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Isolation of high-affinity phosphate transporters *SbPT1* AND *SbPT2* in *Sorghum bicolor* and their characterization in contrasting genotypes

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ABSTRACT. Phosphate (Pi) availability is highly limited in the acidic soils of the Brazilian savannahs (Cerrado) used for sorghum cultivation. Although several sorghum genotypes contrasting for P use-efficiency have been developed from natural genetic variants, the Pi transport pathway mechanisms in these plants remain unclear. High-affinity Pi transporters play a pivotal role in Pi acquisition by roots and its subsequent mobilization to aerial parts of the plant. We investigated the potential roles of high-affinity Pi transporters in Pi use efficiency in contrasting genotypes of sorghum. A cDNA library prepared from Pi-deprived sorghum seedlings was screened with heterologous *Zea mays* (maize) Pi transporters *ZmPT*s, leading to isolation of two homologous sorghum genes referred to as *SbPT 1* and *SbPT2*. Southern analysis revealed that a small gene family represents the *SbPT*s genes in the sorghum genome. There were significant increases in the transcription levels of *SbPT1* and S*bPT2* in roots of Pi-deprived seedlings of both Pi-use efficient (101B) and Pi-use inefficient (136B) genotypes. A decrease in the transcript levels of these transporters in 101B and 136B upon Pi replenishment

suggested their transcriptional regulation by Pi. Although *SbPT1* and *SbPT2* were induced in the roots, and in young and old leaves of Pi-deprived sorghum, high transcription levels were observed exclusively in the stems of Pi-efficient genotype 101B under Pi-deprivation. This suggests a role of *SbPT*s in the efficient mobilization of Pi from the root to the shoot, which could be one of the factors conferring higher Pi-use efficiency in this genotype.

Key words: *Sorghum bicolor*; Pi-use efficiency; Contrasting genotypes; High-affinity Pi transporters; Northern analysis

INTRODUCTION

Phosphorus (P), an essential macronutrient for all living organisms, is a constituent of various biologically relevant molecules and serves as a metabolite in a broad spectrum of biological processes (Marschner, 1995; Motomura et al., 2018). P is mostly made available for the plant rhizospheres in the form of inorganic phosphate (Pi) (Raghothama, 1999; Shen et al., 2011). Due to slow diffusion rates of Pi and its interaction with various soil constituents, its availability is considerably limiting, leading to adverse effects on crop growth and development (Wu et al., 2013; Baveye, 2015).

In Latin America, many crops are raised on vast stretches of severely Pi deficient soils (Yan et al., 1996; Fageria et al., 2014). In Brazil, soils of the Cerrado (savanna type regions) are highly acidic, with toxic levels of aluminum (Al) and low levels of P. Thus, Pi availability is a major yield-limiting factor for crops in the region (Schaffert et al., 2001; Chen et al., 2019). In conventional agriculture, Pi deficiency is alleviated by the application of Pi fertilizers. However, this practice is expensive and unsustainable due to dwindling reserves of non-renewable rock Pi (Kisko et al., 2018). There is also increasing evidence that fertilizers alone cannot sustain yields for extended periods (Tilman et al., 2002; Liverpool-Tasie et al., 2017; Mtangadura et al., 2017). Among the strategies to minimize this problem, there is (i) screening the diversity of sorghum germplasm with better Pi-use efficiency under Pi-deprived conditions and (ii) decipher the underlying morphophysiological and/or molecular mechanisms regulating this trait. Several crop species show inter-and intra-specific variation in their ability to grow under Pi-limiting conditions (Lynch and Beebe, 1995; Hammond et al., 2004; Leiser et al., 2015; Schneider et al., 2019). These variations are primarily correlated with an increased length and density of root hairs, facilitating the exploration of soil by the roots (Bates and Lynch, 2001; Gahoonia et al., 2001; Bernardino et al., 2019). Formation of cortical aerenchyma in the roots of some elite bean genotypes has also been attributed to their adaptation to Pi-deficient soil (Fan et al., 2003; Anis et al., 2018). Concurrently, quantitative trait loci (QTL) mapping of rice populations (Wissuwa and Ae, 2001; Anis et al., 2018; Jewel et al., 2019) showed that about 80% of the variations between Pi-use efficient and inefficient genotypes were involved a single QTL *Pup1* (*Phosphorus uptake 1*) (Wissuwa et al., 2002), which was later identified as *PSTOL1* (*Phosphorus Starvation Tolerance 1*) encoding Pup1 protein kinase (Gamuyao et al., 2012). Introgression of *Pup1* into rice varieties significantly raised the Pi uptake and grain yield in soils with low Pi (Wissuwa and Ae, 2001). However, the QTL-based approach for identifying a candidate gene is generally time-consuming and labor-intensive.

Alternatively, microarray, RNA sequencing (RNA-Seq), proteomics and metabolomics have sped up identifying an array of phosphate starvation-responsive (PSR) molecular determinants that play critical roles in maintaining Pi homeostasis (Misson et al., 2005; Bielecka et al., 2015; Heuer et al., 2017). Among these PSR molecular determinants, extensive work has been carried out on the PHT1 family of plasma membrane-localized Pi transporters (Park et al., 2007; Nussaume et al., 2011). Homolog genes of PHT1 transporters have been identified and characterized in several members of the grass family (Nagy et al., 2006; Zhang et al., 2015; Roch et al., 2019). Studies using two Pi transporter promoters regulating three reporter genes (green fluorescent protein, luciferase, and β-glucuronidase) showed their spatial regulation ranging from different parts of roots, and aerial tissues (vegetative and reproductive organs) (Karthikeyan et al., 2002; 2009; Zhang et al., 2015). These results suggested that their broad-spectrum role is not only the Pi acquisition from rhizosphere by roots but also its allocation from source to sink tissues. Loss-of-function mutations in *Pht1;1* and *Pht1;4* transporters in Arabidopsis resulted in significant attenuation in Pi uptake capacity of the mutants (Shin et al., 2004). Whereas loss-of-function mutations in *Pht1;5* resulted in an attenuated allocation of Pi to Pi-deprived shoots (Nagarajan et al., 2011). Further, *OsPT4* has been shown to play critical roles in the acquisition and mobilization of Pi and the development of the embryo in rice (Zhang et al., 2015). Therefore, it is logical to assume their likely role in higher Pi-use efficiency of some of the elite genotypes. The study by Davies et al. (2002), which demonstrated the differential expression pattern of different Pht1 transporters in different wheat genotypes and responded differently to Pi deprivation, provides some creditability to this assumption.

Sorghum bicolor (sorghum) is a drought-resistant crop grown around the world. The crop has a lower cost of production than maize and other competing crops, cultivated for both grain and forage. Since 2001 until today, the Brazilian Embrapa Maize and Sorghum Center (Embrapa) has extensively evaluated and selected the diversity of sorghum populations from different geographical locations for Pi-acquisition, Pi-use efficiency, and grain yield (Schaffert et al., 2001). Several diallel crosses and inbred lines were generated with varying levels of Pi-use efficiency and Pi-responsiveness (Schaffert et al., 2001). However, the molecular mechanism governing Pi-use efficiency in contrasting genotypes of sorghum has not yet been deciphered.

We isolated and characterized *SbPT1* and *SbPT2* encoding high-affinity Pi transporters in sorghum. Contrasting Pi-use efficient and Pi-inefficient sorghum genotypes from Embrapa were used for comparative analysis of expression profiles in various tissues under contrasting Pi regimes.

MATERIAL AND METHODS

Plant materials and growth conditions

Sorghum bicolor genotypes with contrasting responses to Pi-use efficiency were evaluated at Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil. They were classified into Pi-efficient (101B, ATF14B, and ATF53B) and Pi-inefficient (ATF16B, 116R, 136B, and 187R) relative to grain yield performance in the field. Seeds of Pi-use efficient and Pi-inefficient sorghum genotypes were germinated in seedling trays containing Scott's ready earth plug mix (Scotts Co., Marysville, OH) grown in the greenhouse for one week. One-

week-old seedlings were gently washed with water to remove the soil medium from the roots and transferred to one half-strength modified Hoagland's nutrient solution for one week before stress application (Liu et al., 1998). To study the effects of different concentrations of Pi on the expression of *SbPT1* and *SbPT*2 genes, the seedlings were transferred to Hoagland's nutrition solution containing different concentrations of Pi (0, 5, 10, 25, 50, 100 and 250 μM). During the treatments, nutrient solutions were replaced with a freshly prepared nutrient solution on alternate days. After 15 days, the roots were harvested from the plants, frozen in liquid nitrogen, and stored at –80ºC for later use in Northern analysis. The absence (0 μM Pi) and the presence (250 μM Pi) concentrations were treated as Pi-deficient (P-) and Pi-sufficient (P+), respectively. For the time-course study, roots from both P+ and P- treatments were harvested sequentially after 1, 3, 5, 6, 7, 8, 12, and 15 d of growth. After 15 days of growth under P+ and P- conditions, roots, stem, and young and old leaves were harvested separately to evaluate the spatial expression of Pi transporters. In another set of experiments, plants starved of Pi for 15 days were replenished with 250 μM of Pi and harvested after 1, 2, 3, 4, and 5 days of treatment to determine the role of Pi in the transcriptional regulation of Pi transporters in sorghum.

Construction and screening of cDNA library

The total RNA was isolated from the roots of Pi-use efficient sorghum genotype starved of Pi for 15 days by using a hot phenol and lithium chloride precipitation method (Pawlowski et al., 1994). The mRNA was isolated using the PolyATtract® mRNA kit (Promega Corporation, Madison, WI). The quality of mRNA was determined on a denatured agarose gel, and 5 μg was used to prepare the cDNA library. A cDNA library was constructed in the *Eco*RI-*Xho*I site of the Uni-ZAP XR vector following the manufacturer's instructions (Stratagene, CA). The library was screened with ^{32}P-labeled high-affinity Pi transporters from maize according to standard procedures (Nagy et al*.,* 2006). Based on the insert size and restriction mapping, the representative clones were sequenced at the Purdue Genomic Facility (West Lafayette, IN).

Rapid amplification of cDNA ends (RACE-PCR)

The full-length clones were obtained by 5'-RACE-PCR using the first strand of sorghum cDNA as a template. The reaction was carried out according to the manufacturer's instructions (Stratagene, CA). The RACE-PCR products were cloned using the TOPO TA cloning kit and TOP10F chemically and competent *E.coli* (Invitrogen, CA). The two clones isolated were named as *SbPT1* and *SbPT2*, sequenced at Purdue Genomic Facility (West Lafayette, IN). The nucleotide sequences were deposited in the GenBank database at NCBI and received the following accession numbers MH333040 and MH333041, respectively.

Southern analysis

Genomic DNA was extracted from the leaves of sorghum, as described by Dellaporta et al. (1983). Twenty micrograms of genomic DNA was digested with *Bam*HI, *Eco*RI, and *Hind*III and separated on 1% (w/v) agarose gel by electrophoresis. Southern analysis was carried out on supported nylon membranes, and the DNA was cross-linked and

hybridized with the ^{32}P labeled cDNA probes of *SbPT1* and *SbPT*2. The filters were initially washed twice for 10 min with a low stringency solution consisting of 2X SSC and 0.2% SDS (v/v), followed by a high stringency wash with 0.1X SSC and 0.1% SDS (v/v) at 42^{o}C for 10 min. Membranes were exposed to Kodak XAR-5 films.

RNA isolation and Northern blot analysis

The total RNA was extracted by the hot phenol and lithium chloride precipitation method (Pawlowski et al., 1994). Ten micrograms of total RNA was electrophoretically separated on 1.2% (w/v) denaturing formaldehyde agarose gel and blotted onto a nylon membrane following the manufacturer's instructions (MAGNA Osmonics Inc., Minnetonka, MN),. After blotting, the RNA was immobilized on the membrane by UV cross-linking (120 mJ) in a UV Stratalinker (Stratagene, La Jolla, CA, USA). The pre-hybridization was carried out for 2 to 4 h at 42^{o}C in a solution containing 50% (v/v) formamide, 5X Denhardt's solution, 0.1% (w/v) SDS, 6X SSPE and 150 µg/mL denatured salmon sperm DNA. DNA fragments labeled with 32P-dCTP using the DECA prime IITM DNA labeling kit (Ambion, Austin, TX) was used to probe the membranes. Hybridization was carried out with 106 cpm of the gene A1 probe/mL at 42^{o}C for 16 h in a fresh pre-hybridization buffer. The wash conditions were the same as described for Southern blot.

Data analysis

The nucleotide sequences of sorghum *SbPT1* and *SbPT2* genes were used in a TBLASTN query against all nucleotide sequences, and the sorghum taxID sequences deposited in the NCBI/GenBank database. The evolutionary analyses of translated amino acid sequences comparisons among *SbPT1*, *SbPT2*, and the fifteen predicted genes encoding for sorghum phosphate transporters were conducted in MEGA X (Kumar et al., 2018), and the evolutionary history was inferred using the UPGMA method. Neighbor-joining trees were constructed using the Jones-Taylor-Thornton (JTT) substitution rate matrix for distance computation. All ambiguous positions were removed for each sequence pair, and there were 631 positions in the final dataset. The evolutionary distances were computed using the Poisson correction method (Zuckerkandl and Pauling, 1965) and are in the units of the number of amino acid substitutions per site. Branch lengths (drawn in the horizontal dimension) are proportional to phylogenetic distances. The programs TMHMM, TMpred, and TopPred (http://www.expasy.ch/proteomics) were used to predict the putative transmembrane (TM) domains for proteins coded by *SbPT1* and *SbPT2* genes.

RESULTS

Isolation of sorghum Pi transporters *SbPT1* and *SbPT2*

Two-week-old seedlings of the Pi-use efficient genotype (101B) were grown hydroponically under P- (0 µM Pi) condition for 15 d in the greenhouse. Then, they were used to prepare the cDNA library, which was subsequently screened by heterologous probes of *ZmPT*s. Two partial clones were isolated from the cDNA library constructed with RNA extracted from Pi-deprived (0 µM Pi) plants. These two clones are hereafter referred to

as *SbPT* (Sorghum bicolor Phosphate Transporter) *1* and *2*. Southern analysis revealed that the two *SbPT*s genes are represented by a gene family in the sorghum genome (Figure 1). The full-length clone of *SbPT1* obtained by RACE PCR was 2,130 bp long and contained a 1,623 bp open reading frame (ORF). The ORF is flanked at 5', and 3' ends by 232 and 275 bp (including the poly-A tail). *SbPT2* is a partial clone with 1,623 bp long and the terminal 3'end. The nucleotide sequences deposited in the GenBank has received the numbers MH333040 (*SbPT1*) and MH333041 (*SbPT2*), and the similarity among the sorghum *pht1* genes deposited in the Genbank are shown in Figure 2. The predicted proteins coded by the open reading frames (ORF) from the clones MH333040 and MH333041 were 541 and 408 amino acids, respectively. Both proteins display the pht1 signature sequence GGDYPLSATIxSE between the TM4 and TM5 transmembrane domains. Sorghum Pi transporter proteins showed variable levels of homology (59 - 87%) with Pi transporter proteins of some species in the grass family (Table 1).

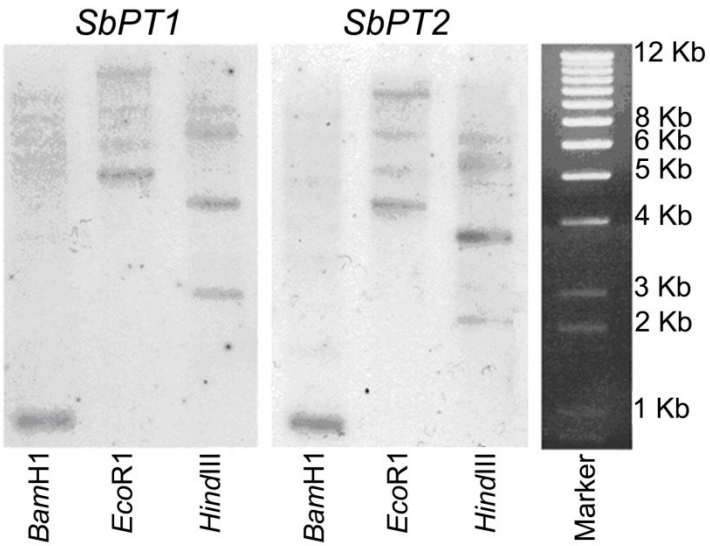

Figure 1. Southern analysis of *SbPT1* and *SbPT2*. Sorghum genomic DNA was digested with *Bam*H1, *Eco*R1 and *Hind*III and probed with ^{32}P-labeled *SbPT1* and *SbPT2*. The DNA ladder was used as a size marker.

Table 1. Homology (in %) of *SbPT1* and *SbPT2* with Pi transporters from the grass family.

Maize Pi transporters (*ZmPT*s)													
	ZmPT1	*ZmPT2*	*ZmPT3*	*ZmPT4*	*ZmPT5*	*ZmPT6*							
SbPT1	87	87	72	85	60	72							
SbPT2	86	86	78	83	60	73							
Barley Pi transporters (*HvPT*s)													
	HvPT1	*HvPT2*	*HvPT4*	*HvPT5*	*HvPT6*	*HvPT7*	*HvPT8*						
SbPT1	76	76	84	80	78	78	71						
SbPT2	75	75	83	81	77	75	70						
Rice Pi transporters (*OsPT*s)													
	OsPT1	*OsPT2*	*OsPT3*	*OsPT4*	*OsPT5*	*OsPT6*	*OsPT7*	*OsPT8*	*OsPT9*	*OsPT10*	*OsPT11*	*OsPT12*	*OsPT13*
SbPT1	78	77	78	80	78	78	77	86	59	59	59	80	61
SbPT2	77	75	76	80	78	78	76	85	59	60	60	80	65

Percent homology of *SbPT*s with Pi transporters from Zea *mays* (*ZmPT*s), *Oryza sativum* (*OsPT*s) and *Hordeum vulgaris* (*HvPT*s). The homology was determined using the FASTA program in GCG (Madison, WI) package and the protein sequences were retrieved in FASTA format using BLAST.

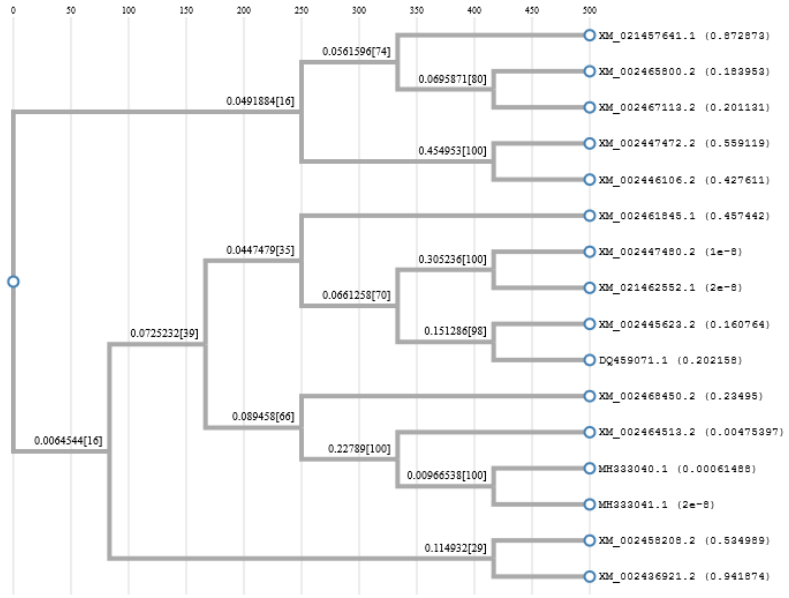

Figure 2. Phylogenetic analysis of sorghum *pht1* transporters genes.

Selection of contrasting genotypes for Pi-use efficiency

Sorghum is an essential crop in Brazil, often cultivated in Pi-deficient acidic soils of the Cerrado. Currently, about 90% of the Pi fertilizer is obtained as mined phosphate rock, a non-renewable resource, and the excessive use of P-fertilizers is a serious risk to the environment. Thus, the selection and development of Pi-use efficient sorghum varieties using a higher proportion of P-fixed in the soil is critical for the long-term sustainability of the agricultural system. For the last three decades, the Sorghum Breeding Program of the Embrapa has screened several diallel crosses and inbred lines from natural genetic variants of sorghum collected from different geographical locations of Brazil. The selected genotypes show higher Pi-use efficiency and above-average yields compared with Pi-use inefficient genotypes performing in acidic soils either completely deprived of Pi (0 µM Pi) or corrected with Pi to a critical level. Exhaustive screening based on their field performance for grain yield resulted in the identification of several contrasting genotypes for Pi-use efficiency. It referred to as Pi-use efficient (ATF14B, ATF53B, 101B) and Pi-use inefficient genotypes (ATF16B, 116R, 136B, 187R).

*SbPT*s are induced in response to Pi deficiency in contrasting genotypes

The effects of Pi deprivation on the expression of *SbPT 1* and *2* genes were evaluated by using three efficient (ATF14B, ATF53B and 101B), and four inefficient (ATF16B, 116R, 136B, 187R) sorghum genotypes were raised hydroponically under 250 mM Pi (P+) and 0 mM Pi (P-) conditions for 15 days. Pi-deficiency induced accumulation of *SbPT1*, and two transcripts were observed in the roots of all the efficient and inefficient

genotypes (Figure 3). The study indicated that the Pi-efficient and Pi-inefficient genotypes respond to Pi stress by inducing the expression of Pi transporters in the roots.

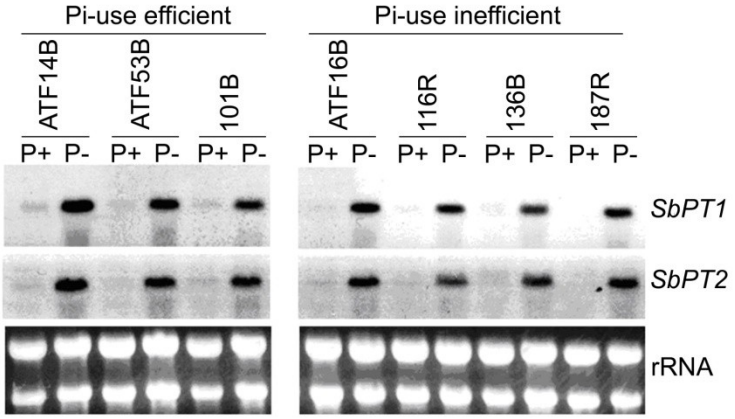

Figure 3. Phospate (Pi) deficiency-mediated induction of *SbPT*s. Northern blot analysis of the roots of Pi use-efficient and-inefficient sorghum genotypes grown hydroponically under P+ (250 µM) and P- (0 µM) conditions for 15 d. Blots were hybridized with ^{32}P-labeled *SbPT1* and *SbPT2*. Equivalence of RNA loading in all the lanes is shown by ethidium bromide-stained rRNA (bottom panel).

Transcriptional regulation of *SbPT*s by Pi in contrasting genotypes

To determine whether there are any differences in the transcript accumulations of *SbPTs* in response to varying Pi concentrations (0, 5, 10, 25, 50, 100, 250 µM) in contrasting genotypes, 101B and 136B were selected as representatives of Pi-use efficient and Pi-inefficient, respectively. In both genotypes, the abundance of *SbPT1* of the two transcripts significant decline at higher Pi concentrations (50-100 µM Pi) and was barely detected at 250 µM Pi (Figure 4).

Therefore, to determine the rapidity of Pi deficiency-mediated transcript accumulation of *SbPT*s in roots, 101B, and 136B genotypes were grown hydroponically under P+ (250 µM) and P- (0 µM) conditions for different time intervals (1, 3, 5, 6, 7, 8, 10 12, and 15 d) and evaluated by Northern blot analysis (Figure 5A). After one day of Pi starvation, no significant increase in transcript levels of *SbPT1* was detected in both 101B and 136B. Transcript levels of *SbPT2* were also comparable in 136B under P+ and P- conditions. However, significant accumulation of *SbPT2* could be detected in 101B starved of Pi for one day. These results suggested an apparent lack of functional redundancy between *SbPT1* and *SbPT2*. Transcript levels of *SbPT*s continued to increase with prolonged Pi starvation for up to 5 days in both contrasting genotypes until stabilization.

We further determined the role of Pi in the transcriptional regulation of *SbPT*s in Pi-use efficiency (101B) and Pi-inefficiency (136B). The genotypes were grown hydroponically under Pi-deprived condition for 15 days, replenished with Pi (250 uM). Then the roots were harvested sequentially from 1 day to 5 days and then used for Northern analysis (Figure 5B). Upon replenishment, with Pi, a noticeable decrease in the expression of *SbPT1* in 101B was observed by the fourth day of replacement. Relatively, its suppression in the expression in 136B could be detected only on the fifth day. This

observation gives evidence of the ability of 101B to recuperate faster than the 136B upon replenishment with the Pi-replete medium. Interestingly, reductions in the *SbPT2* transcripts in 101B and 136B could be detected as early as on the third day of Pi replenishment and further provided evidence for the lack of functional redundancy between *SbPT1* and *SbPT2*.

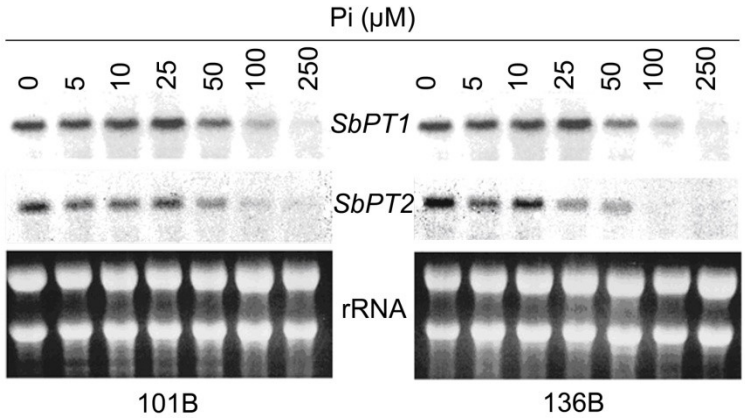

Figure 4. Effect of phosphate (Pi) concentration on transcript abundance of *SbPT*s. Northern blot analysis of the roots of Pi use-efficient (101B) and-inefficient (136B) sorghum genotypes grown hydroponically in Hoagland's solution supplemented with different concentrations of Pi (0 μM Pi to 250 μM Pi) for 15 d. Blots were hybridized with ^{32}P-labeled *SbPT1* and *SbPT2*. Equivalence of RNA loading in all the lanes is shown by ethidium bromide-stained rRNA (bottom panel).

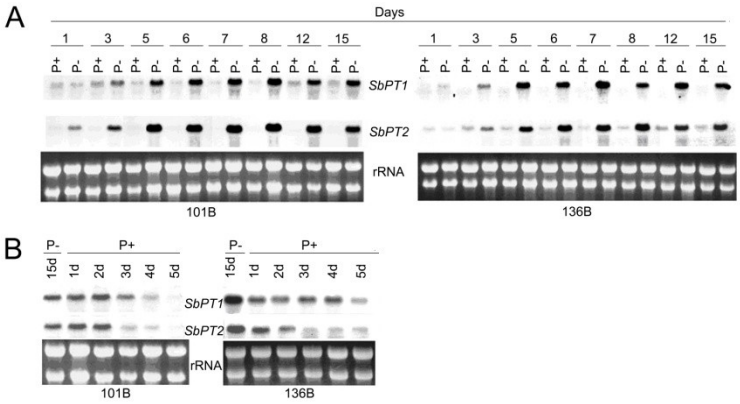

Figure 5. *SbPT*s are transcriptionally regulated by phosphate (Pi). Pi use-efficient (101B) and-inefficient (136B) sorghum genotypes were grown hydroponically under (A) P+ (250 μM) and P- (0 μM) conditions for different time intervals and (B) starved of Pi for 15 d followed by replenishment with Pi for different period. (A and B) Northern blots were prepared from root samples and hybridized with ^{32}P-labeled *SbPT1* and *SbPT2*. Equivalence of RNA loading in all the lanes is shown by ethidium bromide-stained rRNA (bottom panel).

Differential spatial expression of *SbPT*s in Pi-use efficient and-inefficient genotypes

Sorghum genotypes 101B and 136B were grown hydroponically under P+ and P- conditions for 15 d and roots, stem, young and old leaves were harvested for Northern

analysis (Figure 6). The expression levels of *SbPT*s were comparable in Pi-deprived roots, and young and old leaves of 101B and 136B. Based on its presumed function and tissue localization, the results suggested the dual role of *SbPT*s in Pi acquisition by roots, subsequent mobilization for young leaves, and remobilization from old leaves in contrasting genotypes.

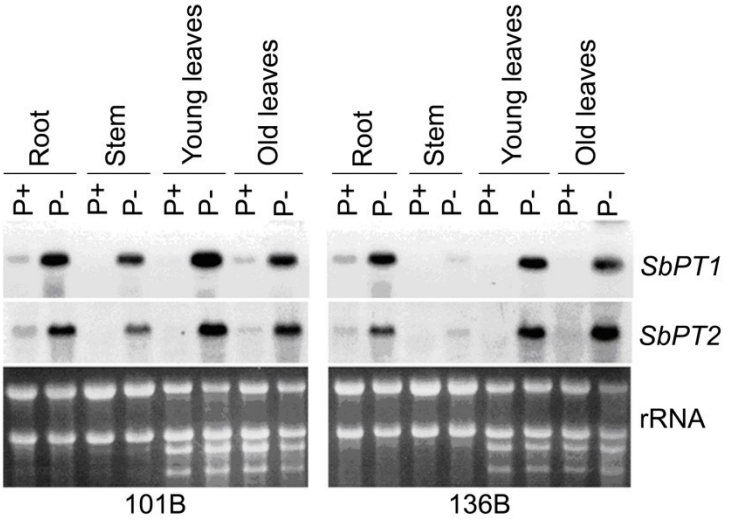

Figure 6. Differential spatial expression of *SbPT*s. Phosphate (Pi) use-efficient (101B) and-inefficient (136B) sorghum genotypes were grown hydroponically under P+ (250 µM) and P- (0 µM) conditions for15 d. Northern blots of different parts (root, stem, young and old leaves) were probed with ^{32}P-labeled *SbPT1* and *SbPT2*. Equivalence of RNA loading in all the lanes is shown by ethidium bromide-stained rRNA (bottom panel).

DISCUSSION

The aim of our work was to investigate the potential roles of high-affinity Pi transporters in Pi use efficiency in contrasting genotypes of sorghum naturally adapted to Pi-deficient soils. Therefore, our strategy was to first isolate high-affinity Pi transporters from a sorghum cDNA library and to characterize its function in Pi-sufficient and Pi-insufficient conditions.

The two clones, *SbPT1* and *SbPT2*, isolated from a cDNA library sowed high homology (83 to 87 %) with the phosphate transporter proteins *OsPT8* (Paszkowski et al., 2002), *HvPT4* (Rae et al., 2003), and *ZmPT*s (Nagy et al., 2006) from rice, barley, and maize, respectively. These results are consistent with earlier studies that found high sequence similarities among the Pi transporter families from a range of plant species (Raghothama, 1999). The isolation of *SbPT1* and *2* are a useful addition to a repertoire of identified *PHT1* transporters from monocotyledonous species (Nagy et al., 2006). These transporters play a pivotal role in Pi uptake from soils and/or mobilization to different vegetative and reproductive organs (Nussaume et al., 2011).

The selection and development of Pi-use efficient sorghum varieties using a higher proportion of P-fixed in the soil is critical for sorghum cultivation in acidic soils. For the last three decades, the Sorghum Breeding Program of the Embrapa has screened several

diallel and inbred lines from natural genetic variants of sorghum collected from different geographical locations of Brazil. The selected genotypes show higher Pi-use efficiency and above-average yields compared with Pi-use inefficient genotypes performing in acidic soils (Schaffert et al., 2001). Similar approaches have also been successfully exploited in developing new rice cultivars efficient for using a higher proportion of fixed P in the soil (Wissuwa and Ae, 2001; Wissuwa et al., 2002). Studies with other crops such as barley and soybean also reported genotypic differences under Pi deficient conditions (Gahoonia and Nielsen, 2004). Several studies have shown Pi deficiency-mediated induction of genes encoding PHT1 transporters in diverse plant species **(**Karthikeyan et al., 2002; Misson et al., 2005; Nagy et al., 2006; Park et al., 2007; Zhang et al., 2015). Transgenic rice (*Oryza sativa* L.) constitutively expressing the tobacco (*Nicotiana tabacum* L.) high-affinity phosphate transporter gene (NtPT1) showed high Pi uptake levels in both low and high Pi concentration (Park et al., 2007). The authors also demonstrated that phosphorus (P) accumulation in the leaves, shoots, and seeds increased significantly, and the PT activity transcriptional controlled pi uptake and accumulation in NtPT1 transgenic rice. However, in elite sorghum, whether these genes have any role in the higher Pi-use efficiency compared with their contrasting counterparts with lower performance in Pi-deficient soils remains a matter of conjecture. In this context, Pi-use efficient and Pi-inefficient sorghum genotypes developed at Embrapa may provide a useful tool for deciphering the role of *PHT1* transporters genes *SbPT1* and *SbPT2*.

Our results on *PHT1* expression in roots and shoots are consistent with earlier studies showing that Pi deficiency causes robust expression of Pi transporters in roots of almost members of PHT1 families of di- and monocotyledonous. (Nagy et al., 2006; Park et al., 2007; Zhang et al., 2015). Eight of the nine Arabidopsis *PHT1* genes are expressed at least in roots highlighting their crucial role in the Pi uptake from the soil (Karthikeyan et al., 2002). The expression of Pi transporters is primarily restricted to the epidermis and root hair zone (Raghothama, 2000; Karthikeyan et al., 2002). In Arabidopsis, transcriptional fusions between promoters of *Pht1;1* and *Pht 1;4* with reporter genes (GUS and GFP) revealed tissue-specific expression pattern in roots. *Pht 1;1* showed a lack of expression in root tips while *Pht1;1* expressed in all cells of the undifferentiated segments of the root, including the tip region (Karthikeyan et al., 2002). This data suggested similar but non-redundant functions of different members of the Pht1 family in the Pi acquisition from soils. Whether there exists any functional redundancy between *SbPT1* and *SbPT2*, it warrants further studies. Considering that the change in transcript abundance of both *SbPT* genes is nearly the same in both Pi-use efficient and Pi-inefficient sorghum, it would be reasonable to conclude that these genes are not relevant for Pi-use efficiency. However, the higher expression of *SbPT* in shoots may be related to an increased number of transporters involved in Pi translocation in organs. Since phosphate use efficiency is determined by several factors, such as uptake, mobility, and recycling, the increased abundance of *SbPT* transcripts in shoots is presumed to contribute towards P efficiency. In sorghum, the post-transcriptional regulation of the transporters and their biochemical properties may be more critical for their function than the fine-tuning of their gene expression (Walder et al., 2015). Thus, studies are needed to prove this assumption in sorghum.

Our finds suggested that variation in Pi concentration in the medium promotes a rapid modulation of *SPT*s transcripts in both contrasting genotypes. Earlier studies showed the ability of different plant species to adjust the level of *PHT1* transcripts in a broad

spectrum of Pi concentrations that often exceeds the expected range in the soils (typically below 10μM) (Muchhal and Raghothama, 1999; Karthikeyan et al., 2002; Misson et al., 2004). In an earlier study, mRNA accumulation and appearance of Pi transporter protein in tomato (*Lycopersicon esculentum*) have been detected within 12 to 24 h after the removal of Pi from the nutrient medium (Liu et al., 1998). A similar temporal trend in transcript accumulation of *Pht1;1* and *Pht1;4* was observed in Pi-deprived Arabidopsis (Karthikeyan et al., 2002).

In Arabidopsis, significant progress has been made in unraveling the role of different transcription factors (TFs) in the transcriptional regulation of various members of the *PHT1* family (Nussaume et al., 2011; Sega and Pacak, 2019). PHOSPHATE STARVATION RESPONSE1 in *Arabidopsis thaliana* (*AtPHR1*) and *OsPHR2* in *Oryza sativa* belong to the MYB-coiled-coil (MYB-CC) related family. It has been the most extensively studied TF in regulating the expression of PSR genes including the members of *PHT1* family in Arabidopsis and *PHT2* in rice (Rubio et al., 2001; Misson et al., 2005; Bari et al., 2006; Zhou et al., 2008; Bustos et al., 2010). Analysis of the conserved sequences located upstream of the transcription start site revealed the presence of cis-regulatory elements *PHR1*-binding sequence (P1BS) in seven out of nine members of the *PHT1* family (Misson et al., 2005). Loss-of function mutation in *PHR1* resulted in significant attenuation in the expression levels of *Pht1;1*, *Pht1;4*, *Pht1;8* and *Pht1;9* (Rubio et al., 2001; Bari et al., 2006). These studies highlighted the role of *PHR1* in regulating the expression of different members of the *PHT1* family. Further, genetic dissection of the *Pht1;4* promoter resulted in the identification of putative binding sequences of TFs Myb2, Myc2, and WRKY (Karthikeyan et al., 2009). Also, RNAi-mediated silencing of *WRKY75* (Devaiah et al., 2007a) or overexpression of *ZAT6* (Devaiah et al., 2007b) and *MYB62* (Devaiah et al., 2009) resulted in the attenuated expression levels of *Pht1;1* and *Pht1;4*. Further, the trafficking of Pi transporters and their post-translational modifications indicate the plethora of molecular mechanisms that govern the optimal functionality of Pi transporters (Nussaume et al., 2011). In this context, further in-depth studies are warranted for deciphering different molecular entities that may have a pivotal role in regulating the function of *SbPT*s in Pi-use efficient and Pi-inefficient sorghum. The robust expression of a promoter-driven reporter gene in senescent leaves of Pi-deprived Arabidopsis, showed that the high-affinity Pi transporter *Pht1;4* is also determinant for Pi remobilization from senescent leaves to younger leaves (Karthikeyan et al., 2002).

It has been suggested that members of the *PHT1* family in *Medicago truncatula* form higher-order structures, potentially dimers or tetramers, and loss of one of the Pi transporter proteins could affect the activity of the whole transporter complex (Chiou et al., 2001). Sieve elements-localized sucrose transporters also form oligomeric complexes due to their capacity to interact with each other (Reinders et al., 2002; Krügel and Kühn, 2013). Further, the double mutant of *Pht1;1* and *Pht1;4* showed about 75% reduction in Pi uptake capacity relative to the wild-type under Pi-deficient condition (Shin et al., 2004). This suggests that members of the *PHT1* family could function together during the acquisition and mobilization of Pi. Whether *SbPT*s operate collectively or independently in maintaining Pi homeostasis is a matter of speculation at present.

Future studies involving the development of transgenic sorghum with RNAi-mediated knockdown of *SbPT1* and *SbPT2* are warranted. However, *SbPT1* and *SbPT2* showed distinct accumulation in the stems of Pi-deprived plants of 101B, and their levels

were barely detectable in 136B. This result suggested the potential roles of *SbPT*s in relatively more efficient Pi mobilization in 101B compared with 136B. These could be due to molecular determinants contributing towards higher Pi use efficiency of 101B genotype. Differences in the expression pattern of Pi transporters have also been reported in the selected wheat varieties (Davies et al., 2002; Grün et al., 2018).

Pi-use efficiency is a complex trait controlled by many induced or suppressed genes, forming a regulatory network interacting with the entire cellular content and the external environment, likely influenced by epistatic interactions. Efforts have been made to elucidate the molecular mechanisms underlying genotypic differences in Pi uptake from P-deficient soils (Zhang et al., 2016; Hasan et al., 2016; Wang et al., 2017; Vasconcelos et al., 2018). Recent studies identified major and minor QTLs associated with P-deficiency in three cereal crops: rice (Anis et al., 2018; Jewel et al., 2019), wheat (Yang et al., 2018), soybean (Yang et al., 2019), and sorghum (Bernardino et al., 2019). Major QTLs for relative shoot dry weight (RSDW) and relative root dry weight (RRDW) were mapped in rice chromosome 12, and several minor QTLs on chromosomes 1, 6, and 9 in plants grown under P deficient conditions (Ni et al., 1998). Recently, Anis et al. (2018) confirmed these data and reported other QTLs associated to low Pi tolerance on chromosomes 2, 3, 4, 5, and 10. A QTL mapping of rice population, developed from a cross between Indica landrace 'Kasakath' (high P uptake) with the *japonica* cultivar Nipponbare (low P uptake), revealed that about 80% of the variation between genotypes was due to a single QTL which is referred to as Pup1 (Phosphorus uptake 1) (Wissuwa and Ae 2001; Wissuwa et al., 2002). In sorghum, Bernardino et al. (2019) identified QTLs on chromosomes 3 and 7 homolog of the rice OsPSTOL1 gene coding for a serine/threonine kinase, which are involved root morphology and grain yield by improving the sorghum P uptake under low-P availability.

For further insight into the role of S*bPT*s in the Pi-efficiency of the sorghum, efforts are underway at Embrapa to develop near-isogenic lines (NILs) from a cross between the genotypes 101B and 136B. *SbPT*s could then be used as molecular markers for linkage mapping and QTL analysis of genes involved in Pi-use efficiency in sorghum.

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CONTRIBUTIONS

K.G. Raghothama developed the ideas and supervised all of the experiments. Experiments were designed and performed by M. J. V. Vasconcelos, M. F. Oliveira, A. Jain and R. E. Schaffert. Manuscript drafts were prepared by M.J.V. Vasconcelos, A. Jain, J.E.F. Figueiredo, and K.G. Raghothama. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Anis GB, Zhang Y, Wang H, Li Z, et al. (2018). Genomic regions analysis of seedling root traits and their regulation in responses to phosphorus deficiency tolerance in CSSL population of elite super hybrid rice. *Int. J. Mol. Sci*. 19(5): 1460. http://doi.org/10.3390/ijms19051460.

Bari R, Pant BD, Stitt M and Scheible WR (2006). Pho2, microRNA399, and PHR1 define a phosphate-signaling pathway in plants. *Plant Physiol*. 141: 988-999. https://doi.org/10.1104/pp.106.079707.

Bates RT and Lynch JP (2001). Roots hairs confer a competitive advantage under low phosphorus availability. *Plant Soil*. 236: 243-250. http://doi.org/10.1023/A:1012791706800.

Baveye P (2015). Grand challenges in the research on soil processes. *Front. Environ. Sci*. 3: 10. https://doi.org/10.3389/fenvs.2015.00010.

Bernardino KC, Pastina MM, Menezes CB, Sousa SM, et al. (2019). The genetic architecture of phosphorus efficiency in sorghum involves pleiotropic QTL for root morphology and grain yield under low phosphorus availability in the soil. *BMC Plant Biol*. 19: 87. http://doi.org/10.1186/s12870-019-1689-y.

Bielecka M, Watanabe M, Morcuende R, Scheible WR, et al. (2015). Transcriptome and metabolome analysis of plant sulfate starvation and resupply provides novel information on transcriptional regulation of metabolism associated with sulfur, nitrogen and phosphorus nutritional responses in Arabidopsis. *Front. Plant Sci*. 6: 1-18. http://doi.org/doi:10.3389/fpls.2014.00805.

Bustos R, Castrillo G, Linhares F, Puga MI, et al. (2010). A central regulatory system largely controls transcriptional activation and repression responses to phosphate starvation in Arabidopsis. *PLoS Genet*. 6: e1001102. https://doi.org/10.1371/journal.pgen.1001102.

Chen Q, Wu W, Zhao T, Tan W, et al. (2019). Complex gene regulation underlying mineral nutrient homeostasis in soybean root response to acidity Stress. *Genes*. 10: 402. http://doi.org/10.3390/genes10050402.

Chiou T-J, Liu H and 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. http://doi.org/10.1046/j.1365-313x.2001.00963.x.

Davies TGE, Ying J, Xu Q, Li ZS, et Al. (2002). Expression analysis of putative high-affinity phosphate transporters in Chinese winter wheats. *Plant Cell Environ*. 25: 1325-1339. https://doi.org/10.1046/j.1365-3040.2002.00913.x.

Dellaporta SL, Wood J and Hicks JB (1983). A plant DNA minipreparation: version II. *Plant Mol. Biol. Rep*. 1: 19-21. https://doi.org/10.1007/BF02712670.

Devaiah BN, Karthikeyan AS and Raghothama KG (2007a). WRKY75 transcription factor is a modulator of phosphate acquisition and root development in Arabidopsis. *Plant Physiol*. 143: 1789-1801. http://doi.org/10.1104/pp.106.093971.

Devaiah BN, Nagarajan VK and Raghothama KG (2007b). Phosphate homeostasis and root development in Arabidopsis are synchronized by the zinc finger transcription factor ZAT6. *Plant Physiol*. 145: 147-159. https://doi.org/10.1104/pp.107.101691.

Devaiah BN, Madhuvanthi R, Karthikeyan AS and Raghothama KG (2009). Phosphate starvation responses and gibberellic acid biosynthesis are regulated by the *MYB62* transcription factor in *Arabidopsis*. *Mol. Plant*. 2: 43-58. http://doi.org/10.1093/mp/ssn081.

Fageria NK and Nascente AS (2014). Management of soil acidity of South American soils for sustainable crop production. *Adv. Agron*. 128: 221-275. https://doi.org/10.1016/B978-0-12-802139-2.00006-8.

Fan M, Zhu J, Richards C, Brown KM, et al. (2003). Physiological roles for aerenchyma in phosphorus-stressed roots. *Funct. Plant Biol*. 30: 493-506. http://doi.org/10.1071/FP03046.

Gahoonia TS and Nielsen NE (2004). Root traits as tools for creating phosphorus efficient crop varieties. *Plant Soil*. 260: 47-57. https://doi.org/10.1023/B:PLSO.0000030168.53340.bc.

Gahoonia TS, Nielsen NE, Joshi PA and Jahoor A (2001). A root hairless barley mutant for elucidating genetic of root hairs and phosphorus uptake. *Plant Soil*. 235: 211-219. https://doi.org/10.1023/A:1011993322286.

Gamuyao R, Chin J, Pariasca-Tanaka J, Pesaresi P, et al. (2012). The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. *Nature*. 488: 535-539. http://doi.org/10.1038/nature11346.

Grün A, Buchner P, Broadley MR and Hawkesford MJ (2018). Identification and expression profiling of Pht1 phosphate transporters in wheat in controlled environments and in the field. *Plant Biol*. 20(2): 374-389. http://doi.org/10.1111/plb.12668.

Hasan MM, Hasan MM, Silva JAT and Li X (2016). Regulation of phosphorus uptake and utilization: transitioning from current knowledge to practical strategies. *Cell Mol. Biol. Lett*. 21: 7. http://doi.org/10.1186/s11658-016-0008-y.

Hammond JP, Broadley MR and White PJ (2004). Genetic responses to phosphorus deficiency. *Ann. Bot*. 94: 323-332. http://doi.org/10.1093/aob/mch156.

Heuer S, Gaxiola R, Schilling and Herrera-Estrela L (2017). Improving phosphorus use efficiency: a complex trait with emerging opportunities. *Plant J*. 90: 868-885. https://doi.org/10.1111/tpj.13423.

Jewel ZA, Ali J, Mahender A and Hernandez J (2019). Identification of quantitative trait loci associated with nutrient use efficiency traits, using SNP markers in an early backcross population of rice (*Oryza sativa* L.). *Int. J. Mol. Sci.* 20: E900. http://doi.org/10.3390/ijms20040900.

Karthikeyan AS, Ballachanda DN and Raghothama KG (2009). Promoter deletion analysis elucidates the role of *cis* elements and 5'UTR intron in spatiotemporal regulation of *AtPht1;4* expression in Arabidopsis. *Physiol. Plant.* 136: 10-18. https://doi.org/10.1111/j.1399-3054.2009.01207.x.

Karthikeyan AS, Varadarajan DK, Mukatira UT, D'Urzo MP, et al. (2002). Regulated expression of Arabidopsis phosphate transporters. *Plant Physiol*. 130: 221-233. https://doi.org/10.1104/pp.020007.

Kisko M, Shukla V, Kaur M, Bouain N, et al. (2018). Phosphorus transport in arabidopsis and wheat: emerging strategies to improve p pool in seeds. *Agriculture*. 8: 27. https://doi.org/10.3390/agriculture8020027.

Kumar S, Stecher G, Li M, Knyaz C, et al. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol*. 35: 1547-1549. https://doi.org/10.1093/molbev/msy096.

Krügel U and Kühn C (2013) Post-translational regulation of sucrose transporters by direct protein–protein interactions. *Front. Plant Sci*. 4: 237. https://doi.org/10.3389/fpls.2013.00237.

Leiser WL, Rattunde H, Frederick W and Piepho HP (2015). Phosphorous efficiency and tolerance traits for selection of sorghum for performance in phosphorous-limited environments. *Crop Sci*. 55: 1152-1162. http://doi.org/10.2135/cropsci2014.05.0392.

Liu C, Muchhal US, Uthappa M, Konowicz AK, et al. (1998). Tomato phosphate transporter genes are differentially regulated in plant tissue by phosphorus. *Plant Physiol*. 116: 91-99. https://doi.org/10.1104/pp.116.1.91.

Liverpool-Tasie SO, Omonona BT, Sanou A and Ogunleye WO (2017). Is increasing inorganic fertilizer use for maize production in SSA a profitable proposition? Evidence from Nigeria. *Food Policy*. 67: 41-51. http://doi.org/10.1016/j.foodpol.2016.09.011.

Lynch JP and Beebe S (1995). Adaptation of beans (*Phaseolus vulgaris* L.) to low phosphorus availability. *Hort. Science*. 30: 1165-1171. https://doi.org/10.21273/HORTSCI.30.6.1165.

Marschner H (1995). Mineral Nutrition of Higher Plants. Academic Press, London. https://doi.org/10.1016/C2009-0-63043-9.

Mtangadura TJ, Mtambanengwe F, Nezomba H, Rurinda J, et al. (2017). Why organic resources and current fertilizer formulations in Southern Africa cannot sustain maize productivity: Evidence from a long-term experiment in Zimbabwe. *PLoS One*. 12(8): e0182840. https://doi.org/10.1371/journal.pone.0182840.

Misson J, Raghothama KG, Jain A, Jouhet J, 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. http://doi.org/10.1073/pnas.0505266102.

Misson J, Thibaud MC, Bechtold N, Raghothama KG, et al. (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. https://doi.org/10.1007/s11103-004-1965-5.

Motomura K, Sano K, Watanabe S, Kanbara A, et al. (2018). Synthetic phosphorus metabolic pathway for biosafety and contamination management of cyanobacterial cultivation. *Synth. Biol*. 7(9): 2189-2198. https://doi.org/10.1021/acssynbio.8b00199.

Muchhal US and Raghothama KG (1999). Transcriptional regulation of plant phosphate transporters. *Proc. Natl. Acad. Sci. USA*. 96: 5868-5872. http://doi.org/10.1073/pnas.96.10.5868.

Nagarajan VK, Jain A, Poling MD, Lewis AJ, et al. (2011). Arabidopsis Pht1;5 mobilizes phosphate between source and sink organs and influences the interaction between phosphate homeostasis and ethylene signaling. *Plant Physiol*. 156: 1149-1163. http://doi.org/10.1104/pp.111.174805.

Nagy R, Vasconcelos MJV, Zhao S, McElver J, et al. (2006). Differential Regulation of five Pht1 phosphate transporters from maize (*Zea mays* L.). *Plant Biol*. 8: 186-197. http://doi.org/10.1055/s-2005-873052.

Ni J, Wu P, Senadhira D and Huang N (1998). Mapping QTLs for phosphorus deficiency tolerance in rice (*Oryza sativa* L.). *Theor. Appl. Genet*. 97: 1361-1369. https://doi.org/10.1007/s001220051030.

Nussaume L, Kanno S, Javot H, Marin E, et al. (2011). Phosphate import in plants: focus on the PHT1 transporters. *Front. Plant Sci*. 2: 83. https://doi.org/10.3389/fpls.2011.00083.

Park MR, Baek S, de los Reyes BG and Yun SJ (2007). Overexpression of a high-affinity phosphate transporter gene from tobacco (*NtPT1*) enhances phosphate uptake and accumulation in transgenic rice plants. *Plant Soil*. 292: 259-269. https://doi.org/10.1007/s11104-007-9222-8.

Paszkowski U, Kroken S, Roux C and 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. https://doi.org/10.1073/pnas.202474599.

Pawlowski K, Kunze R, De Vries S and Bisseling T (1994). Isolation of total, poly (A) and polysomal RNA from plant tissues. In : Gelvin SB, Shilperoort RA, eds, Plant Molecular Biology Manual, Kluwer Academic Publishers, Dordecht, The Netherlands, pp 1-3. http://doi.org/10.1007/978-94-011-0511-8_16.

Raghothama KG (2000). Phosphate transport and signaling. *Curr. Opin. Plant Biol.* 3: 182-187. https://doi.org/10.1016/S1369-5266(00)80063-1.

Raghothama KG (1999). Phosphate acquisition. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50: 665-693. https://doi.org/10.1007/s11104-004-2005-6.

Rae AL, Cybinski DH, Jarmey JM and Smith FW (2003). Characterization of two phosphate transporters from barley; evidence for diverse function and kinetic properties among members of the Pht1 family. *Plant Mol. Biol.* 53: 27-36. https://doi.org/10.1023/B%3APLAN.0000009259.75314.15.

Reinders A, Schulze W, Kühn C, Barker L, et al. (2002). Protein–protein interactions between sucrose transporters of different affinities colocalized in the same enucleate sieve element. *Plant Cell*. 14: 1567-577. https://doi.org/10.1105/tpc.002428.

Roch GV, Maharajan T, Ceasar SA and Ignacimuthu S (2019). The role of PHT1 family transporters in the acquisition and redistribution of phosphorus in plants. *Critical Rev. Plant Sci.* 38(3): 171-198. https://doi.org/10.1080/07352689.2019.1645402.

Rubio V, Linhares F, Solano R, Martin AC, et al. (2001). A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Gene Dev.* 15: 2122-2133. http://doi.org/10.1101/gad.204401.

Schaffert RE, Alves VMC, Pitta GVE, Bahia AFC, et al. (2001). Genetic variability in sorghum for P efficiency and responsiveness. In: Plant Nutrition – Food Security and Sustainability of Agroecosystems, Kluwer Academic Publishers, Netherlands, pp72-73. https://doi.org/10.1007/0-306-47624-X_34.

Schneider KD, Martens JRT, Zvomuya F, Reid DK, et al. (2019). Options for improved phosphorus cycling and use in agriculture at the field and regional scales. *J. Environ. Qual.* 48: 1247-1264. https://doi.org/10.2134/jeq2019.02.0070.

Sega P and Pacak P (2019). Plant PHR transcription factors: put on a map. *Genes* (Basel). 10: 1018. http://doi.org/10.3390/genes10121018.

Shen J, Yuan L, Zhang J, Li H, et al. (2011). Phosphorus dynamics: From soil to plant. *Plant Physiol.* 156: 997-1005. http://doi.org/10.1104/pp.111.175232.

Shin H, Shin H-S, Dewbre GR and 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. https://doi.org/10.1111/j.1365-313X.2004.02161.x.

Tilman D, Cassman KG, Matson PA, Naylor R, et al. (2002). Agricultural sustainability and intensive production practices. *Nature*. 418: 671-677. https://doi.org/10.1038/nature01014.

Vasconcelos MJV, Figueiredo JEF and Raghothama KG (2018). Expression analysis of anthocyanin gene induced under phosphorus starvation in maize genotypes with contrasting phosphorus use efficiency. *Genet. Mol. Res.* 17: gmr18036. http://doi.org/10.4238/gmr18036.

Walder F, Brule D, Koegel S, Wiemken A, et al. (2015). Plant phosphorus acquisition in a common mycorrhizal network: regulation of phosphate transporter genes of the Pht1 family in sorghum and flax. *New Phytologist*. 205: 1632-1645. https://doi.org/10.1111/nph.13292.

Wang D, Lv S, Jiang P and Li Y (2017). Roles, regulation, and agricultural application of plant phosphate transporters. *Front. Plant Sci.* 8: 817. http://doi.org/10.3389/fpls.2017.00817.

Wissuwa M and Ae N (2001). Genotypic variation for tolerance to phosphorus deficiency in rice and the potential for its exploitation in rice improvement. *Plant Breed.* 120: 43-48. https://doi.org/10.1046/j.1439-0523.2001.00561.x.

Wissuwa M, Wegner J, Ae N and Yano M (2002). Substitution mapping of *Pup1*: a major QTL increasing phosphorus uptake of rice from phosphorus-deficient soil. *Theor. Appl. Genet.* 105: 890-897. https://doi.org/10.1007/s00122-002-1051-9.

Wu P, Shou H, Xu G and Lian X (2013). Improvement of phosphorus efficiency in rice on the basis of understanding phosphate signaling and homeostasis. *Curr. Opin. Plant Biol.* 16: 205-12. https://doi.org/10.1016/j.pbi.2013.03.002.

Yan X, Lynch JP and Beebe SE (1996). Utilization of phosphorus substrates by contrasting common bean genotypes. *Crop Sci.* 36: 936-941. https://doi.org/10.2135/cropsci1996.0011183X003600040020x.