Phosphate Deprivation in Maize: Genetics and Genomics[1]

Carlos Calderón-Vázquez, Ruairidh J.H. Sawers, and Luis Herrera-Estrella*

Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Instituto Politécnico Nacional, Unidad Sinaloa, Guasave, 81101 Sinaloa, Mexico (C.C.-V.); and Laboratorio Nacional de Genómica para la Biodiversidad (Langebio), Centro de Investigación y Estudios Avanzados, Campus Guanajuato, 36500 Irapuato, Guanajuato, Mexico (R.J.H.S., L.H.-E.)

Plants acquire phosphorus (P) from the soil in order to reach the intracellular concentrations necessary for growth and reproduction. To prevent P starvation, both monocotyledonous and dicotyledonous plants have evolved well-regulated systems for inorganic phosphate (Pi) scavenging, acquisition, and recycling. Phosphate starvation responses (PSR) in plants include release of organic acids and hydrolytic enzymes to scavenge soil Pi from organic and inorganic sources, metabolic modifications to bypass ATP requirements, recycling and mobilization of internal P resources, modifications of root system architecture to increase soil exploratory capacity, and, for many plant species, the establishment of symbiotic relations with arbuscular mycorrhizal fungi (Bucher, 2007).

Maize (*Zea mays*) is one of the most widely cultivated crop plants, for both staple food and industrial usage, in tropical and temperate soils worldwide. Under cultivation, especially in acidic and alkaline soils, large quantities of P fertilizer are applied to maize fields in order to maximize yields. P fertilizers, however, are becoming increasingly costly owing to dwindling natural reserves of rock Pi and an increase in the costs of extracting what remains; it is projected that we have already moved beyond the point of peak production (Cordell et al., 2011) and that reserves will be exhausted before the end of the century (Khasawneh et al., 1986). Recent progress in maize genetics and genomics has furthered characterization of the molecular basis of maize PSR, with the potential to provide important information in the development of maize varieties with enhanced P use efficiency.

GENERAL MOLECULAR RESPONSES TO LOW PI AVAILABILITY

The most pertinent questions in the study of maize PSR are as follows: How similar is the maize response to what is known in other plant species? And how is the maize response typical of cereals in general? Exhaustive microarray analyses performed in the model plant Arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*), together with a small but increasing number of reports concerning the molecular basis of PSR in maize as well as other cereals, are beginning to shed light on these questions. Furthermore, the recent completion of maize genome sequencing (Schnable et al., 2009; Vielle-Calzada et al., 2009) has opened new possibilities for the comparative study of maize and other model species. In Table I, we summarize current knowledge of maize PSR and compare this with what is known from Arabidopsis. Sequencing of the maize genome has revealed maize gene families to typically be more expanded than their counterparts in Arabidopsis, and we have consistently found multiple maize genes that exhibit close homology to any given Arabidopsis sequence. This level of gene duplication raises the possibility for paralog subfunctionalization and, consequently, both fine-tuning and greater plasticity in maize PSR.

Several key regulatory components involved in the regulation of P deprivation response have been characterized in Arabidopsis, including transcription factors such as PHOSPHATE STARVATION RESPONSE1 (Rubio et al., 2001), PHR1-LIKE1 (Bustos et al., 2010), WRKY75 and ZAT6 (Devaiah et al., 2007a, 2007b), IPS1/At4 (Shin et al., 2006; Franco-Zorrilla et al., 2007), the microRNA miR399 (Bari et al., 2006; Chiou et al., 2006; Pant et al., 2008), and members of the PHO family (Aung et al., 2006; Stefanovic et al., 2007). In addition, global gene expression studies in Arabidopsis have revealed that the responses to P deprivation include alterations in many biochemical and signaling pathways, regulated by a complex network involving members of a range of transcription factor gene families (Hammond et al., 2004; Misson et al., 2005; Morcuende et al., 2007). Microarray analysis and large-scale sequencing projects in a number of species have reinforced this idea and have widened our knowledge and uncovered new strategies exploited by plants to cope with P starvation, revealing how particular species have evolved specific mechanisms. Valuable genetic and genomic information has been obtained from Arabidopsis, rice, bean (*Phaseolus vulgaris*), and lupine (*Lupinus albus*), for which large-scale studies have permitted a detailed characterization of

[1] This work was supported by SAGARPA (grant no. Zea-2006), the Howard Hughes Medical Institute (grant no. 55005946), and the Consejo Nacional de Ciencia y Tecnología (grant no. 299/43979).

* Corresponding author; e-mail lherrera@ira.cinvestav.mx.

www.plantphysiol.org/cgi/doi/10.1104/pp.111.174987

Table I. *Pi starvation-related genes in Arabidopsis and their orthologs from rice, maize, sorghum, and Brachypodium distachyon*

Gene models from Arabidopsis (The Arabidopsis Information Resource version 10), rice (release 6.1), maize (release 4a.53, filtered set), *Brachypodium* (release 1.0), and sorghum (release 1.4) were considered for identification of the putative orthologs by using the OrthoMCL pipeline (Li et al., 2003) with default parameters except for *miR399*, *At4*, and *IPS1*. Adapted from Lin et al. (2009). ND, No data; NPOI, no putative ortholog identified.

Gene	Arabidopsis	Rice	Maize	Sorghum	*Brachypodium*	Description of Function	References
PHR1	At4g28610	LOC_Os03g21240 LOC_Os07g25710	GRMZM2G006477 GRMZM2G162409	Sb01g036440 Sb02g010520	Bradi1g63530 Bradi1g28920	A MYB transcription factor; it activates a subset of Pi starvation-induced genes by binding to the P1BS element; *phr1* shows reduced Pi concentration	Rubio et al. (2001); Nilsson et al. (2007); Zhou et al. (2008)
SIZ1	At5g60410	LOC_Os05g03430 LOC_Os03g50980	GRMZM2G155123 GRMZM2G002999	Sb09g002225 Sb08g000380	Bradi2g38030 Bradi4g45080	SUMO E3 ligase; it facilitates the sumoylation of PHR1 and regulates the expression of several Pi starvation-responsive genes; *siz1* shows higher shoot Pi concentration	Miura et al. (2005)
PHF1	At3g52190	LOC_Os07g09000	GRMZM2G158489	Sb02g005080	Bradi1g55000	An endoplasmic reticulum-located SEC12-related protein; it facilitates the trafficking of PHT1 proteins to plasma membrane regulated by PHR1; *phf1* shows reduced Pi content	González et al. (2005)
PHO1	At3g23430	LOC_Os02g56510	GRMZM2G466545	Sb04g036730	Bradi3g54920	A protein with SPX and EXS domains; it is involved in Pi loading into the xylem; *pho1* displays Pi starvation symptoms with reduced shoot Pi	Poirier et al. (1991); Hamburger et al. (2002); Stefanovic et al. (2007)
PHO2 (*UBC24, LTN1*)	At2g33770	LOC_Os05g48390	GRMZM2G381709 GRMZM2G464572	Sb09g028110	Bradi2g16960	Ubiquitin E2 conjugase; it regulates Pi uptake, allocation, and remobilization; a target gene of miR399s; *pho2* displays Pi toxicity with excessive shoot Pi	Delhaize and Randall (1995); Dong et al. (1998); Aung et al. (2006); Bari et al. (2006); Hu et al. (2011)
miR399	At1g29265, At1g63005, At5g62162, At2g34202, At2g34204, At2g34208	osa-MIR399a osa-MIR399b osa-MIR399c osa-MIR399d osa-MIR399e osa-MIR399f osa-MIR399g osa-MIR399h osa-MIR399i osa-MIR399j osa-MIR399k	zma-MIR399a zma-MIR399b zma-MIR399c zma-MIR399d zma-MIR399e zma-MIR399f zma-MIR399g zma-MIR399h zma-MIR399i zma-MIR399j	sbi-MIR399a sbi-MIR399b sbi-MIR399c sbi-MIR399d sbi-MIR399e sbi-MIR399f sbi-MIR399g sbi-MIR399h sbi-MIR399i sbi-MIR399j sbi-MIR399k	ND	A microRNA; it negatively regulates PHO2 and serves as a shoot-derived long-distance signal; regulated by PHR1 overexpression of miR399; mimics the Pi toxic phenotype of *pho2*	Jones-Rhoades and Bartel (2004); Fujii et al. (2005); Bari et al. (2006); Chiou et al. (2006); Lin et al. (2008); Pant et al. (2008); Zhang et al. (2009)
PHO3 (*SUC2*)	At1g22710	NPOI				A Suc/H^+ symporter; it regulates Pi starvation responses; *pho3* shows reduced total P concentration	Lloyd and Zakhleniuk (2004); Zakhleniuk et al. (2001)
PHT1;1 (*PT1*) *PHT1;4* (*PT2*)	At5g43350 At2g38940	LOC_Os04g10800 LOC_Os04g10750 LOC_Os04g10690 LOC_Os03g05610 LOC_Os10g30790	GRMZM2G170208 GRMZM2G045473 GRMZM2G154090	Sb06g002560 Sb06g002800 Sb01g046900 Sb01g020570	Bradi5g02750 Bradi5g02730 Bradi3g27680	High-affinity Pi transporters involved in Pi acquisition; *pht1;1* mutant shows reduced Pi uptake and shoot Pi content; increased Pi uptake by overexpression of PHT1;1 in tobacco cells; *pht1;1/pht1;4* double mutant shows significant decrease in Pi uptake, shoot Pi content, and fresh weight	Mitsukawa et al. (1997); Misson et al. (2004); Shin et al. (2004)

(*Table continues on following page.*)

Table I. (*Continued from previous page.*)

Gene	Arabidopsis	Rice	Maize	Sorghum	*Brachypodium*	Description of Function	References
PHT2;1	At3g26570	LOC_Os02g38020	GRMZM2G092780	Sb04g024630	Bradi3g47550	A low-affinity Pi transporter in chloro plast; it is involved in Pi allocation between roots and shoots and Pi remobilization between old and young leaves	Daram et al. (1999); Versaw and Harrison (2002)
At4, IPS1	At5g03545 At3g09922	LOC_Os03g05334 LOC_Os01g0838350	GRMZM2G086179 GRMZM5G843352 GRMZM2G436295	ND	ND	Noncoding RNAs; they regulate Pi allocation between roots and shoots; in hibit the cleavage of miR399 to PHO2 mRNA by mimicking the target sequence of miR399	Hou et al. (2005); Shin et al. (2006); Franco-Zorrilla et al. (2007)
PTF1	NPOI	LOC_Os06g09370	GRMZM2G024530	Sb10g006250	Bradi1g46700	A bHLH transcription factor; it regulates the expression of several Pi starvation-responsive genes; overex pression of OsPTF1 in creases Pi content, r oot growth, and tiller number; overexpres sion in maize leads to improved tolerance of low-P stress	Yi et al. (2005); Li et al. (2011)
WRKY75	At5g13080	LOC_Os05g45230	NPOI			A WRKY transcription factor; it positively regulates Pi starvation responses but negatively regulates lateral root and root hair growth	Devaiah et al. (2007a)
ZAT6	At5g04340	LOC_Os03g32230	GRMZM2G106026	Sb01g031900	NPOI	A C2H2 zinc finger transcription factor; it regulates several Pi starvation-responsive genes; controls root architecture, Pi up take, and Pi accumulation	Devaiah et al. (2007b)
BHLH32	At3g25710	LOC_Os03g15440 LOC_Os01g06640	GRMZM2G043854 GRMZM2G088443	Sb01g040450 Sb03g005250	Bradi1g67500 Bradi2g03830	A bHLH transcription factor; it is a negative regulator of Pi starvation responses; *bhlh32* has hypersen sitive Pi starvation responses and increased Pi concentration	Chen et al. (2007)
SQD2	At5g01220	LOC_Os07g01030 LOC_Os01g04920 LOC_Os03g15840	GRMZM2G100652 NPOI GRMZM2G049190	Sb02g000240 Sb03g006480 Sb01g040150	Bradi1g59860 Bradi2g02800 Bradi1g67200	Involved in sulfolipid biosynthesis; *sqd2* mutant shows reduced growth under Pi-deficient conditions	Yu et al. (2002)
PLDζ1	At3g16785	LOC_Os05g29050 LOC_Os01g20860	GRMZM2G066485 Sb03g012720	Sb09g017850 Bradi2g11810	Bradi2g27950	Involved in phospholipid degradation and synthesis of galactoli pid in roots; regulates root architecture in response to Pi starvation	Cruz-Ramírez et al. (2006); Li et al. (2006a, 2006b)
CAX1	At1g08960	LOC_Os11g05070	GRMZM2G004414	Sb08g002860	Bradi4g42870 Bradi4g42880	Tonoplast Ca^{2+}/H^{+} antiporters; *cax1/cax3* double mutant has increased shoot Pi content	Cheng et al. (2003, 2005)
CAX3	At3g51860	LOC_Os01g37690 LOC_Os02g21009	GRMZM2G011592 NPOI	Sb03g024820 Sb04g010130	Bradi2g41090 NPOI		

(*Table continues on following page.*)

Table I. (*Continued from previous page.*)

Gene	Arabidopsis	Rice	Maize	Sorghum	*Brachypodium*	Description of Function	References
IPK1	At5g42810	LOC_Os04g56580	GRMZM2G150496	Sb06g031650	Bradi5g24890	An inositol polyphosphate kinase; reduced phytate but increased Pi concentration in *ipk1* mutant seeds; *ipk1* mutant has high leaf Pi concentration	Stevenson-Paulik et al. (2005)
SPX1	At5g20150	LOC_Os06g40120	GRMZM2G171423	Sb04g006990 Sb10g023590	Bradi1g36610 Bradi3g07470	SPX domain-containing proteins; regulates the expression of several Pi starvation-responsive genes involved in Pi uptake, allocation, and remobilization	Duan et al. (2008); Wang et al. (2009); Liu et al. (2010)
SPX3	At2g45130	LOC_Os10g25310 LOC_Os03g29250	GRMZM2G370780 NPOI	Sb01g032880 Sb01g023270	Bradi1g60250 NPOI		
PDR2	At5g23630	LOC_Os05g33390	GRMZM2G060824	Sb09g019760	Bradi2g25860	P5-type ATPase; mediates developmental responses of root meristems to Pi availability	Ticconi et al. (2004, 2009)
MYB62	At1g68320	LOC_Os01g03720	GRMZM2G096358 GRMZM2G162709	Sb03g007360	Bradi2g01960	Transcription factor; overexpression represses the expression of GA biosynthetic and PSR genes; controls root architecture and Pi uptake	Devaiah et al. (2009)
ARP6	At3g33520	LOC_Os01g16414	GRMZM2G088487	Sb08g021780	Bradi2g10130	Component in the SWR1 (SWI/SNF related) chromatin-remodeling complex; suppresses PSR gene expression if Pi concentration is optimal	Smith et al. (2010)
LPR1	At1g23010	LOC_Os01g03530 LOC_Os01g03549 LOC_Os01g03620	GRMZM2G086727 GRMZM2G054050 NPOI	Sb03g007480 Sb03g007470 Sb03g007440	NPOI Bradi2g01850 NPOI	A multicopper oxidase; mediates the arrest in primary root growth when the root tip is in contact with low-Pi medium	Svistoonoff et al. (2007)

genetic responses to P starvation (Uhde-Stone et al., 2003; Wasaki et al., 2003; Misson et al., 2005; Hernández et al., 2007; Morcuende et al., 2007). For maize, using a microarray platform to characterize a P starvation-tolerant maize genotype, a comprehensive view of P starvation responses in maize roots was obtained, showing that the steady-state level of over 1,100 transcripts (2% of those analyzed) is modulated by P availability, and among those, at least 33% do not have a significant match with an ortholog in the Arabidopsis genome (Calderon-Vazquez et al., 2008). Such analysis allowed the identification of some biochemical pathways either not reported previously as P responsive in other plant species or at least not as responsive as in maize roots, including a putative shift in nitrogen (N) metabolism to preserve N-containing metabolites, phenylpropanoids, lignin, and fatty acid biosynthesis, in parallel with an increase in the β-oxidation (Calderon-Vazquez et al., 2008). Furthermore, a proteomic analysis of root tips identified changes in protein accumulation following long-term P starvation (Li et al., 2007), indicating modifications in carbohydrate metabolism, amino acid and nucleotide synthesis and degradation, and secondary metabolism. Thus, to date, it is known that coordinated gene expression is necessary to acquire and utilize P efficiently in maize and that a number of regulatory components are also P regulated. In this Update, recent findings and opportunities for further understanding the regulation of maize PSR are summarized.

VARIATION AMONG MAIZE VARIETIES IN TOLERANCE TO LOW PI AVAILABILITY

The wide diversity of maize germplasm includes many varieties adapted to grow in low-P soils, although the basis of this adaptation remains to be studied in detail. Commercial maize breeding efforts and large-scale public programs have placed relatively little emphasis on Pi relations per se; efficient N use and drought tolerance remain the major goals of targeted efforts to alleviate abiotic stress. Nonetheless, a number of screens of diverse plant material have been performed, taking an important step toward the development of varieties better able to acquire and use

available Pi resources. Furthermore, screens of this type provide the starting point for a characterization of the genetic architecture of maize responses to different Pi availabilities. Given the high level of phenotypic variation present in maize, there may be difficulties in detecting relevant genetic variation on the basis of a direct comparison of performance under low-Pi stress (Sawers, 2009). For this reason, many studies have made a relative comparison of performance between low- and high-Pi conditions. A standard screening methodology is well illustrated by the study of Da Silva and Gabelman (1992). In that work, seedling performance was assessed in a panel of maize inbred lines, and a substantial range of performance was observed under both low- and high-Pi availability. Furthermore, the ranking of the lines differed between the two growth conditions, indicative of a specific effect related to Pi, as opposed to more general variation in plant performance. Such studies provide strong evidence that significant variation exists in the ability of maize to exploit the P in its environment, a prerequisite for selection-based improvement.

Performance screens are not by themselves informative with regard to the number of genes that underlie the variation observed or the nature of their effects. Consequently, researchers have used quantitative trait loci (QTL) analyses to provide further information on the genetic architecture of variation in Pi response. A number of studies have employed a B73-Mo17 recombinant inbred line mapping population to investigate variation in overall performance (Kaeppler et al., 2000) and specific root developmental traits (Zhu et al., 2005). Significantly, when analyses have been performed under low- and high-Pi availability, certain QTL have been common to both conditions, while others have been specific to one or the other environment. A further QTL study using a population derived from the Chinese varieties 082 and Ye107 examined a broader range of traits, including root characteristics, vegetative growth, and the quantity of plant exudates (Chen et al., 2008). A number of chromosomal regions were associated with QTL for multiple traits, indicative of tight linkage of causative genes or pleiotropy. Such QTL are candidates for general regulators of PSR. Collectively, QTL analyses support the existence of multiple loci linked to variation in Pi relations. Although, to date, none of the genes underlying one of these QTL has been identified, a combination of further mapping and transcriptome analysis may facilitate future gene identification and further our understanding of the relationship between Pi availability and maize performance.

MOLECULAR RESPONSES TO P STARVATION IN MAIZE

When comparing P starvation responses among plants, we can define a basic set of shared responses. This "core" is constituted by the modification of the mobilization, uptake, and transport of P. These mechanisms were first identified in dicotyledonous species, and they have also been observed in maize genotypes tolerant to P starvation. Here, the current knowledge on how different genes may act during Pi starvation is contrasted to what is hypothesized in maize.

Typical plant PSR include release of Pi from organic and inorganic sources by increasing the synthesis and secretion of organic acids, acid phosphatases, and ribonucleases from roots, in parallel with the up-regulation of genes encoding high-affinity Pi transporters (Bariola et al., 1994; Li and Anderson, 1997; Daram et al., 1998; Liu et al., 1998; del Pozo et al., 1999; Neumann and Römheld, 1999; Raghothama, 1999; Baldwin et al., 2001; Chiou et al., 2001; Li et al., 2002). Several genes that code for Pi-responsive transporters have been described in rice (Paszkowski et al., 2002), barley (*Hordeum vulgare*; Schünmann et al., 2004a), wheat (*Triticum aestivum*; Tittarelli et al., 2007; Miao et al., 2009), and maize (Nagy et al., 2006), and it is known that in maize and rice roots, the up-regulation of P transport- and P recycling-related genes was the most extensive adaptation under Pi starvation, as reflected by the up-regulation of several putative Pi transporters, phosphatase, and RNase genes (Wasaki et al., 2003; Calderon-Vazquez et al., 2008).

Despite that the root architecture differs significantly between monocotyledonous and dicotyledonous plants (Hochholdinger and Zimmermann, 2008), both systems under P starvation present a set of developmental modifications that tend to increase the exploratory capacity of the plant, particularly in the P-rich upper layers of the soil. In Arabidopsis and bean, P starvation results in a modification of root hair growth (Bates and Lynch, 1996) but also in a reduction in the length of the primary root and changes in the angle of growth and the diameter of lateral roots (Bonser et al., 1996; Lynch and Brown, 2001; Williamson et al., 2001; López-Bucio et al., 2002; Hodge, 2004). In wheat and barley, root hair growth is also an adaptive modification (Gahoonia et al., 1997). In maize, it has been reported that P starvation induces alterations in the postembryonic root system, including modification in the angle, length, and number of shoot-borne and lateral roots (for review, see Calderón-Vázquez et al., 2009). However, in contrast to Arabidopsis, it appears that the primary root system in maize, which is important for early stages of development, is not affected by Pi availability, probably because cereal grains contain large quantities of the P (O'Dell et al., 1972) required during seedling establishment. Development- and hormone-related genes have been reported in Arabidopsis as mediators of changes in root system architecture in response to P starvation (López-Bucio et al., 2002; Ma et al., 2003; Pérez-Torres et al., 2009). Likewise, *PHOSPHATE DEFICIENCY RESPONSE2* (*PDR2*) and *LOW PHOSPHATE ROOT1* (*LPR1*) and *LPR2* have been proposed as regulators of the root-meristem response to P-starvation in Arabidopsis (Ticconi et al., 2004,

2009; Reymond et al., 2006; Svistoonoff et al., 2007), playing a key role in root cap P sensing (Ticconi et al., 2009). In maize roots, SHORTROOT- and SCARECROW-like transcription factors, which are involved in determining meristem identity and root morphology (Nakajima and Benfey, 2002; Lim et al., 2005), exhibit altered expression patterns under P starvation (Calderon-Vazquez et al., 2008). The same is true for the root development genes ENHANCER OF GLABRA3, TRANSPARENT TESTA1, NAC (for NAM [No Apical Meristem], ATAF1/2, Cup-Shaped Cotyledons2), APETALA1, and APETALA2 (Xie et al., 2000, 2002; Hardtke, 2006) and transcripts responsive to abscisic acid and auxins, including members of the Auxin-Responsive Factor and AUX/IAA gene families (Vieten et al., 2007; Calderon-Vazquez et al., 2008). In the same way, trehalose has been proposed to regulate a number of developmental processes in both Arabidopsis and maize (Ramon and Rolland, 2007). Its biosynthesis is mediated by the enzymes trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP). In maize, the TPP enzyme, coded by the RAMOSA3 (RA3) gene, controls inflorescence architecture (Satoh-Nagasawa et al., 2006). Three maize transcripts encoding TPP enzymes have been observed to change in accumulation under P starvation (Calderon-Vazquez et al., 2008). Two of those *TPP* transcripts are highly similar to the RA3. Despite that there is not an ortholog of RA3 in Arabidopsis (Satoh-Nagasawa et al., 2006), the mutant *tps1* showed a severe reduction in root length and meristematic region, suggesting a role of trehalose or its intermediaries in root meristem activity in Arabidopsis (Ramon and Rolland, 2007). Considering the proposed role of RA3 in the establishment of axillary meristem identity and determinacy by either mediating sugar signaling or as a transcriptional regulator in maize inflorescence architecture (Satoh-Nagasawa et al., 2006), and the fact that meristematic activity but also sugar signaling determine root developmental responses in Arabidopsis to P starvation (Chiou and Lin, 2011), it is possible that RA3 may be a regulator of root developmental responses in maize.

When plants evoke the recycling and mobilization of internal P and other metabolic resources during P starvation, the synthesis and activity of intracellular phosphatases is increased to mobilize vacuolar P and additional P from other subcellular compartments in source tissues, such as old leaves or seeds (Duff et al., 1994), and a series of bypass reactions are established to avoid Pi- or ATP-dependent enzymes (Duff et al., 1989; Theodorou and Plaxton, 1993). In maize roots, carbon (C) intermediates are also differentially distributed between shoots and roots (Usuda and Shimogawara, 1991) and glycolysis appears to be also modified by bypassing reactions that require P, as suggested by the high increase in expression of genes encoding phospho*enol*pyruvate carboxylase and phospho*enol*-pyruvate carboxylase kinase (Calderon-Vazquez et al., 2008). Modifications in their expression may sustain C supply through the tricarboxylic acid cycle or provide C skeletons for continuing C metabolism. Similarly, the modification of metabolic pathways in maize can be seen at the protein and enzymatic activity levels (Gaume et al., 2001; Li et al., 2008), including the increase in accumulation of pyruvate dehydrogenase complex and tricarboxylic acid cycle-related proteins, possibly to increase the flow of C through this cycle and to generate organic acids for exudation (Li et al., 2008), as reported for maize (Gaume et al., 2001) and rice (Hoffland et al., 2006). These results seem somehow contradictory to the concentrations of organic acid levels found in both shoots and roots of P-starved barley plants (Huang et al., 2008). However, the authors consider that barley is a P-inefficient plant, which represents a difference from what has been reported for maize and rice (Wasaki et al., 2003; Li et al., 2007; Calderon-Vazquez et al., 2008; Huang et al., 2008). It is worth mentioning that the up-regulation of the C-metabolism genes has been reported in maize roots, whereas an important reduction in the activity of such enzymes has been reported in leaves of plants under P starvation in parallel with a strong decrease in the rate of photosynthesis (Usuda and Shimogawara, 1992).

Modifications in N assimilation and amino acid metabolism have been considered as an indirect consequence of P starvation, and it has been proposed that such changes bear a resemblance to the response to low-C conditions (Morcuende et al., 2007). In maize roots, N assimilation and amino acid metabolism are also regulated by P starvation. A significant decrease of nitrate reductase and Gln synthetase was observed under moderate P limitation (Calderon-Vazquez et al., 2008). Although metabolite quantifications and enzyme activity measurements are still pending for maize, the general down-regulation of enzymes involved in amino acid degradation and the specificity for synthesis of some amino acids suggest that maize retains both C and N economies, at the same time maintaining the synthesis of secondary metabolites directing C toward organic acid synthesis. In the same way, barley plants under P starvation seem to use amino acids as an alternative C source, especially in root, as proposed by Huang et al. (2008). These results are in contrast to those reported for Arabidopsis, where modifications in the abundance of transcripts involved in amino acid metabolism are limited (Misson et al., 2005; Morcuende et al., 2007).

In plants in general, it is known that membrane phospholipids are degraded and replaced by sulfolipids and/or galactolipids, allowing the release of P (Essigmann et al., 1998; Andersson et al., 2003). Lipid metabolism is deeply regulated by P starvation in maize. At least 59 transcripts related to lipid metabolism changed expression levels, including genes putatively involved in the recycling of C intermediates from the membrane. A similar shift could be inferred in rice roots and in a very limited fashion in leaves from an expression experiment using an array of

approximately 9,000 cDNAs (Wasaki et al., 2003, 2006). Although this array did not represent the full genome, it showed the change in expression of lipid-related genes, particularly an increase in transcript levels for genes involved in sulfolipid synthesis. In contrast to Arabidopsis, where phospholipid degradation is mediated by transcriptional regulation of phospholipases C and D (Misson et al., 2005; Tjellström et al., 2008), maize roots under P stress did not present a modification in the expression of these genes. Instead, a strong induction of phospholipase A2 and glycerophosphodiesterases, also related to phospholipid degradation, was reported, suggesting triacylglycerol breakdown (Calderon-Vazquez et al., 2008). An induction of β-oxidation in conjunction with fatty acid synthesis was also observed in maize, possibly to provide C intermediates for energy production, thus reflecting a facultative use of C compounds in maize roots to fulfill requirements during P starvation (Calderon-Vazquez et al., 2008).

A number of P starvation responses have been identified by gene expression profiling in maize that have not been identified in other species. A significant modulation of the phenylpropanoid pathway and transcripts encoding components of the lignin biosynthesis pathway were either up- or down-regulated under P starvation. Although metabolomics data for maize would reinforce the idea, this shift in expression suggests a rearrangement in the structural integrity of maize roots (Calderon-Vazquez et al., 2008). A similar modulation of cell wall structure under P starvation has also been suggested in rice, although not by modulation of lignin biosynthesis itself (Wasaki et al., 2003).

ASSOCIATION WITH MYCORRHIZA

Maize, as opposed to Arabidopsis, is efficiently colonized by arbuscular mycorrhizal fungi (Paszkowski and Boller, 2002), and, as in other species, this interaction significantly increases shoot growth rate, P content, dry matter accumulation, and rate of photosynthesis under P-limiting conditions (Parniske, 2008). In maize, the benefit to the plant can vary depending on the fungal species, soil P status, and genotype (Gavito and Varela, 1995; Kaeppler et al., 2000; Bressan and Vasconcellos, 2002; Wright et al., 2005; Sawers et al., 2010). Studies realized with maize inbred lines indicate that mycorrhizal responsiveness of maize genotypes under low P, rather than the interaction with the mycorrhizal fungus as such, is a key factor in determining benefit (Kaeppler et al., 2000), although further analyses have suggested that maize germplasm may indeed harbor significant variation in the ability to profit from mycorrhizal interaction per se (Sawers et al., 2010).

The establishment of mycorrhizal association is preceded by a molecular dialogue between fungus and plant host, including the root exudation of strigolactones (Akiyama et al., 2005; Steinkellner et al., 2007) and the modification of plant epidermal cells (Genre et al., 2005). Once symbiosis is established, a series of membrane transport systems are activated to facilitate the exchange of metabolites, including a set of mycorrhizally specific P-uptake transporters. Such transporters have been reported in a number of species, including the cereals rice, wheat, barley, and maize (Paszkowski et al., 2002; Glassop et al., 2005; Nagy et al., 2006).

PUTATIVE REGULATORS OF P STARVATION RESPONSES IN MAIZE

Although conserved regulatory patterns, as well as common effectors that mediate the biochemical and developmental responses to P starvation, have been identified among several plant species, it is possible that species-specific, or even variety-specific, responses have also evolved. That could be particularly true for maize, given the complexity of the responses to P deprivation among diverse maize varieties. Research in Arabidopsis has demonstrated that the regulation of PSR involves several elements (for review, see Chiou and Lin, 2011), including the transcription factors *PHR1* and *PHR1-LIKE1* (Nilsson et al., 2007; Bustos et al., 2010), SIZ1, a SUMO E3 ligase also proposed as a regulator of P starvation responses including root architecture (Miura et al., 2005), *PHO2*, an E2 ubiquitin-conjugating enzyme (UBC) involved in P loading and redistribution from roots to shoots (Bari et al., 2006), miR399, a microRNA that targets *PHO2* mRNA and thus regulates long-distance Pi homeostasis (Aung et al., 2006; Pant et al., 2008), the At4/Mt4 family that blocks miR399 function (Franco-Zorrilla et al., 2007), and PHO/SPX/EXS domain-containing proteins (Stefanovic et al., 2007; Secco et al., 2010).

Interesting results have been obtained in monocots by analyzing rice, barley, and wheat responses. Two potential rice orthologs of AtPHR1 can be identified in the rice genome database, OsPHR1 and OsPHR2. Both OsPHR1 and OsPHR2 are involved in P availability signaling by regulating the expression of P starvation-induced genes. In addition, OsPHR2, but not OsPHR1, is related to modifications in root architecture in both systemic and local pathways (Zhou et al., 2008). In barley, Schünmann et al. (2004a) identified the P1BS element in promoters of P-regulated PHT1 transporters. P1BS, an imperfect palindromic DNA sequence, was previously identified as the PHR1 target in promoters of P-regulated genes in Arabidopsis (Rubio et al., 2001). Furthermore, P1BS mediates P starvation expression in barley (Schünmann et al., 2004b), whereas in wheat, the P1BS motif was found in the promoter of the gene *TaPHT1.2_D1* (Tittarelli et al., 2007; Miao et al., 2009), a Pi transporter regulated by P starvation in roots, suggesting that PHR1-like elements could be regulating P starvation responses in cereals.

The ortholog of AtSPX3, OsSPX1, stands also as an important regulator of the response to P starvation in cereals (Wang et al., 2009). Work done by Liu et al. (2010) has demonstrated that OsSPX1 negatively regulates OsPHR2 and miR399 in the up-regulation of OsPT2; however, OsPHR2 positively regulates OsSPX1, thus generating a loop in the regulation of P response pathways in rice (Wang et al., 2009; Liu et al., 2010). In the same way, *LEAF TIP NECROSIS1* (*LTN1*) was reported as the ortholog of Arabidopsis *PHO2* (Hu et al., 2011). Its corresponding mutant, *ltn1*, exhibited increased Pi uptake and translocation and was also regulated by OsmiR399 in the same fashion as *PHO2* in Arabidopsis (Aung et al., 2006; Bari et al., 2006; Pant et al., 2008; Hu et al., 2011).

Searches in the maize genome have identified at least two sequences highly similar to PHR1 (Table I), suggesting that the PHR1 signaling pathway may be common in both monocotyledonous and dicotyledonous species. The predicted maize PHR1 genes open up the possibility of subfunctionalization at the level of maize PSR developmental stage and/or tissue specificity. However, it remains to be determined if those sequences are a result of duplication or are members of a multigene family. Interestingly, the rice genome contains two PHR1-like genes, both reported as involved in Pi starvation responses (Zhou et al., 2008). More research is needed to clarify the function of the homologs of PHR1, not only in maize but also in other plants.

Another gene that stands as a potential regulator of P starvation in monocots is the rice transcription factor OsPTF1. To date, no Arabidopsis homolog has been identified. OsPTF1 overexpression enhances the tolerance to P starvation and also increases root surface. A microarray analysis of the overexpressing lines showed at least 158 genes as up-regulated, all harboring OsPTF1-binding elements in their promoter region. These findings suggest that OsPTF1 coordinates a mechanism that allows increased tolerance to P starvation (Yi et al., 2005). The maize genome contains at least two sequences highly homologous to OsPTF1 (Table I). Their possible role in regulating the expression of other genes involved in Pi deficiency responses in maize remains to be investigated. However, transgenic maize lines overexpressing a ZmPTF1 have been recently reported to exhibit improved tolerance of low-P stress and had an increased expression of key genes in Suc synthesis and C assimilation in leaves, including Fru-1,6-bisphosphatase and Suc Pi synthase 1 (Li et al., 2011). These results show that low Pi tolerance in maize can be modulated by altering the expression Pi-responsive genes that have been previously characterized in other plant species.

Two other families highly expressed in maize roots in response to P starvation are the Mt4-like and SPX domain-encoding genes. Three Mt4-like and nine SPX domain transcripts were strongly induced in maize roots, and one of them, highly similar to a member of the PHO1 family, was found among the most highly expressed genes in response to Pi deprivation. In the same way, four PHO2-like sequences and 43 transcription factors were also reported as regulated by P starvation (Calderon-Vazquez et al., 2008). In general, it is possible that some of the mechanisms controlling P responses are conserved in maize and Arabidopsis, including the microRNA miR399-derived pathway (the maize genome contains at least 10 miR399 precursors; Zhang et al., 2009).

CONCLUDING REMARKS

Maize is fundamental as a staple food but also as a source of primary compounds for industrial innovations. The identification of P starvation-tolerant genotypes and knowledge of how they respond are keys to fully take advantage of this cereal. As described above, transcriptomics and proteomics are tools that have been used to provide a general overview of the maize PSR. However, more detailed and comprehensive studies that could uncover the diversity of responses in maize germplasm are still pending. The task is now to focus on a gene functional analysis of the maize homologs to those that have been reported as key components in P starvation signaling pathways in other plants by overexpression and transposon insertion mutants as well as complementation studies in other model systems. In the same way, by taking advantage of high-throughput DNA sequencing technology together with the recently available information on the maize genome (Schnable et al., 2009; Vielle-Calzada et al., 2009), it is possible now to resequence and analyze maize genotypes with contrasting P starvation responses and P utilization efficiency to set up genome-wide strategies for mapping and marker development. Using these tools, we will have a much deeper understanding of the physiological, biochemical, and molecular adaptations to P starvation in maize and attempt to associate QTLs of Pi utilization efficiency with genes identified by genome mining.

Received February 22, 2011; accepted May 24, 2011; published May 26, 2011.

LITERATURE CITED

Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature **435:** 824–827

Andersson MX, Stridh MH, Larsson KE, Liljenberg C, Sandelius AS (2003) Phosphate-deficient oat replaces a major portion of the plasma membrane phospholipids with the galactolipid digalactosyldiacylglycerol. FEBS Lett **537:** 128–132

Aung K, Lin SI, Wu CC, Huang YT, Su CL, Chiou TJ (2006) pho2, a phosphate overaccumulator, is caused by a nonsense mutation in a microRNA399 target gene. Plant Physiol **141:** 1000–1011

Baldwin JC, Karthikeyan AS, Raghothama KG (2001) LEPS2, a phosphorus starvation-induced novel acid phosphatase from tomato. Plant Physiol **125:** 728–737

Bari R, Datt Pant B, Stitt M, Scheible WR (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

Bates T, Lynch J (1996) Stimulation of root hair elongation in Arabidopsis thaliana by low phosphorus availability. Plant Cell Environ **19:** 529–538

Bonser AM, Lynch J, Snapp S (1996) Effect of phosphorus deficiency on growth angle of basal roots in Phaseolus vulgaris. New Phytol **132:** 281–288

Bressan W, Vasconcellos CA (2002) Alteracoes morfológicas no sistema radicular do milho induzidas por fungos micorrízicos e fósforo. Pesq Agropec Brasil **37:** 509–517

Bucher M (2007) Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. New Phytol **173:** 11–26

Bustos R, Castrillo G, Linhares F, Puga MI, Rubio V, Pérez-Pérez J, Solano R, Leyva A, Paz-Ares J (2010) A central regulatory system largely controls transcriptional activation and repression responses to phosphate starvation in Arabidopsis. PLoS Genet **6:** e1001102

Calderón-Vázquez C, Alatorre-Cobos F, Simpson-Williamson J, Herrera-Estrella L (2009) Maize under phosphate limitation. *In* JL Bennetzen, SC Hake, eds, Handbook of Maize: Its Biology. Springer, New York, pp 381–404

Calderon-Vazquez C, Ibarra-Laclette E, Caballero-Perez J, Herrera-Estrella L (2008) Transcript profiling of Zea mays roots reveals gene responses to phosphate deficiency at the plant- and species-specific levels. J Exp Bot **59:** 2479–2497

Chen J, Cai Y, Xu J (2008) QTL mapping of phosphorous efficiency and relative biologic characteristics in maize (Zea mays L.) at two sites. Plant Soil **313:** 251–266

Chen ZH, Nimmo GA, Jenkins GI, Nimmo HG (2007) BHLH32 modulates several biochemical and morphological processes that respond to Pi starvation in Arabidopsis. Biochem J **405:** 191–198

Cheng NH, Pittman JK, Barkla BJ, Shigaki T, Hirschi KD (2003) The *Arabidopsis cax1* mutant exhibits impaired ion homeostasis, development, and hormonal responses and reveals interplay among vacuolar transporters. Plant Cell **15:** 347–364

Cheng NH, Pittman JK, Shigaki T, Lachmansingh J, LeClere S, Lahner B, Salt DE, Hirschi KD (2005) Functional association of Arabidopsis CAX1 and CAX3 is required for normal growth and ion homeostasis. Plant Physiol **138:** 2048–2060

Chiou TJ, Aung K, Lin SI, Wu CC, Chiang SF, Su CL (2006) Regulation of phosphate homeostasis by microRNA in *Arabidopsis*. Plant Cell **18:** 412–421

Chiou TJ, Lin SI (2011) Signaling network in sensing phosphate availability in plants. Annu Rev Plant Biol **62:** 185–206

Chiou TJ, Liu H, Harrison MJ (2001) The spatial expression patterns of a phosphate transporter (MtPT1) from Medicago truncatula indicate a role in phosphate transport at the root/soil interface. Plant J **25:** 281–293

Cordell D, Rosemarin A, Schröder JJ, Smit AL (March 16, 2011) Towards global phosphorus security: a systems framework for phosphorus recovery and reuse options. Chemosphere http://dx.doi.org/10.1016/j.chemosphere.2011.02.032

Cruz-Ramírez A, Oropeza-Aburto A, Razo-Hernández F, Ramírez-Chávez E, Herrera-Estrella L (2006) Phospholipase DZ2 plays an important role in extraplastidic galactolipid biosynthesis and phosphate recycling in Arabidopsis roots. Proc Natl Acad Sci USA **103:** 6765–6770

Daram P, Brunner S, Persson BL, Amrhein N, Bucher M (1998) Functional analysis and cell-specific expression of a phosphate transporter from tomato. Planta **206:** 225–233

Daram P, Brunner S, Rausch C, Steiner C, Amrhein N, Bucher M (1999) Pht2;1 encodes a low-affinity phosphate transporter from *Arabidopsis*. Plant Cell **11:** 2153–2166

Da Silva A, Gabelman W (1992) Screening maize inbred lines for tolerance to low-P stress condition. Plant Soil **146:** 181–187

Delhaize E, Randall PJ (1995) Characterization of a phosphate-accumulator mutant of *Arabidopsis thaliana*. Plant Physiol **107:** 207–213

del Pozo JC, Allona I, Rubio V, Leyva A, de la Peña A, Aragoncillo C, Paz-Ares J (1999) A type 5 acid phosphatase gene from Arabidopsis thaliana is induced by phosphate starvation and by some other types of phosphate mobilising/oxidative stress conditions. Plant J **19:** 579–589

Devaiah BN, Karthikeyan AS, Raghothama KG (2007a) WRKY75 transcription factor is a modulator of phosphate acquisition and root development in Arabidopsis. Plant Physiol **143:** 1789–1801

Devaiah BN, Madhuvanthi R, Karthikeyan AS, Raghothama KG (2009) Phosphate starvation responses and gibberellic acid biosynthesis are regulated by the MYB62 transcription factor in Arabidopsis. Mol Plant **2:** 43–58

Devaiah BN, Nagarajan VK, Raghothama KG (2007b) Phosphate homeostasis and root development in Arabidopsis are synchronized by the zinc finger transcription factor ZAT6. Plant Physiol **145:** 147–159

Dong B, Rengel Z, Delhaize E (1998) Uptake and translocation of phosphate by pho2 mutant and wild-type seedlings of Arabidopsis thaliana. Planta **205:** 251–256

Duan K, Yi K, Dang L, Huang H, Wu W, Wu P (2008) Characterization of a sub-family of Arabidopsis genes with the SPX domain reveals their diverse functions in plant tolerance to phosphorus starvation. Plant J **54:** 965–975

Duff SM, Moorhead GB, Lefebvre DD, Plaxton WC (1989) Phosphate starvation inducible 'bypasses' of adenylate and phosphate dependent glycolytic enzymes in *Brassica nigra* suspension cells. Plant Physiol **90:** 1275–1278

Duff SMG, Sarath G, Plaxton WC (1994) The role of acid phosphatase in plant phosphorus metabolism. Physiol Plant **90:** 791–800

Essigmann B, Güler S, Narang RA, Linke D, Benning C (1998) Phosphate availability affects the thylakoid lipid composition and the expression of SQD1, a gene required for sulfolipid biosynthesis in Arabidopsis thaliana. Proc Natl Acad Sci USA **95:** 1950–1955

Franco-Zorrilla JM, Valli A, Todesco M, Mateos I, Puga MI, Rubio-Somoza I, Leyva A, Weigel D, García JA, Paz-Ares J (2007) Target mimicry provides a new mechanism for regulation of microRNA activity. Nat Genet **39:** 1033–1037

Fujii H, Chiou TJ, Lin SI, Aung K, Zhu JK (2005) A miRNA involved in phosphate-starvation response in Arabidopsis. Curr Biol **15:** 2038–2043

Gahoonia T, Care D, Nielsen N (1997) Root hairs and phosphorus acquisition of wheat and barley cultivars. Plant Soil **191:** 181–188

Gaume A, Machler F, De Leon C, Narro L, Frossard E (2001) Low-P tolerance by maize (Zea mays L.) genotypes: significance of root growth, and organic acids and acid phosphatase root exudation. Plant Soil **228:** 253–264

Gavito ME, Varela L (1995) Response of "criollo" maize to single and mixed species inocula of arbuscular mycorrhizal fungi. Plant Soil **176:** 101–105

Genre A, Chabaud M, Timmers T, Bonfante P, Barker DG (2005) Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. Plant Cell **17:** 3489–3499

Glassop D, Smith SE, Smith FW (2005) Cereal phosphate transporters associated with the mycorrhizal pathway of phosphate uptake into roots. Planta **222:** 688–698

González E, Solano R, Rubio V, Leyva A, Paz-Ares J (2005) PHOSPHATE TRANSPORTER TRAFFIC FACILITATOR1 is a plant-specific SEC12-related protein that enables the endoplasmic reticulum exit of a high-affinity phosphate transporter in *Arabidopsis*. Plant Cell **17:** 3500–3512

Hamburger D, Rezzonico E, MacDonald-Comber Petétot J, Somerville C, Poirier Y (2002) Identification and characterization of the *Arabidopsis PHO1* gene involved in phosphate loading to the xylem. Plant Cell **14:** 889–902

Hammond JP, Broadley MR, White PJ (2004) Genetic responses to phosphorus deficiency. Ann Bot (Lond) **94:** 323–332

Hardtke CS (2006) Root development: branching into novel spheres. Curr Opin Plant Biol **9:** 66–71

Hernández G, Ramírez M, Valdés-López O, Tesfaye M, Graham MA, Czechowski T, Schlereth A, Wandrey M, Erban A, Cheung F, et al (2007) Phosphorus stress in common bean: root transcript and metabolic responses. Plant Physiol **144:** 752–767

Hochholdinger F, Zimmermann R (2008) Conserved and diverse mechanisms in root development. Curr Opin Plant Biol **11:** 70–74

Hodge AJ (2004) The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytol **162:** 9–24

Hoffland E, Wei C, Wissuwa M (2006) Organic anion exudation by lowland rice (Oryza sativa L.) at zinc and phosphorus deficiency. Plant Soil **283:** 155–162

Hou X, Wu P, Jiao F, Jia Q, Chen H, Yu J, Song X, Yi K (2005) Regulation of the expression of OsIPS1 and OsIPS2 in rice via systemic and local Pi signalling and hormones. Plant Cell Environ **28:** 353–364

Hu B, Zhu C, Li F, Tang J, Wang Y, Lin A, Liu L, Che R, Chu C (2011) *LEAF TIP NECROSIS1* plays a pivotal role in regulation of multiple phosphate starvation responses in rice. Plant Physiol **156:** 1101–1115

Huang CY, Roessner U, Eickmeier I, Genc Y, Callahan DL, Shirley N, Langridge P, Bacic A (2008) Metabolite profiling reveals distinct changes in carbon and nitrogen metabolism in phosphate-deficient barley plants (Hordeum vulgare L.). Plant Cell Physiol **49:** 691–703

Jones-Rhoades MW, Bartel DP (2004) Computational identification of plant microRNAs and their targets, including a stress-induced miRNA. Mol Cell **14:** 787–799

Kaeppler SM, Parke JL, Mueller SM, Senior L, Stuber C, Tracy WF (2000) Variation among maize inbred lines and detection of quantitative trait loci for growth at low phosphorus and responsiveness to arbuscular mycorrhizal fungi. Crop Sci **40:** 358–364

Khasawneh FE, Sample EC, Kamprath EJ, editors (1986) The Role of Phosphorus in Agriculture. American Society of Agronomy, Madison, WI

Li AD, Anderson LE (1997) Expression and characterization of pea chloroplastic glyceraldehyde-3-phosphate dehydrogenase composed of only the B-subunit. Plant Physiol **115:** 1201–1209

Li D, Zhu 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

Li K, Xu C, Li Z, Zhang K, Yang A, Zhang J (2008) Comparative proteome analyses of phosphorus responses in maize (Zea mays L.) roots of wild-type and a low-P-tolerant mutant reveal root characteristics associated with phosphorus efficiency. Plant J **55:** 927–939

Li K, Xu C, Zhang K, Yang A, Zhang J (2007) Proteomic analysis of roots growth and metabolic changes under phosphorus deficit in maize (Zea mays L.) plants. Proteomics **7:** 1501–1512

Li L, Stoeckert CJ Jr, Roos DS (2003) OrthoMCL: identification of ortholog groups for eukaryotic genomes. Genome Res **13:** 2178–2189

Li M, Qin C, Welti R, Wang X (2006a) Double knockouts of phospholipases Dζ1 and Dζ2 in Arabidopsis affect root elongation during phosphate-limited growth but do not affect root hair patterning. Plant Physiol **140:** 761–770

Li M, Welti R, Wang X (2006b) Quantitative profiling of Arabidopsis polar glycerolipids in response to phosphorus starvation: roles of phospholipases D ζ1 and D ζ2 in phosphatidylcholine hydrolysis and digalactosyldiacylglycerol accumulation in phosphorus-starved plants. Plant Physiol **142:** 750–761

Li Z, Gao Q, Liu Y, He C, Zhang X, Zhang J (2011) Overexpression of transcription factor ZmPTF1 improves low phosphate tolerance of maize by regulating carbon metabolism and root growth. Planta **233:** 1129–1143

Lim J, Jung JW, Lim CE, Lee MH, Kim BJ, Kim M, Bruce WB, Benfey PN (2005) Conservation and diversification of SCARECROW in maize. Plant Mol Biol **59:** 619–630

Lin SI, Chiang SF, Lin WY, Chen JW, Tseng CY, Wu PC, Chiou TJ (2008) Regulatory network of microRNA399 and PHO2 by systemic signaling. Plant Physiol **147:** 732–746

Lin WY, Lin SI, Chiou TJ (2009) Molecular regulators of phosphate homeostasis in plants. J Exp Bot **60:** 1427–1438

Liu C, Muchhal US, Uthappa M, Kononowicz AK, Raghothama KG (1998) Tomato phosphate transporter genes are differentially regulated in plant tissues by phosphorus. Plant Physiol **116:** 91–99

Liu F, Wang Z, Ren H, Shen C, Li Y, Ling HQ, Wu C, Lian X, Wu P (2010) OsSPX1 suppresses the function of OsPHR2 in the regulation of expression of OsPT2 and phosphate homeostasis in shoots of rice. Plant J **62:** 508–517

Lloyd JC, Zakhleniuk OV (2004) Responses of primary and secondary metabolism to sugar accumulation revealed by microarray expression analysis of the Arabidopsis mutant, pho3. J Exp Bot **55:** 1221–1230

López-Bucio J, Hernández-Abreu E, Sánchez-Calderón L, Nieto-Jacobo MF, Simpson J, Herrera-Estrella L (2002) Phosphate availability alters architecture and causes changes in hormone sensitivity in the Arabidopsis root system. Plant Physiol **129:** 244–256

Lynch JP, Brown KM (2001) Topsoil foraging: an architectural adaptation to low phosphorus availability. Plant Soil **237:** 225–237

Ma Z, Baskin TI, Brown KM, Lynch JP (2003) Regulation of root elongation under phosphorus stress involves changes in ethylene responsiveness. Plant Physiol **131:** 1381–1390

Miao J, Sun J, Liu D, Li B, Zhang A, Li Z, Tong Y (2009) Characterization of the promoter of phosphate transporter TaPHT1.2 differentially expressed in wheat varieties. J Genet Genomics **36:** 455–466

Misson J, Raghothama KG, Jain A, Jouhet J, Block MA, Bligny R, Ortet 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

Misson J, Thibaud MC, Bechtold N, Raghothama K, Nussaume L (2004) Transcriptional regulation and functional properties of Arabidopsis Pht1;4, a high affinity transporter contributing greatly to phosphate uptake in phosphate deprived plants. Plant Mol Biol **55:** 727–741

Mitsukawa N, Okumura S, Shirano Y, Sato S, Kato T, Harashima S, Shibata D (1997) Overexpression of an Arabidopsis thaliana high-affinity phosphate transporter gene in tobacco cultured cells enhances cell growth under phosphate-limited conditions. Proc Natl Acad Sci USA **94:** 7098–7102

Miura K, Rus A, Sharkhuu A, Yokoi S, Karthikeyan AS, Raghothama KG, Baek D, Koo YD, Jin JB, Bressan RA, et al (2005) The Arabidopsis SUMO E3 ligase SIZ1 controls phosphate deficiency responses. Proc Natl Acad Sci USA **102:** 7760–7765

Morcuende R, Bari R, Gibon Y, Zheng W, Pant BD, Bläsing O, Usadel B, Czechowski T, Udvardi MK, Stitt M, et al (2007) Genome-wide reprogramming of metabolism and regulatory networks of Arabidopsis in response to phosphorus. Plant Cell Environ **30:** 85–112

Nagy R, Vasconcelos MJV, Zhao S, McElver J, Bruce W, Amrhein N, Raghothama KG, Bucher M (2006) Differential regulation of five *Pht1* phosphate transporters from maize (*Zea mays* L.). Plant Biol (Stuttg) **8:** 186–197

Nakajima K, Benfey PN (2002) Signaling in and out: control of cell division and differentiation in the shoot and root. Plant Cell (Suppl) **14:** S265–S276

Neumann G, Römheld V (1999) Root excretion of carboxylic acids and protons in phosphorus-deficient plants. Plant Soil **211:** 121–130

Nilsson L, Müller R, Nielsen TH (2007) Increased expression of the MYB-related transcription factor, PHR1, leads to enhanced phosphate uptake in Arabidopsis thaliana. Plant Cell Environ **30:** 1499–1512

O'Dell B, de Boland A, Koirtyohann S (1972) Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. J Agric Food Chem **20:** 718–721

Pant BD, Buhtz A, Kehr J, Scheible WR (2008) MicroRNA399 is a long-distance signal for the regulation of plant phosphate homeostasis. Plant J **53:** 731–738

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol **6:** 763–775

Paszkowski U, Boller T (2002) The growth defect of lrt1, a maize mutant lacking lateral roots, can be complemented by symbiotic fungi or high phosphate nutrition. Planta **214:** 584–590

Paszkowski U, Kroken S, Roux C, Briggs SP (2002) Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci USA **99:** 13324–13329

Pérez Torres CA, López Bucio J, Herrera Estrella L (2009) Low phosphate signaling induces changes in cell cycle gene expression by increasing auxin sensitivity in the Arabidopsis root system. Plant Signal Behav **4:** 781–783

Poirier Y, Thoma S, Somerville C, Schiefelbein J (1991) Mutant of Arabidopsis deficient in xylem loading of phosphate. Plant Physiol **97:** 1087–1093

Raghothama KG (1999) Phosphate acquisition. Annu Rev Plant Physiol Plant Mol Biol **50:** 665–693

Ramon M, Rolland F (2007) Plant development: introducing trehalose metabolism. Trends Plant Sci **12:** 185–188

Reymond M, Svistoonoff S, Loudet O, Nussaume L, Desnos T (2006) Identification of QTL controlling root growth response to phosphate starvation in Arabidopsis thaliana. Plant Cell Environ **29:** 115–125

Rubio V, Linhares F, Solano R, Martín AC, Iglesias J, Leyva A, Paz-Ares J (2001) A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. Genes Dev **15:** 2122–2133

Satoh-Nagasawa N, Nagasawa N, Malcomber S, Sakai H, Jackson D (2006) A trehalose metabolic enzyme controls inflorescence architecture in maize. Nature **441:** 227–230

Sawers RJ (2009) Breeding for reaction. *In* N Huttunen, T Sinisalo, eds, Plant Breeding. Nova Publishers, New York, pp 127–142

Sawers RJ, Gebreselassie MN, Janos DP, Paszkowski U (2010) Charac-

terizing variation in mycorrhiza effect among diverse plant varieties. Theor Appl Genet **120:** 1029–1039

Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, Pasternak S, Liang C, Zhang J, Fulton L, Graves TA, et al (2009) The B73 maize genome: complexity, diversity, and dynamics. Science **326:** 1112–1115

Schünmann PH, Richardson AE, Smith FW, Delhaize E (2004a) Characterization of promoter expression patterns derived from the Pht1 phosphate transporter genes of barley (Hordeum vulgare L.). J Exp Bot **55:** 855–865

Schünmann PH, Richardson AE, Vickers CE, Delhaize E (2004b) Promoter analysis of the barley Pht1;1 phosphate transporter gene identifies regions controlling root expression and responsiveness to phosphate deprivation. Plant Physiol **136:** 4205–4214

Secco D, Baumann A, Poirier Y (2010) Characterization of the rice PHO1 gene family reveals a key role for OsPHO1;2 in phosphate homeostasis and the evolution of a distinct clade in dicotyledons. Plant Physiol **152:** 1693–1704

Shin H, Shin HS, 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

Shin H, Shin HS, Dewbre GR, Harrison MJ (2004) Phosphate transport in Arabidopsis: Pht1;1 and Pht1;4 play a major role in phosphate acquisition from both low- and high-phosphate environments. Plant J **39:** 629–642

Smith AP, Jain A, Deal RB, Nagarajan VK, Poling MD, Raghothama KG, Meagher RB (2010) Histone H2A.Z regulates the expression of several classes of phosphate starvation response genes but not as a transcriptional activator. Plant Physiol **152:** 217–225

Stefanovic A, Ribot C, Rouached H, Wang Y, Chong J, Belbahri L, Delessert S, Poirier Y (2007) Members of the PHO1 gene family show limited functional redundancy in phosphate transfer to the shoot, and are regulated by phosphate deficiency via distinct pathways. Plant J **50:** 982–994

Steinkellner S, Lendzemo V, Langer I, Schweiger P, Khaosaad T, Toussaint JP, Vierheilig H (2007) Flavonoids and strigolactones in root exudates as signals in symbiotic and pathogenic plant-fungus interactions. Molecules **12:** 1290–1306

Stevenson-Paulik J, Bastidas RJ, Chiou ST, Frye RA, York JD (2005) Generation of phytate-free seeds in Arabidopsis through disruption of inositol polyphosphate kinases. Proc Natl Acad Sci USA **102:** 12612–12617

Svistoonoff S, Creff A, Reymond M, Sigoillot-Claude C, Ricaud L, Blanchet A, Nussaume L, Desnos T (2007) Root tip contact with low-phosphate media reprograms plant root architecture. Nat Genet **39:** 792–796

Theodorou ME, Plaxton WC (1993) Metabolic adaptations of plant respiration to nutritional phosphate deprivation. Plant Physiol **101:** 339–344

Ticconi CA, Delatorre CA, Lahner B, Salt DE, Abel S (2004) Arabidopsis pdr2 reveals a phosphate-sensitive checkpoint in root development. Plant J **37:** 801–814

Ticconi CA, Lucero RD, Sakhonwasee S, Adamson AW, Creff A, Nussaume L, Desnos T, Abel S (2009) ER-resident proteins PDR2 and LPR1 mediate the developmental response of root meristems to phosphate availability. Proc Natl Acad Sci USA **106:** 14174–14179

Tittarelli A, Milla L, Vargas F, Morales A, Neupert C, Meisel LA, Salvo GH, Peñaloza E, Muñoz G, Corcuera LJ, et al (2007) Isolation and comparative analysis of the wheat TaPT2 promoter: identification in silico of new putative regulatory motifs conserved between monocots and dicots. J Exp Bot **58:** 2573–2582

Tjellström H, Andersson MX, Larsson KE, Sandelius AS (2008) Membrane phospholipids as a phosphate reserve: the dynamic nature of phospholipid-to-digalactosyl diacylglycerol exchange in higher plants. Plant Cell Environ **31:** 1388–1398

Uhde-Stone C, Zinn KE, Ramirez-Yáñez M, Li A, Vance CP, Allan DL (2003) Nylon filter arrays reveal differential gene expression in proteoid roots of white lupin in response to phosphorus deficiency. Plant Physiol **131:** 1064–1079

Usuda H, Shimogawara K (1991) Phosphate deficiency in maize. I. Leaf phosphate status, growth, photosynthesis and carbon partitioning. Plant Cell Physiol **32:** 497–504

Usuda H, Shimogawara K (1992) Phosphate deficiency in maize. III. Changes in enzyme activities during the course of phosphate deprivation. Plant Physiol **99:** 1680–1685

Versaw WK, Harrison MJ (2002) A chloroplast phosphate transporter, PHT2;1, influences allocation of phosphate within the plant and phosphate-starvation responses. Plant Cell **14:** 1751–1766

Vielle-Calzada JP, Martínez de la Vega O, Hernández-Guzmán G, Ibarra-Laclette E, Alvarez-Mejía C, Vega-Arreguín JC, Jiménez-Moraila B, Fernández-Cortés A, Corona-Armenta G, Herrera-Estrella L, et al (2009) The Palomero genome suggests metal effects on domestication. Science **326:** 1078

Vieten A, Sauer M, Brewer PB, Friml J (2007) Molecular and cellular aspects of auxin-transport-mediated development. Trends Plant Sci **12:** 160–168

Wang C, Ying S, Huang H, Li K, Wu P, Shou H (2009) Involvement of OsSPX1 in phosphate homeostasis in rice. Plant J **57:** 895–904

Wasaki J, Shinano T, Onishi K, Yonetani R, Yazaki J, Fujii F, Shimbo K, Ishikawa M, Shimatani Z, Nagata Y, et al (2006) Transcriptomic analysis indicates putative metabolic changes caused by manipulation of phosphorus availability in rice leaves. J Exp Bot **57:** 2049–2059

Wasaki J, Yonetani R, Kuroda S, Shinano T, Yazaki J, Fujii F, Shimbo K, Yamamoto K, Sakata K, Sasaki T, et al (2003) Transcriptomic analysis of metabolic changes by phosphorus stress in rice plant roots. Plant Cell Environ **26:** 1515–1523

Williamson LC, Ribrioux SP, Fitter AH, Leyser HM (2001) Phosphate availability regulates root system architecture in Arabidopsis. Plant Physiol **126:** 875–882

Wright DP, Scholes JD, Read DJ, Rolfe SA (2005) European and African maize cultivars differ in their physiological and molecular responses to mycorrhizal infection. New Phytol **167:** 881–896

Xie Q, Frugis G, Colgan D, Chua NH (2000) Arabidopsis NAC1 transduces auxin signal downstream of TIR1 to promote lateral root development. Genes Dev **14:** 3024–3036

Xie Q, Guo HS, Dallman G, Fang S, Weissman AM, Chua NH (2002) SINAT5 promotes ubiquitin-related degradation of NAC1 to attenuate auxin signals. Nature **419:** 167–170

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

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

Zakhleniuk OV, Raines CA, Lloyd JC (2001) pho3: a phosphorus-deficient mutant of Arabidopsis thaliana (L.) Heynh. Planta **212:** 529–534

Zhang L, Chia JM, Kumari S, Stein JC, Liu Z, Narechania A, Maher CA, Guill K, McMullen MD, Ware D (2009) A genome-wide characterization of microRNA genes in maize. PLoS Genet **5:** e1000716

Zhou J, Jiao F, Wu Z, Li Y, Wang X, He X, Zhong W, Wu P (2008) OsPHR2 is involved in phosphate-starvation signaling and excessive phosphate accumulation in shoots of plants. Plant Physiol **146:** 1673–1686

Zhu J, Kaeppler SM, Lynch JP (2005) Mapping of QTLs for lateral root branching and length in maize (Zea mays L.) under differential phosphorus supply. Theor Appl Genet **111:** 688–695