Genomic and Genetic Control of Phosphate Stress in Legumes[1]

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Phosphorus (P), an essential element for growth and development, is taken up by plants as phosphate (Pi), but Pi is unevenly distributed and relatively immobile in soils. As a result, more than 30% of the world's arable land requires the application of P fertilizers for cropping (Vance et al., 2003). Unfortunately, P fertilizers are manufactured from nonrenewable resources that are increasingly becoming more costly and less available. Current estimates indicate that easily mined rock Pi reserves could easily be depleted by 2060 (Steen, 1997; Vance, 2001; Vance et al., 2003). Paradoxically, part of the applied P in intensive cropping systems can enter the waterways through runoff and erosion, contributing to pollution of surrounding lakes and marine environments. Improving P acquisition and use by crops is critical to economical and environmentally friendly crop agriculture.

Plants have evolved a variety of adaptive strategies to improve their acquisition, use, and remobilization of P (Vance et al., 2003; Hammond et al., 2004; Lambers et al., 2006). Plant responses to P stress conditions involve changes in root morphology and architecture (Lynch, 1995; Liao et al., 2001; Lynch and Brown, 2001; Yan et al., 2004; Beebe et al., 2006; Hill et al., 2006; Ochoa et al., 2006), as well as changes in shoot and flower development (Bucciarelli et al., 2006). Among the legumes, white lupine (*Lupinus albus*), common bean (*Phaseolus vulgaris*), and to a lesser extent barrel medic (*Medicago truncatula*) and soybean (*Glycine max*) have been the focus of P stress research. White lupine, a nonmycorrhizal species, is adaptable to scarce P and displays a highly synchronous suite of molecular and biochemical adaptations to P stress by developing proteoid (cluster) roots, increasing organic acid exudation, and enhancing the expression of many genes, such as secreted acid phosphatase (*LaSAP1*) and Pi transporters (*LaPT1*; Vance, 2001; Uhde-Stone et al., 2003a, 2003b; Vance et al., 2003, and refs. therein). Common bean is the most important food legume worldwide, and genetic variability for the capacity to produce grain in low soil P conditions has been documented (Broughton et al., 2003; Ochoa et al., 2006). Moreover, several thousand ESTs derived from P-stressed common bean roots have been characterized (Ramírez et al., 2005). Noteworthy in an accompanying article in this legume focus issue, Hernández et al. (2007) have completed a P stress root transcriptome survey in common bean, identifying some 125 genes responsive to P stress. Recent studies of barrel medic, a model legume for plant biology research, showed that P stress delayed: (1) leaf development and leaf expansion along the main and axillary shoots; (2) axillary shoot emergence and elongation, resulting in stunted plants; and (3) timing and frequency of flower emergence (Bucciarelli et al., 2006). P-stressed barrel medic also formed shorter petioles and shorter blade lengths relative to plants in P-sufficient conditions. Whether or not morphological changes seen in P-starved barrel medic plants are attributable to an overall delay in whole plant development or as a P stress response remains to be seen. However, the lack of a standardized approach to describe plant growth and phenotypic responses to P stress (Bucciarelli et al., 2006), together with the plastic nature of plant morphological traits (Beebe et al., 2006; Ochoa et al., 2006), makes result comparisons from different laboratories difficult.

Because of the subterranean nature of growth, plant roots have been recalcitrant to phenotypic study. Root adaptations to P limitations include reduced extension of primary roots, highly branched roots with increased lateral roots, and an increased density of root hair formation. Consistent with a general stress response by plants, however, P-stressed plants tend to allocate a greater proportion of biomass to root dry matter compared to P-sufficient plants (López-Bucio et al., 2002, 2003; Hammond et al., 2004; Hill et al., 2006). Most pasture species studied showed reduced total root mass as a response to P stress conditions. On average, a 32% to 86% reduction in root mass was observed in

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most pasture species with decreasing P concentrations (Hill et al., 2006). Owing to inherent root architecture differences, some pasture species did not respond with root mass reduction to P stress (Hill et al., 2006). Similarly, there were no root architecture differences between barrel medic plants grown under P-sufficient and P-deficient conditions until 28 d after planting, when lateral root length and number of P-limited plants showed a decline (Bucciarelli et al., 2006). By contrast, alfalfa (*Medicago sativa*) roots show changes in architecture when grown under P stress. Genetic regulation of root architecture changes due to P stress within and among species is not understood and offers a fruitful area of emphasis for future research.

Molecular genetic, biochemical, physiological, and morphological responses of plants subjected to P stress have been the subject of many recent reviews (Vance, 2001; López-Bucio et al., 2003; Vance et al., 2003; Franco-Zorrilla et al., 2004; Hammond et al., 2004; Raghothama and Karthikeyan, 2005; Lambers et al., 2006). This *Update* summarizes the ongoing plant biology research toward the understanding of P stress in legumes such as white lupine, common bean, barrel medic, and soybean. Additional studies using model plant systems such as Arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*) have also provided valuable genomic and genetic evidence in understanding plant responses and adaptations to P limitations (Hammond et al., 2003; Yi et al., 2005; Aung et al., 2006; Bari et al., 2006; Müller et al., 2007).

QUANTITATIVE TRAIT LOCI ASSOCIATED WITH ROOT ARCHITECTURE FOR P STRESS TOLERANCE AND ADAPTATION

Genetic variability with contrasting degree of root architecture responses to P-limiting conditions has been known for a wide range of plant species (Chevalier et al., 2003; Rubio et al., 2003; Yan et al., 2004). Lynch (1995) has noted a direct correlation between plant productivity and root architecture. As a result, P stress tolerance and adaptation have begun to be analyzed in common bean and Arabidopsis through the identification of quantitative trait loci (QTL) approach (Beebe et al., 2006; Ochoa et al., 2006; Reymond et al., 2006). Ochoa et al. (2006) generated recombinant inbred lines (RIL) from a cross of two common bean accessions with contrasting root architecture traits for adventitious roots. Screening 86 $F_{5:7}$ RIL under P stress and P-sufficient conditions resulted in the identification of 19 QTLs for adventitious root formation (Ochoa et al., 2006). Because Pi availability is expected to be greater in topsoil compared to subsoil, selection for root trait QTL markers associated with adventitious rooting and topsoil foraging may enhance P acquisition. In a previous study, QTL analysis applied to RIL of a cross of G19833 and DOR 364 common beans showed that root hair formation and root organic acid exudation are important traits for marker-assisted selection and breeding of P stress tolerance and adaptation (Yan et al., 2004). Common bean G19833 is a landrace of the Andean gene pool with superior growth and yield in P stress conditions, and DOR 364 is a Mesoamerican gene pool with low P accumulation efficiency in P-limiting conditions. Recently, a composite interval mapping approach identified 26 more QTLs associated with basal root development and greater P acquisition efficiency in P stress conditions (Beebe et al., 2006). In a recent study involving a RIL population of Arabidopsis, three QTLs involved in root growth response to P stress were identified (Reymond et al., 2006). One of the QTLs, *LPR1*, explained 52% of the variation associated with primary root length response. QTL analysis of P stress tolerance appears to be a useful approach in determining which root traits are associated with P uptake. With the soon-to-be-completed sequencing of the genomes of *Medicago* and *Lotus* accompanied by the strikingly conserved synteny among legume genomes, using positional cloning, it should be possible to identify specific genes that contribute to QTLs affecting adaptation to P stress. The selection and development of P-efficient legume plants using QTL markers would not only be beneficial to low-input agricultural systems but also would enhance environmentally friendly cropping in intensively cultivated systems.

P STRESS-INDUCED ESTS

Following international collaborations in recent years, more than 25,000 partially sequenced cDNA inserts or ESTs derived from P-starved tissues of four legume species (barrel medic, soybean, common bean, and white lupine) are currently deposited in the public domain (http://compbio.dfci.harvard.edu/tgi/). Microarray and macroarray analysis of P stress in plants showed increased transcript abundance of genes with homology to Pi transporters, organic acid synthesis, purple acid phosphatase, mulitdrug and toxin efflux (MATE), transcription factors, signaling, and defense (Hammond et al., 2003; Uhde-Stone et al., 2003a, 2003b; Wu et al., 2003; Misson et al., 2005; Ramírez et al., 2005; Müller et al., 2007). Gene indices at http://compbio.dfci.harvard.edu/tgi/ derived from EST sequencing efforts of P-stressed tissues have been used as tools for gene discovery, molecular marker generation, and gene transcript pattern analysis. By evaluating available microarray and macroarray data and utilizing bioinformatic analysis of publicly available EST sequencing projects, Graham et al. (2006) identified 52 candidate genes clustered in 22 groups that appear to respond in common to P stress in four legume species and Arabidopsis. This in silico analysis identified P stress-responsive genes that are overrepresented in the gene indices. Transcripts identified annotate to various important functional categories, including MYB and WRKY transcription factors, signal transduction proteins (Ser/Thr kinases, mitogen-activated protein kinases, and calcium-dependent protein kinases), transporters (Pi

transporter and ATP-binding cassette transporter family), and purple acid phosphatases. Current research is aimed at: (1) using P stress-induced ESTs in marker-assisted selection for genotypes having improved tolerance to P deficiency; (2) characterizing the functional significance of P stress-induced genes; and (3) identifying candidate genes that may be used to enhance P efficiency.

P STRESS-RESPONSIVE TRANSCRIPTION FACTORS IN LEGUMES

Research in Arabidopsis and rice suggests that an important step in regulation of gene expression during plant stress appears to be the transcriptional activation or repression of genes (Chen et al., 2002; Wu et al., 2003). Transcription factors are key global regulators of gene expression and are known to play critical roles in many biological processes, including the regulation of plant responses to numerous biotic and abiotic stresses (Rubio et al., 2001; Tang et al., 2001; Chen et al., 2002; Singh et al., 2002). In Arabidopsis alone, approximately 6% (about 1,800) of the total number of genes are composed of transcription factors, including about 72 WRKY family of genes, more than 600 zinc finger proteins, and 133 MYB transcription factors (Eulgem et al., 2000; Riechmann et al., 2000; Stracke et al., 2001; Guo et al., 2005). In a microarray analysis, approximately 30% of the 333 transcription factor genes included in the array were up- or down-regulated 2-fold or more during P stress in Arabidopsis (Wu et al., 2003). Misson et al. (2005) and Müller et al. (2007) also reported up to 80 P stress-responsive transcription factor genes in Arabidopsis. P stress-responsive transcription factors belong to several families, including MYB, SCARECROW, APETALA2 domain, homeobox, WRKY, and zinc fingers. A recent bioinformatic analysis of legume gene indices (*Medicago*, *Glycine*, *Phaseolus*, and *Lupinus*) queried for genes overrepresented in P-stressed tissue revealed the annotation of several putative transcription factor genes, including WRKY, MYB, and zinc finger family of genes (Graham et al., 2006). Furthermore, leaves and root tissues showed nonoverlapping sets of transcription factor genes (Wu et al., 2003). In addition, distinct sets of genes that may function as early- and late-responsive genes during P stress were observed (Hammond et al., 2003; Misson et al., 2005).

bHLH Transcription Factors

Recently, a bHLH transcription factor involved in Pi stress in rice (*OsPTF1*) was cloned and characterized (Yi et al., 2005). Normally, *OsPTF1* is constitutively expressed in shoots, but transcript accumulation was induced in roots during P starvation of rice plants (Yi et al., 2005). Under P-limiting conditions, overexpression of OsPTF1 in transgenic rice using the constitutive cauliflower mosaic virus 35S promoter resulted in increased P uptake compared to untransformed rice (Yi et al., 2005). Transgenic rice also displayed longer total root length and larger root surface area, resulting in a 30% higher root and shoot biomass than untransformed rice (Yi et al., 2005). An *OsPTF1* gene promoter fused to GUS reporter gene showed strong GUS staining in lateral roots, primary root elongation zone, and leaves of transgenic rice in P-limited conditions (Yi et al., 2005).

HD-Zip and MYB Family of Transcription Factors

Two other transcription factors involved in signaling P-responsive gene expression include the homeodomain Leu zipper (HD-ZIP) protein in soybean (Tang et al., 2001) and the MYB transcription factor in Arabidopsis (Rubio et al., 2001). Although the HD-ZIP protein binds to the sequence 5′-CATTAATTAG-3′ present in vacuolar glycoprotein acid phosphatases (Tang et al., 2001), the Arabidopsis MYB transcription factor with sequence homology to a Pi starvation response (*PHR1*) gene in *Chlamydomonas reinhardtii* was shown to bind to an imperfect palindromic consensus sequence, 5′-GNATATNC-3′ (Rubio et al., 2001). Although transcript abundance of *PHR1* gene was not greatly influenced by Pi status (Rubio et al., 2001), a recent report indicated that *PHR1* defines a Pi-signaling network in Arabidopsis (Bari et al., 2006). There is limited experimental evidence on the regulation of P stress-responsive genes in legumes. However, we and others have observed the *PHR1* imperfect palindromic consensus sequence motif within the 5′-upstream region of many P stress-induced genes, including the white lupine *LaPT1* and *LaSAP1* (Liu et al., 2001; Miller et al., 2001; Hammond et al., 2003; Müller et al., 2007). Also noteworthy is that contigs encoding orthologs of *PHR1* occur in the common bean, *Medicago*, and soybean gene indices.

Zinc Finger Family of Transcription Factors

The *Phaseolus* gene index at The Institute for Genomic Research contains 12 ESTs that show homology to zinc finger transcription factor. These P-responsive ESTs were clustered in two tentative consensus sequences (TC564 and TC1862) and eight singletons derived from P stress roots. Using semiquantitative reverse transcription-PCR analysis of 13 ESTs encoding zinc finger transcription factors, we observed increased transcript abundance for two of the ESTs in P-starved roots of common bean (R. Schirmer and C. Vance, unpublished data). The functional importance of P-responsive zinc finger transcription factors remains to be established.

The WRKY Superfamily of Transcription Factors

Another group of transcription factors among the publicly available EST sequences of P-stressed tissues of white lupine, common bean, soybean, and barrel

medic showed homology to the WRKY family of genes. This family of genes, defined by a DNA-binding domain that contains the strictly conserved amino acid sequence WRKY, appears to be unique to plants (Eulgem et al., 2000). Upon binding to their cognate W-box binding motif (C/T)TGAC(C/T), members of this family of genes have been shown to be up-regulated in response to a diverse set of stresses, including infection by pathogens and wounding, as well as during senescence (Eulgem et al., 2000). In Arabidopsis microarray analysis, a transcription factor *WRKY75* gene was found to be up-regulated during Pi starvation (Misson et al., 2005). A recent study by Devaiah et al. (2007) demonstrated that *WRKY75* is a nuclear localized, Pi-responsive transcription factor gene in Arabidopsis. However, RNAi-silenced *WRKY75* mutants showed increased lateral root numbers and length and root hair number in both Pi-limited and P-sufficient conditions, indicating that the regulatory effect on root architecture may not be related to P stress. Preliminary observations in our laboratory have found that transcript abundance of selected *WRKY* genes could be either up-regulated or down-regulated in P-limited root tissues of common bean (Fig. 1). In addition, a recombinant WRKY protein derived from a common bean *WRKY* gene binds to the 5′-upstream promoter of P stress-induced genes from several species (J. Liu and C. Vance, unpublished data). Given the complexity of the WRKY family of transcription factors, these findings were in agreement with suggestions by others that the WRKY family of genes may act as both transcriptional activators and repressors and that their binding sites may differ from the consensus W-box. Many plant genes that respond to P stress contain WRKY boxes in the 5′-upstream regions. Inclusively, there is a wide array of transcription factors involved in modulating gene expression in P-stressed plants. More than likely, regulation of transcription in P stress will result from cross talk between transcription factors. Although significant advances have been made in understanding transcriptional regulation during P stress, much work remains to define the complex interactions involved in transducing P cascade events. The challenge for plant biologists is to identify putative downstream targets for P-responsive transcription factors and determine whether they can be manipulated to improve P tolerance in plants, as suggested by overexpression of the *OsPTF1* gene in rice.

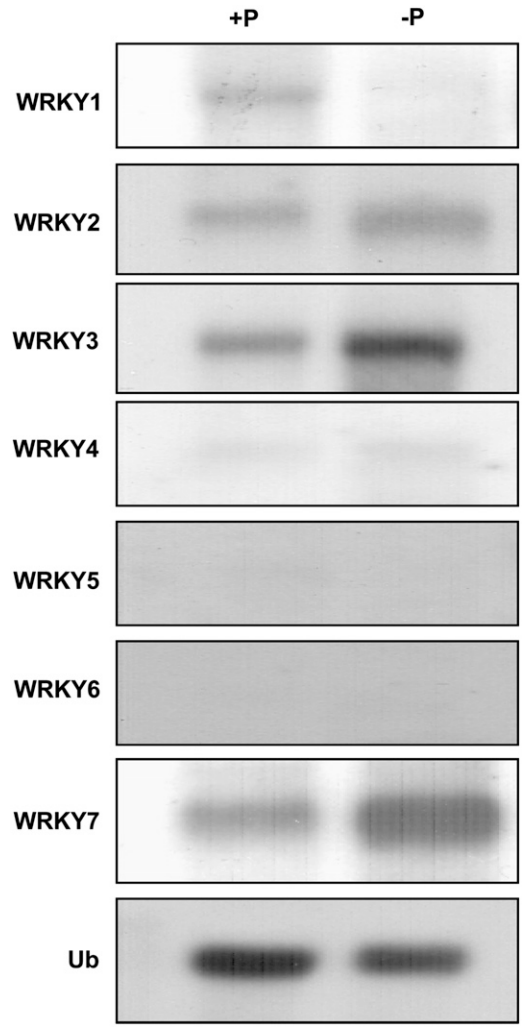

Figure 1. P-regulated expression of the WRKY family of transcription factor genes in common bean roots. For RNA blots, 15 μg of total RNA was isolated from roots of 3-week-old plants grown under P-sufficient (+P) or P-deficient (−P) conditions as described previously (Ramírez et al., 2005).

P STRESS-RESPONSIVE MICRORNAS

Computational and molecular cloning approaches revealed a group of endogenous noncoding small RNAs that may play important roles in the control of many developmental processes in plants and animals (Bartel, 2004; Jones-Rhodes and Bartel, 2004; Xie et al., 2005; Chen et al., 2006). MicroRNAs (miRNAs) are one such small RNA, about 18 to 32 nucleotides in length in plants, which function as posttranscriptional negative regulators or repressors through base pairing to complementary or partially complementary sequences to the target mRNA cleavage (Reinhart et al., 2002; Rhoades et al., 2002; Bartel, 2004; Sunkar and Zhu, 2004). Most known miRNAs in plants are predicted to target the expression of several classes of genes, including transcription factors, indicating their importance in regulating various plant developmental aspects (Bartel and Bartel, 2004). Recently, miR399, first identified in Arabidopsis and rice (Sunkar and Zhu 2004), was shown to be induced by P stress after 24 and 48 h of P starvation (Fujii et al., 2005; Chiou et al., 2006). Transcript abundance of miR399 declines rapidly following the addition of Pi in the medium (Bari et al., 2006) and is not detected at all under P-sufficient conditions (Fujii et al., 2005; Aung et al., 2006; Bari et al., 2006; Chiou et al., 2006). On the other hand, miR399 is

predicted to show five binding targets on the ubiquitin-conjugating E2 enzyme (*UBC*) gene sequence through 5′-untranslated region (UTR) interactions (Fujii et al., 2005; Chiou et al., 2006). Apparently, transcript accumulation of the *UBC* gene was suppressed in P-starved Arabidopsis plants (Fujii et al., 2005). Constitutive expression of miR399 in transgenic Arabidopsis using the cauliflower mosaic virus 35S promoter resulted in suppression of UBC mRNA under P-sufficient conditions and overaccumulation of inorganic Pi in Arabidopsis shoots (Fujii et al., 2005; Bari et al., 2006; Chiou et al., 2006). The observed phenotypes were similar to those displayed by Arabidopsis mutants of the UBC gene from T-DNA insertions (Fujii et al., 2005; Chiou et al., 2006), as well as to a previously described whole-plant phenotype of a Pho2 mutant from an ethyl methanesulfonate-mutagenized pool of Arabidopsis (Aung et al., 2006; Bari et al., 2006). Utilizing map-based cloning approaches, a region toward the C terminus of *Pho2* gene was shown to correspond to the UBC domain of E2 enzyme (Bari et al., 2006). As the mutation in Pho2 was found to be a result of a single nucleotide substitution that resulted in an early termination of the *UBC* gene, *UBC* transcripts were not detected in RNA blots of the Pho2 mutant (Aung et al., 2006; Bari et al., 2006). The *Pho2* gene spanning about a 5.5-kb region is predicted to encode a 4.1-kb transcript, including a 1.1-kb 5′-UTR (Bari et al., 2006). Consistent with the earlier computational prediction of five miRNA399 binding sites in *UBC*, Pho2 sequences also contain five binding sites for miRNA399 in the 5′-UTR (Bari et al., 2006). Interestingly, transcript abundance of miRNA399 was not influenced by other nutrient stress conditions such as potassium or N limitation (Fujii et al., 2005). Taken together, these results have shown that Pho2 encodes UBC and that miR399 induced under P stress appears to control Pi homeostasis by regulating the expression of UBC in Arabidopsis. A *UBC* ortholog is up-regulated in common bean P-stressed roots.

Putative miRNA399 and *UBC* homologs have been computationally identified in barrel medic and *Lotus* (Sunkar and Zhu, 2004; Bari et al., 2006). A potential binding site for miRNA164 has been predicted for the transcription factor NAC1 gene (Rhoades et al., 2002), and we have noted *NAC1* EST in white lupine P-stressed proteoid root expression libraries (P. Smith, C. Atkins, and C. Vance, unpublished data). We also have observed up-regulated expression of a *NO APICAL MERISTEM* gene (a *NAC* gene family) in P-starved roots of barrel medic and alfalfa (M. Tesfaye and C. Vance, unpublished data). *NAC1* gene transduces auxin signals for lateral root development by activating the expression of two downstream auxin-responsive genes, *DBP* and *AIR3*, in Arabidopsis (Xie et al., 2000). It remains to be seen if miRNA399, miRNA164, or other plant miRNAs play a significant role in the regulation of P-responsive genes in legumes. The P stress response of plants is often considered to be a systemic event, and it will be informative to ascertain whether miRNAs are the effectors of such systemic responses.

SUGARS MODULATE THE EXPRESSION OF P STRESS-RESPONSIVE GENES

Our laboratory and others have shown that increased transcript abundance of several genes coincides with P starvation in white lupine and *Medicago*. Transcript accumulation of white lupine *LaPT1*, *MATE*, and *LaSAP1* genes was increased in stems and in normal and cluster roots of P-stressed plants, although there was virtually no transcript accumulation of these genes in P-sufficient plants (Liu et al., 2001, 2005; Uhde-Stone et al., 2005). The direct involvement of *LaPT1*, *MATE*, and *LaSAP1* genes in P starvation has been verified by studies using transgenic lupine and alfalfa plants that contained the promoters and 5′-untranslated intron fused to a reporter GUS gene (Liu et al., 2005; Uhde-Stone et al., 2005). Roots of transgenic alfalfa plants grown under P limitation showed strong GUS staining and enzyme activity. Similar to wild-type P-sufficient alfalfa and lupine plants, no GUS staining was visible in root segments of transgenic plants grown in P-sufficient medium. Other abiotic stress treatments, such as nitrogen (N) starvation, aluminum toxicity, or addition of naphthylacetic acid, did not produce GUS staining or show reporter gene enzyme activity in transgenic plants containing *LaPT1* and *LaSAP1* genes. However, the expression of the *MATE* gene was not unique to P stress, as increased transcript accumulation of *MATE* in proteoid roots was shown during aluminum phytotoxicity as well as in iron, N, and manganese stress conditions (Uhde-Stone et al., 2005).

In white lupine, *LaPT*1 transcripts are readily detectable at 5 d after germination of plants grown at 16 h of light and 8 h of darkness in a 24-h cycle. However, dark-grown white lupine seedlings showed considerably reduced transcript accumulation of the Pi transporter gene, indicating the importance of light in P metabolism. Sugars, apart from being metabolites, are also recognized as signal molecules in plants. Research in lupine and Arabidopsis revealed that Pi and sugars are integrally related to P deficiency-induced expression of Pi transporter genes (Liu et al., 2005; Karthikeyan et al., 2006). Exogenous application of Suc induced the accumulation of Pi transporter genes in dark-grown and P-sufficient seedlings. Stem-girdling studies in white lupine have also revealed that photosynthates/sugars play a role in mediating increased expression of Pi-responsive genes under Pi deficiency conditions (Liu et al., 2005). Expression of P stress-induced *LaPT1*, *LaSAP1*, and *LaMATE* in cluster roots was reduced in girdled plants. Moreover, when P-stressed plants grown in a 16-/8-h photoperiod were placed in the dark for 24 h, P stress-induced gene expression in roots was abolished but recovered upon 16-h reexposure to light (Liu et al., 2005). To further investigate the relationship between P stress and photosynthates in proteoid roots, we recently evaluated transcript abundance of selected sugar sensing and metabolism genes, including hexokinase, Suc synthase, fructokinase, PPi-dependent phosphofructokinase-1, and trehalose-6-P synthase under dark and light conditions in P-starved white lupine

plants. Expression of these genes was enhanced greater than 2-fold in P stress-induced cluster roots of white lupine (Uhde-Stone et al., 2003a). Results in Figure 2 show that the expression of sugar-sensing and metabolism genes was repressed in P-deficient proteoid roots of white lupine after 48 h of continuous dark conditions. Expression of these genes recovered in P-starved proteoid roots of white lupine when dark-treated plants were returned to full light or when leaves were partially exposed to light for 48 h (Fig. 2). These data show a direct cross talk between sugar metabolism and P stress.

The influences of Pi and Suc on root architecture and root growth have been studied recently in Arabidopsis. In Arabidopsis, exogenous application of Pi and Suc in growth medium regulate root growth and root architecture (Nacry et al., 2005; Karthikeyan et al., 2006). Fresh weight of Arabidopsis seedlings decreased by up to 10-fold when grown in either P- or Suc-limited conditions (Karthikeyan et al., 2006). Lateral root density increased significantly as long as Suc was present in P-starved Arabidopsis plants. Lateral root density of Arabidopsis seedlings was decreased by 50% in medium lacking Suc in P stress conditions and by nearly 5-fold in medium lacking Suc in P-sufficient conditions (Karthikeyan et al., 2006). These observations have substantiated the interplay between sugar metabolism and/or sensing and P stress signaling in plants. A systemic signal for Pi status in the shoot and the subsequent regulation of P-responsive genes seems to be transduced, at least in part, by the altered levels of sugars and/or sugar metabolites in plants. A recent microarray analysis by Müller et al. (2007) provides additional lines of evidence for a cross talk between P acquisition and sugars. It was revealed that more than 640 genes were up- or down-regulated 2-fold or more in Arabidopsis leaf sections following Suc treatment (Müller et al., 2007). Also, a large number of genes (approximately 150) showed enhanced expression by both Pi and Suc treatments (Müller et al., 2007), indicating an interplay between Pi and sugars in coregulating gene expression during P metabolism. Other P-responsive white lupine genes, including *MATE* and *LaSAP*, also showed similar expression patterns to *LaPT1* and selected genes involved in sugar sensing and metabolism (Liu et al., 2005). Although it is now clear that many genes expressed in response to P stress require sugar for maximum expression, it remains to be established how these processes interact.

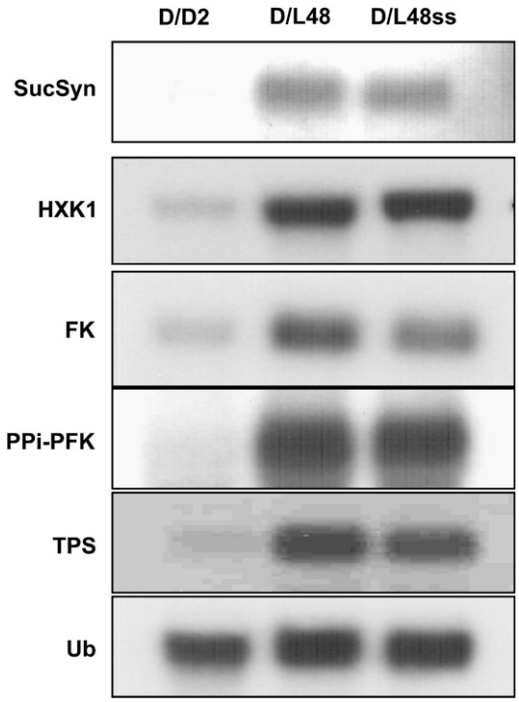

Figure 2. Pi stress and light affect expression of sugar-related genes in proteoid roots of white lupine. Total RNA isolated from proteoid roots of P-deficient white lupine plants at 14 d after emergence under different light regimes. Treatments: D/D2, plants shaded in continuous dark for 48 h; D/L48, dark-treated plants reexposed to continuous light for 48 h; D/L48ss, one-half of the shoot of dark-grown plants reexposed to light and the other one-half remaining in the dark. SucSyn, Suc synthase; HXK1, hexokinase; FK, fructokinase; PPi-PFK, PPi-dependent phosphofructokinase-1; TPS, trehalose-6-P synthase; Ub, polyubiquitin. Growth conditions were described previously (Liu et al., 2005).

PHYTOHORMONES AND P STRESS ADAPTATION

The plant hormone auxin (principally indole-3-acetic acid [IAA]) has been implicated in the regulation of many aspects of plant growth and root development, including P stress-induced proteoid (cluster) root development (Gilbert et al., 2000). Nacry et al. (2005) showed that during Pi starvation, IAA concentration increases in the whole primary root and in young lateral roots of Arabidopsis. Without IAA, only primary root growth was observed in Arabidopsis (Karthikeyan et al., 2006). Regardless of Pi status and/or Suc availability, exogenous application of auxin enhanced lateral root formation and suppressed primary root elongation in Arabidopsis (López-Bucio et al., 2002; Karthikeyan et al., 2006). The effect of IAA on lateral root formation was striking under P-sufficient conditions that did not contain Suc (Karthikeyan et al., 2006). Furthermore, exogenous application of auxin to P-sufficient white lupine mimics proteoid cluster root formation as seen under P-deficient conditions (Gilbert et al., 2000). Auxin transport inhibitors added to P-deficient plants dramatically reduced the formation of cluster roots. The data strongly suggest that cluster root development in response to P deficiency in white lupine is controlled by auxin availability.

In Arabidopsis, a *NAC1* gene is expressed at high levels in root tips and lateral root initiation sites and at low levels in stems and leaves (Xie et al., 2000). Arabidopsis plants expressing antisense *NAC1* showed reduced lateral root emergence, and the lateral root stimulation effect of auxin treatment could not be observed in roots of *NAC1*-silenced transgenic plants (Xie et al.,

2000). Ectopic expression of *NAC1* cDNA in transgenic Arabidopsis coincided with increased *AIR3* and *DBP* gene expression and promoted the production of lateral roots (Xie et al., 2000). Evidence suggested that auxin and *NAC1* gene expression could have synergistic effects on lateral root initiation in Arabidopsis. Exogenously applied auxin resulted in significantly more lateral roots formed in *NAC1* overexpressing plants than wild-type plants. It remains to be seen if the *NAC1* gene has a similar role in root architecture traits associated with P stress adaptations in plants.

P deficiency stress is known to stimulate ethylene production in many plant species, including bean (Borch et al., 1999), lupine (Gilbert et al., 2000), and Arabidopsis (Ma et al., 2003). Ethylene regulates the rate of root elongation in both bean and Arabidopsis. Under P deficiency stress, ethylene application maintains primary and lateral root elongation but does not affect lateral root density. Interestingly, P deficiency can restore lateral root density to Arabidopsis ethylene-insensitive mutants. Root hair formation appears to be regulated, in part, by ethylene. Stimulation of ethylene production results in an increase in root hair density and length (Grierson et al., 2001; López-Bucio et al., 2003). It is noteworthy that bean, lupine, and barrel medic plants exposed to P stress have increased density and length of root hairs. Some 40 genes are suggested to be involved in root hair development (Grierson et al., 2001). Graham et al. (2006) reported that a key gene, 1-aminocyclopropane-1-carboxylic acid oxidase, in ethylene biosynthesis is overrepresented in the ESTs derived from P-stressed roots of lupine, bean, and *Medicago*. These results taken together indicate that ethylene production and/or plant responsiveness to ethylene plays a role in root adaptation to P deficiency.

The role of cytokinins in root growth and P deficiency stress is not resolved. Traditionally, cytokinins are thought to be negative regulators of root growth while having positive effects on shoot growth (Werner et al., 2003; Aloni et al., 2006). Application of cytokinin inhibits root development and abolishes the auxin effect of increased lateral root development. Plants that overexpress the cytokinin oxidase (*CKX*) genes have reduced cytokinin content and show enhanced root growth due to more lateral and adventitious root formation (Lohar et al., 2004). Both P and N deficiency result in decreased cytokinin content (López-Bucio et al., 2003), accompanied by increased lateral root formation. Exogenously applied cytokinin represses the expression of P stress-induced genes in Arabidopsis roots (Martin et al., 2000). In P-stressed lupine proteoid roots, *CKX* gene expression showed a 3- to 5-fold increase in expression (Vance et al., 2003). Moreover, application of cytokinin to P-deficient white lupine inhibits proteoid root formation, and kinetin content is increased in proteoid roots (Neumann et al., 2000). Aloni et al., (2006) have proposed a mechanism for lateral root initiation in P-sufficient plants that involves the interaction of auxin, cytokinin, and ethylene. They propose that factors that modulate root tip cytokinin production allow ethylene and auxin to increase at lateral root initiation sites, giving rise to new laterals. This hypothesis would be consistent with the effect that low P has on inhibition of primary root tip growth, thereby reducing or releasing cytokinin-dependent root apical dominance accompanied by increased lateral root formation. It is fairly obvious that changes in phytohormone balance modulate root developmental plasticity in response to nutrient stress. However, the overriding question remains: How do plants integrate P stress signaling, phytohormone balance, and gene induction to achieve plasticity?

ARBUSCULAR MYCORRHIZAL SYMBIOSES

Mycorrhizal symbioses, which evolved with land plants more than 400 million years ago, are the most important adaptation for plants to acquire scarce P (Parniske, 2004; Harrison, 2005; Oldroyd et al., 2005). More than 80% of angiosperm plants have mycorrhizal symbioses. A major rationale for developing barrel medic, *L. japonicus*, and soybean as model species is due to the fact that they have symbiotic associations with arbuscular mycorrhizae (AM) fungi and rhizobial bacteria. Similar to legume-rhizobial symbiosis, the legume-AM symbiosis results from elegant signal exchanges between the fungus and host plant (Harrison, 2005; Requena et al., 2007). The legume releases compounds in root exudates that stimulate fungal growth, hyphal branching, and appressoria formation (Akiyama et al., 2005). After infection, plant signals are thought to stimulate arbuscule formation in root cortical cells. The AM fungus stimulates root growth and branching. AM fungal hyphae extend from roots into the soil profile, allowing the symbiosis to mine P and other nutrients for plant growth, while the plant provides the fungus with carbon. The exchange of nutrients and carbon between the symbionts occurs within root cortical cells in which elaborate multilobed-fungal structures called arbuscules develop. The plant cytosol and fungal arbuscule never come into contact with each other due to their separation by the periarbuscular membrane, which is an extension of the cortical cell plasmalemma. As evidenced by this brief description, legume-AM symbiosis is an exquisite, highly regulated interaction between the legume host and AM fungi, requiring coordinated expression of genes from two vastly different organisms. A complete review of AM symbiosis is beyond the scope of this article, and the reader is referred to several excellent recent reviews (Parniske, 2004; Harrison, 2005; Oldroyd et al., 2005; Krajinski and Frenzel, 2007; Kuster et al., 2007; Requena et al., 2007). Herein only salient features of genomic studies of legume-AM symbioses will be addressed.

In recent years, several genetic loci in legumes that affect rhizobial root nodule and AM symbioses have been characterized through positional cloning. Although these loci were defined initially as root nodulation

mutants, they also limit AM infection (Parniske, 2004; Harrison, 2005; Oldroyd et al., 2005). Genetic loci identified to date impairing AM symbiosis and nodulation include: a Leu-rich, receptor-like kinase (*dmi2*, *LjSYM2*, PsSYM19); a ligand gated ion channel (*dmi1*); and a calcium-calmodulin dependent kinase (*dmi3*, *PsSYM9/30*). Interestingly, orthologs of these loci have been recently identified in nonlegume species (Zhu et al., 2006). Moreover, two proteins with similarity to *dmi1*, designated *CASTOR* and *POLLUX*, and plastid targeted in *L. japonicus* have been shown to be required for symbiosis (Imaizumi-Anraku et al., 2005). Several other genetic loci affecting AM symbiosis have been identified in various legumes, but they await full characterization (Kistner et al., 2005). Without doubt, however, we now know that AM and root nodule symbioses share some common signaling pathways. In addition, there is a backlog of loci that require attention to fully understand legume control of AM symbiosis.

Using macroarray, microarray, and in silico analyses of EST databases, a large number (at least 200) of plant genes have been identified that respond to AM fungal inoculation and infection (Journet et al., 2002; Liu et al., 2003; Wulf et al., 2003; Grunwald et al., 2004; Liu et al., 2004; Manthey et al., 2004; Hohnjec et al., 2005; Kuster et al., 2007). Several gene families were identified repeatedly as being highly expressed during AM symbiosis, including: lectins; PR proteins; glutathione *S*-transferases; sugar; PO_4^- and NO_3^- transporters; MYB and zinc finger proteins; Cys proteinases; annexins; metalothionines; and membrane intrinsic proteins. Although the functional significance of most of the proteins induced in legume roots in response to AM symbiosis has not been defined, current approaches utilizing RNAi, TILLING, and insertional mutagenesis will allow functional significance to be tested. For example, the PO_4^- transporter genes *LjPT3* from *L. japonicus* (Maeda et al., 2006) and *MtPT4* from barrel medic (Javot et al., 2007) were shown via RNAi and/or TILLING mutants to be required for effective AM symbiosis. Similarly, a calcium-dependent protein kinase that mediates root development in barrel medic was shown to impact AM symbiosis (Ivashuta et al., 2005). These functional analyses represent only the tip of the iceberg. As more genes responding to AM are characterized, the ultimate aim would be to identify genetic strategies that regulate AM symbioses and P acquisition.

CONCLUDING REMARKS

P is second to N as the most limiting element for plant growth. With the availability of a wide array of genomic and bioinformatic research platforms, P stress research is moving toward an exciting phase centered around signal transduction, regulation of developmental plasticity, gene function, and increased efficiency of use. Sustainable cropping practices require that plant researchers identify and discover mechanisms in plants that improve P acquisition and exploit these P stress adaptations to make better plants that are efficient at acquiring Pi. Efforts that improve soil P availability to plants contribute greatly to the practice of economical and environmentally friendly crop agriculture.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers CV543965, CV542870, CV543085, CV542465, CV543813, CV543844, and CV543732 (WRKY1–7, respectively); CA410415 (SucSyn); CA410267 (HXK1); CA410623 (FK); CA409542 (PPi-PFK); and CA410467 (TPS).

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