yu B, Xu C, Benning C (2002) Arabidopsis disrupted in SQD2 encoding sulfolipid synthase is impaired in phosphate-limited growth. Proc Natl Acad Sci USA 99: 5732–5737