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Morphological, biochemical and molecular characterization of sorghum (*Sorghum bicolor)* genotypes contrasting for phosphate-use efficiency

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ABSTRACT. We compared three Pi-efficient (ATF-14B, ATF-53B, 101B) and four Pi-inefficient (ATF-16B, 116R, 136B, 187R) sorghum genotypes under different Pi concentrations. There were no significant differences between the groups in Pi-use efficiency under Pi-deprivation for anthocyanin accumulation, dry-weight matter, acid phosphatase activity (APA), and aerenchyma formation. However, both groups showed anthocyanin accumulation under Pi-deprivation. Under Pi-deficiency, there was a significant reduction of dry weight in both groups, with no significant differences between contrasting genotypes. All genotypes exhibited a significant increase in root/shoot ratios during Pi-deficiency, and these changes were not related to Pi-use efficiency. The total Pi content in roots and shoots in all genotypes was similar and represented less than 0.2 % of the total dry weight. For all genotypes, the Pi content in P+ treatment resulted in a significant variation ranging from 0.45 to 0.85% and 0.41 to 0.66% in roots and shoots, respectively. The genotype 187R had the highest P content in roots and shoots. APA activity showed increased activity only in the roots of both groups. The development of aerenchyma was conspicuous in the basal and in the middle root

sections of all genotypes grown under different Pi levels. Two sorghum Pi-transporter genes were strongly overexpressed in the middle part of Pi-deprived roots of 136B genotype. We did not find differences that explain the Pi-use efficiency between efficient and inefficient genotypes. More studies are needed to elucidate the complex mechanism of P-utilization by sorghum plants.

Key words: Aerenchyma; Acid phosphatase; Root/shoot ratio; Pi- content; Northern blot

INTRODUCTION

Phosphorus (Pi) is an essential macronutrient for plant growth and development as a component of many biologically relevant molecules and plays vital functions in metabolic processes (Marschner and Rimmington, 1988; Raghothama and Karthikeyan, 2005; Jain et al., 2007; Malhotra et al., 2018). Pi availability is a major yield-limiting factor for crop production in the Brazilian Cerrado, thus requiring the use of chemical fertilizers. However, there is increasing evidence that fertilizers alone cannot sustain the crop yields for an extended period, indicating the need for improving the Pi-acquisition and Pi-use efficiency by crop and the development of precise methods to monitor crop Pi-status (Hammond et al., 2004; Dwivedi et al., 2016). One way to minimize this problem is to identify or develop superior sorghum genotypes for *Pi acquisition and Pi-use efficiency*.

Considerable research efforts have concentrated on screening of existing cultivars and advanced breeding lines for Pi-use efficiency showing above-average yields with a higher proportion of phosphorus fixed in the soil (Wissuwa and Ae, 2001; Gahoonia and Nielsen, 2004; Leiser et al., 2014; 2015). Leiser et al. (2015), evaluated 70 genotypes of sorghum in Western Africa for grain yield performance under P- and P+ conditions and found significant genetic variation for all Pi-tolerance ratios across multiple sites.

Higher plants have developed several strategies to deal with Pi deprivation by activating adaptive responses to enhance P assimilation to maintain the Pi homeostasis (Raghothama, 2000; Rai et al., 2015). Inter- and intra-specific variations in the ability of plants to grow under Pi-limiting conditions have been reported earlier for several agronomic crop species (Gahoonia and Nielsen, 2004; Aziz et al., 2014; Leiser et al., 2015; Marcante et al., 2016). Researchers at the Embrapa, Brazilian Enterprise for Agricultural Research have pursued the goal of validating sorghum cultivars adapted to low levels of available P in the acidic soil of the Cerrado for the last three decades. The Pi-efficient and Pi-inefficient genotypes of sorghum selected at Embrapa using morphological, biochemical, and molecular parameters may provide useful gene pool for deciphering the underlying mechanism controlling Pi-use efficiency in plants (Schaffert et al., 1999).

Plant P-deficiency responsive genes are separated into two groups based on response time, with genes responding rapidly and generally non-specifically and late genes that change the morphology, physiology, or plant metabolism after long periods of P-deficiency exposure (Hammond et al., 2004). Strong changes occur in plant physiology, root morphology, architecture, and gene expression under Pi deficiency. Among the greatest alterations are anthocyanin accumulation, exudation of low molecular weight organic acids, and secretion of enzymes to solubilize external inorganic P and organic P compounds,

cytosolic glycolysis, mitochondrial electron transport, and tonoplast H+ pumping (Hammond et al., 2004).

Changes in root morphology correlate primarily to an increase in the length of clustered roots and density of root hairs and aerenchyma formation, which facilitate the exploration of the soil by the roots under low Pi availability, increasing the Pi uptake and Pi use efficiency by the plants (Gahoonia et al., 2001; Gahoonia and Nielsen, 2004; Leiser et al., 2015; Bernardino et al., 2019). Other evident modifications in plant architecture are a decline in plant growth and the whole plant dry weight (Malhotra et al., 2018), and plant-mycorrhizal association (Johri et al., 2015). The genetic alterations most commonly observed are the transcription of high-affinity Pi transporters and Pi-deficiency-induced genes (Raghothama and Karthikeyan 2005; Jain et al., 2007; Ascencio, 2015; Leiser et al., 2015; Bernardino et al., 2019). The identification of phosphate stress-responsive genes is crucial for further characterizing the primary mechanisms involved in Pi acquisition and Pi-use efficiency by plants.

Plant responses to Pi deficiency are a complex pathway involving genes and signaling cascades that are still poorly understood (Hammond et al., 2004). Many studies have been made to try to understand the gene regulatory network involved in Pi uptake by plants and the identification of genes and signaling cascades involved in plant responses to Pi deficiency (Muchhal and Raghothama, 1999; Hammond et al., 2004; Gu et al., 2016). Also, concurrently, efforts have been directed towards elucidation of the complex molecular mechanisms underlying genotype differences in Pi uptake from Pi-deficient soils (Hammond et al., 2004; Leiser et al., 2015; Marcante et al., 2016; Schneider and Lynch, 2016; Vasconcelos et al., 2018**)**.

Recent studies identified major and minor QTLs associated with Pi-deficiency in three cereal crops: rice (Anis et al., 2018; Jewel et al., 2019), wheat (Yang et al., 2018) and sorghum (Leiser et al., 2014; Bernardino et al., 2019). Using genome-wide association mapping based on 220,934 single nucleotide polymorphisms (SNPs), Leiser et al. (2014) found a genomic region in the sorghum genome highly associated with grain yield production on chromosome 3, which is coincident with the region of a major Al-tolerance gene in sorghum (*SbMATE*). The *SbMATE* specific SNPs showed very high associations with grain yield production, especially under -P conditions, and a possible pleiotropic role in providing tolerance to Al toxicity and P deficiency (Leiser et al., 2014).

In sorghum, QTLs for root morphology and grain yield under low P availability in the soil are linked to genetic determinants conferring higher root surface area and slight increases in fine root diameter, which may improve the sorghum P uptake under low-P availability and Pi-efficiency (Bernardino et al., 2019). Wang et al. (2019), using bioinformatics tools, identified 27 PHT family members with different expression patterns in sorghum. Two PHT genes (SbPHO1;1 and SbPHT4;3) mapped in the short arm of chromosome 3 were localized in the vicinity of the QTL mapped by Bernardino et al. (2019).

Another approach used to elucidate the genetic response of plants to P deficiency includes Microarray technology. These studies revealed alterations in the expression of various transcription factors with common *cis*-regulatory elements in the plant genome by P deficiency, suggesting that they may be involved in the coordinated plant responses to P deficiency (Hammond et al., 2004; Heuer et al., 2017).

Thus, the differences in Pi-use efficiency among cultivars are due to the mode of action and between the mechanisms mentioned above (Aziz et al., 2014; Marcante et al., 2016). In our study, we focused on evaluating the usefulness of aerenchyma formation, plant dry weight matter, acid phosphatase enzyme (APA), and the expression of phosphate transporter genes for characterizing contrasting Pi-efficient and Pi-inefficient sorghum genotypes.

MATERIAL AND METHODS

Plant materials

In this study, *Sorghum bicolor* genotypes with contrasting responses to Pi-use efficiency were obtaind from the Sorghum Breeding Program at Embrapa, Sete Lagoas, Brazil. They were classified into two categories, namely efficient (CMSXS101B, ATF-14B, ATF-53B) and inefficient (ATF-16B, CMSXS116R, CMSXS136B, CMSXS187R) based on grain yield in soil with low P levels and soil corrected with phosphate fertilizers. The greenhouse and laboratory experiments were conducted at Purdue University, USA. Each treatment consisted of three plants, with three replicates for each treatment.

Growth conditions

Seeds of efficient and inefficient sorghum genotypes were germinated in seedling trays containing Scott's ready earth plug mix (Scotts Co., Marysville, OH) and grown in the greenhouse for one week. Subsequently, seedlings were gently washed with water to remove the soil medium adhering to the roots and then transferred to one half-strength modified Hoagland's nutrient solution for one week. For studying the effects of Pi on the expression of *SbPT*1 and *SbPT*2 transporter genes, plants were transferred to Hoagland's nutrient solution in the absence (0μM) or presence (250μM) of Pi. During the treatment period, nutrient solutions were replaced on alternate days. After 15 days, the roots were harvested, frozen in liquid nitrogen, and stored at -80ºC until further analysis.

Dry weight and root/ shoot ratio

The Pi efficient and inefficient sorghum genotypes were grown hydroponically in the presence (P+) or absence (P-) of Pi and harvested after 15 days of treatment. The roots and shoots of efficient and inefficient sorghum genotypes were separated and dried at 50ºC until achieving a constant weight. The root and shoot weights were used for estimating the dry weight and root/shoot ratio.

Total phosphorus content

The dried roots and shoots of efficient and inefficient sorghum genotypes grown in P+ and P- solutions, were ground in a Wiley mill to pass through a 20-mesh screen and then digested with concentrated nitric acid at 95ºC overnight. The total phosphorus content was determined by inductively coupled plasma-mass spectroscopy ICP-MS (Lahner et al., 2003).

Estimation of anthocyanin content

Anthocyanin contents in roots of efficient and inefficient sorghum genotypes grown in P (+) and P (-) solutions were quantified as described by Abdel-Aal and Hucl (1999).

Quantification of total acid phosphatase activity

Total acid phosphatase activity in hydroponically grown sorghum seedlings was evaluated by p-Nitrophenyl Phosphate (pNPP) hydrolysis assay (Richardson et al., 2000). The samples were extracted from about 30 mg of frozen ground tissue. The enzyme activity was measured at A_{405}. Total protein was estimated separately using Bradford's reagent and the total acid phosphatase activity expressed as mU/mg protein.

Root sectioning

Root segments were sampled from the basal (2 cm from the basal end of the root) and the middle section of the hydroponically grown seedlings. Fresh root samples were fixed in FAA fixative (5% formaldehyde, 5% glacial acetic acid, 90% of 70% ethanol) for 24 hours. Fixed root samples were gradually dehydrated in a graded ethanol series (50-100%, v/v). The roots were sectioned transversally into 10 µm thickness sections, stained with Astra blue and safranin, visualized with a light microscope and photographed.

RNA isolation and Northern blot analysis

The total root RNA was extracted by the hot phenol and lithium chloride precipitation method (Pawlowski et al., 1994), processed as described by Vasconcelos et al., 2018, and hybridized with two phosphate transporter genes (*SbPT1* and *SbPT2*, GenBank accession numbers MH333040 and MH333041, respectively) isolated from sorghum (Vasconcelos and Raghothama, unpublished).

RESULTS

The effect of Pi deficiency treatment for 15 days on plant dry weight was evaluated for three Pi-efficient (ATF-14B, ATF-53B, 101B) and four Pi-inefficient (ATF-16B, 116R, 136B, 187R) genotypes (Figure 1B). There was a significant dry-weight reduction in both Pi-efficient and inefficient genotypes in response to Pi-deficiency treatment. However, no significant correlation existed between Pi-deficiency induced reduction in the dry weight and type of genotypes evaluated. Likewise, all genotypes (efficient and inefficient) exhibited a significant increase in their root/shoot ratios during Pi-deficiency treatment (Figure 1C). Similar to dry weight data, changes in the root/shoot ratio could not be specifically related to the Pi use efficiency of the different genotypes. During Pi deficiency, the total phosphorus content in roots and shoots of all the efficient and inefficient genotypes was comparable and represented less than 0.2 % of the total dry weight of the tissues (Figure 1D and 1E). However, during growth under P (+) condition, a significant variation

ranging from 0.45 – 0.85% of Pi contents in roots and shoots of efficient and inefficient genotypes was noticed (Figure 1D and 1E).

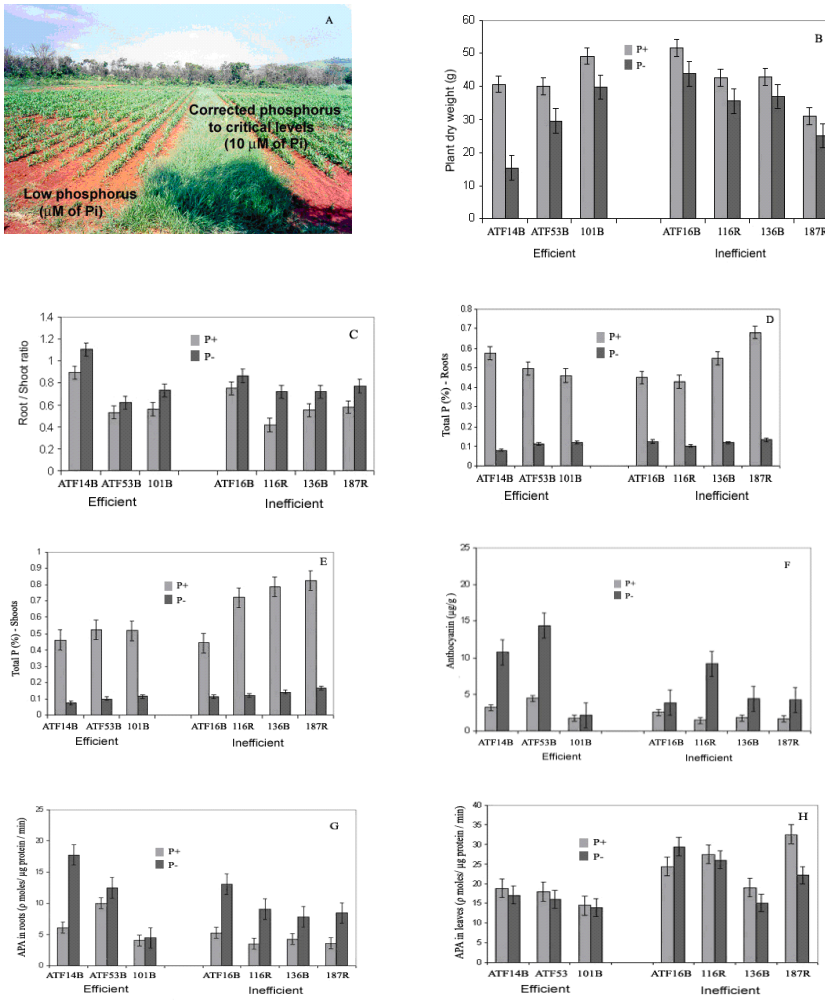

Figure 1. Responses of Pi-efficient and Pi-inefficient sorghum genotypes to Pi-deprivation. (A) Sorghum genotypes contrasting for Pi-use efficiency grown side by side in uncorrected Cerrado soil with low Pi level (2µM) and soil corrected with 10µM Pi, and B to H: Effects of *phosphorus* supply on contrasting genotypes hydroponically grown in the greenhouse under P + (250µM Pi) and P- (0µM Pi) conditions after 15 days of treatment. (B) plant dry weight, (C) root/shoot ratio, (D) total phosphorus (%) on root dry weight, (E) total phosphorus (%) on the shoot dry weight, (F) anthocyanin content in roots, (G) acid phosphatase activity (APA) in roots, and (H) APA in shoots. Each bar represents the mean ± SE (standard error) of three replicates.

Interestingly, one of the inefficient genotypes (187 R) revealed a higher Pi content in both roots and shoots. Since anthocyanin accumulation is a typical plant response under Pi-starvation, this trait was measured in Pi-efficient and Pi-inefficient genotypes (Figure 1F). Significant differences were noted in Pi deficiency-induced anthocyanin accumulation in the roots of Pi-efficient and Pi-inefficient genotypes of sorghum. However, a notable exception was observed for the efficient genotype 101B, which did not accumulate

significant amounts of anthocyanin despite being deprived of Pi. On the contrary, the other two efficient genotypes (ATF-14B and ATF-53B) accumulated much higher amounts of anthocyanin during P (-) treatments. The Pi-inefficient genotypes did not show any specific trend concerning anthocyanin accumulation during P (-) conditions.

Further, acid phosphatase activity (APA) was determined in roots and leaves of the Pi-efficient and Pi-inefficient genotypes grown under P+ and P- conditions (Figure 1G-H). Although Pi deficiency resulted in increased activity of APA in the roots of both Pi-efficient and Pi-inefficient genotypes, no significant difference in APA activity was observed in the efficient genotype 101B (Figure 1G). However, APA activity in the leaves of 101B genotype was comparable to other efficient genotypes grown under different Pi levels (Figure 1H).

Among the efficient genotypes, 101B showed lesser accumulation of anthocyanin and lower APA activity in roots. Thus, it was further characterized for the development of aerenchyma (Figure 2). The development of aerenchyma became conspicuous in the basal section of roots of genotypes grown under different Pi levels. Under P- condition, the development of aerenchyma was pronounced in the middle section of the roots of both Pi-efficient and Pi-inefficient genotypes. However, in P+ condition, the development of aerenchyma in the middle root section was detected only in the Pi-inefficient genotypes. The Northern blot showed that the two sorghum phosphate transporter genes (*SbPT1* and *SbPT2*) were overexpressed in the middle part of Pi-deprived roots of both Pi-efficient (data not shown) and Pi-inefficient genotypes (Figure 3). Our study highlights the usefulness of this trait in differentiating between Pi-efficient and Pi-inefficient genotypes of sorghum grown under Pi-replete conditions.

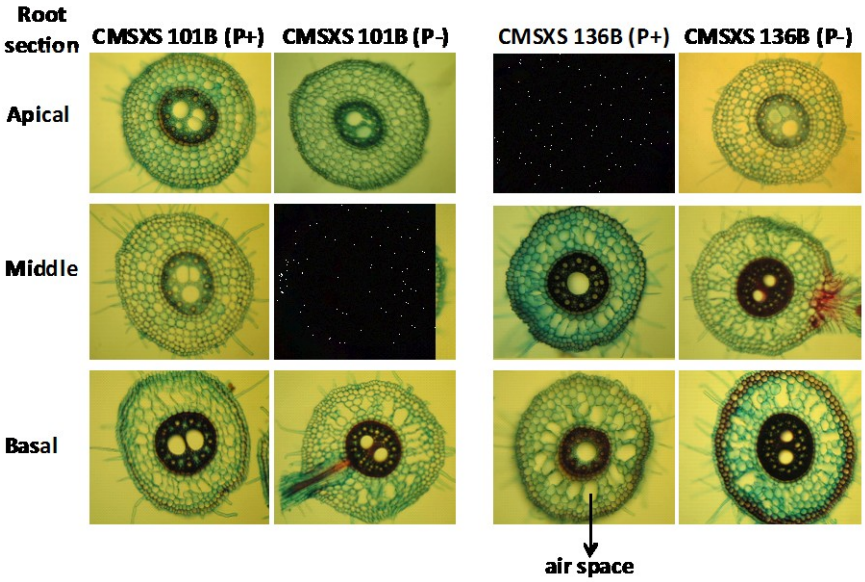

Figure 2. Cross-sections of roots of 101B (Pi-efficient) and 136B (Pi-inefficient) sorghum genotypes grown in hydroponic culture for 10 days in the presence (250μM Pi) or absence (0μM Pi) of phosphate. The effects of Pi content in the medium on aerenchyma formation was evaluated by the visible root cortical air spaces.

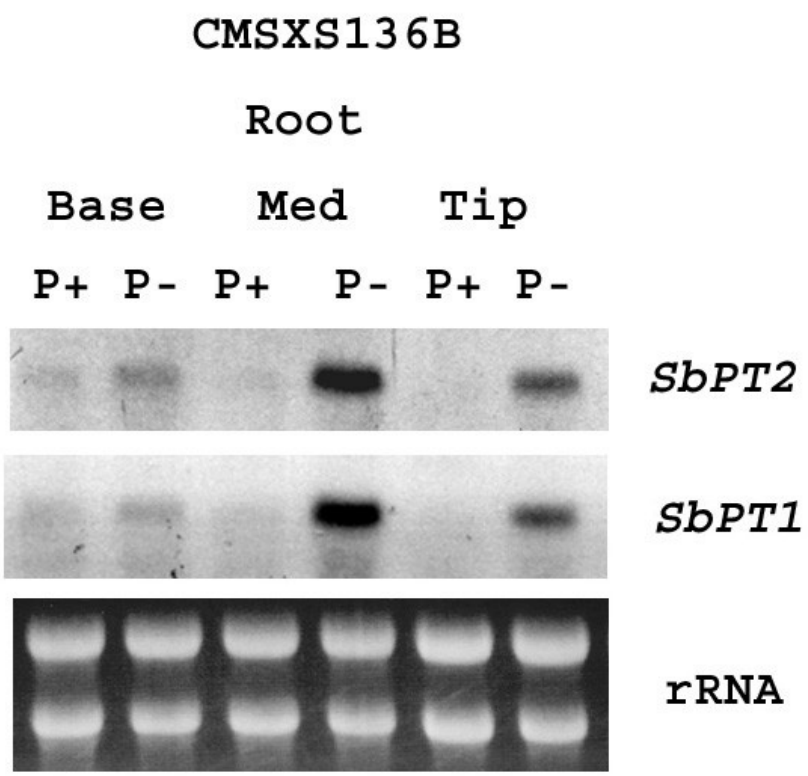

Figure 3. Northern blot of Pi-inefficient sorghum genotype CMSXS136B under Pi sufficiency and Pi-deprived conditions. Both sorghum phosphate transporter genes *SbPT2* and *SbPT1* were only overexpressed in the middle part of Pi-deprived roots.

DISCUSSION

In our study, both Pi-efficient and Pi-inefficient genotypes of sorghum showed a significant reduction in their dry weight, Pi content in roots and shoots, increased root/shoot ratio, and increased anthocyanins accumulation during Pi deprivation. This set of changes is typical of the plant response to Pi deficiency (Raghothama and Karthikeyan 2005; Jain et al., 2007; Ascencio, 2015). Furthermore, there was a significant increase in acid phosphatase activity in the roots of the Pi-efficient and Pi-inefficient genotypes during Pi deprivation. This result was consistent with earlier studies showing that the synthesis of acid phosphatase enzymes (APA) is a universal plant response to Pi deficiency characterized by a remarkable increase in the levels of APA production/secretion in roots (Ascencio, 2015; Zhang et al., 2015; Ciereszko et al., 2017; Gao et al., 2017). However, the leaves of Pi-efficient and Pi-inefficient genotypes under P-deficient conditions did not show any increase in APA activity with a notable exception of the Pi-inefficient genotype ATF-116R. The data suggested that an increased APA activity in ATF-116R might not be associated with Pi-use efficiency. Similar results have been shown in *Phaseolus vulgaris*, wherein the leaf acid phosphatase activity was not associated with either Pi acquisition or its use efficiency (Yan et al., 2001). Although there were considerable variations in Pi deficiency-induced modulation of these traits in the sorghum genotypes, no significant correlation

could be established with their genotypic differences in Pi-use efficiency. The expression data from *SbPT1* and *SbPT2* phosphate transporter genes showing their induction in the middle part of roots in both efficient and Pi-inefficient genotypes are similar to results from Liu et al. (2018) showing that plant phosphate transporters facilitate the acquisition of inorganic phosphate (Pi). However, the expression of the phosphate transporter genes only in the middle part of roots needs additional investigation.

The data indicated an apparent lack of close parallelism between the traits developed during Pi deprivation and those linked to Pi-use efficiency. We suggest that some of the traits associated with Pi-stress responses may not be directly contributing to the genetic variability for Pi-use efficiency in sorghum. This is probably due to the action of other mechanisms not directly involved in the plant response to Pi deprivation, such as the up-regulation of vacuolar and secreted ATPases enzymes that hydrolyze Pi from Pi monoesters, several miRNAs involved in Pi uptake, relocation, and remobilization (Plaxton and Tran, 2011, Nguyen et al., 2015). Alternative interacting metabolic processes for cytosolic glycolysis, mitochondrial electron transport, and tonoplast H+ pumping (Hammond et al., 2004), and pleiotropic QTLs for serine/threonine-protein kinase not directly involved in Pi metabolism may influence Pi acquisition by the plant (Leiser et al., 2014; 2015; Bernardino et al., 2019). Therefore, in our study, there is a possibility that secondary traits overlapped with Pi-use efficiency genes in some sorghum genotypes. For instance, among the Pi-efficient genotypes, the 101B showed an inverse result with the lesser accumulation of anthocyanin and lower acid phosphatase activity in the roots during Pi-deprivation.

In addition to those mentioned above, there are many types of evidence indicating the effect of suboptimal P availability on aerenchyma formation in a well-aerated nutrient solution (Gahoonia et al., 2001; Díaz et al., 2018; Schneider and Lynch, 2018; Sou et al., 2019). There was a higher percentage of aerenchyma formation in the cortex of middle and basal sections of roots of both Pi-efficient and Pi-inefficient genotypes grown under P deficiency conditions. Pi deficiency-induced aerenchyma formation may represent a useful plant adaptation to low phosphorus availability by reducing the respiratory and phosphorus requirements, thus allowing more root surface to scavenge nutrients in the soil (Schneider and Lynch 2018). Interestingly, under P+ condition, Pi-efficient genotype did not develop aerenchyma in the middle section of the root, whereas its development was conspicuous in the Pi-inefficient genotypes. Our data provide evidence of the usefulness of this trait in differentiating between Pi-efficient and Pi-inefficient genotypes grown under Pi-sufficiency conditions.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abdel-Aal ESM and Hucl P (1999). A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. *Cereal Chem*. 76: 350-354.

Anis GB, Wang H, Li Z, Wu W, 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: E1460.

Ascencio J (2015). Acid Phosphatase kinetics as a physiological tool for assessing crop adaptability to phosphorus deficiency. In: Plants for the future (El-Shemy H, ed.). IntechOpen, London, 79-96. Available at: [https://www.intechopen.com/books/plants-for-the-future/acid-phosphatase-kinetics-as-a-physiological-tool-for-assessing-crop-adaptability-to-phosphorus-defi]. Accessed 28 April 2020.

Aziz T, Sabir M, Farooq M, Maqsood MA, et al. (2014). Phosphorus deficiency in plants: responses, adaptive mechanisms, and signaling. In: Plant Signaling: Understanding the Molecular Crosstalk (Hakeem K, Rehman R and Tahir I, eds.). Springer, New Delhi.

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.

Ciereszko I, Balwicka H and Zebrowska E (2017). Acid phosphatases activity and growth of barley, oat, rye and wheat plants as affected by Pi deficiency. *Open Plant Sci. J*. 10: 110-122.

Díaz AS, Aguiar GM, Pereira MP, Castro EM, et al. (2018). Aerenchyma development in different root zones of maize genotypes under water limitation and different phosphorus nutrition. *Biol. Plantarum*. 62: 561-568.

Dwivedi A, Singh A, Naresh RK and Kumar M (2016). Towards sustainable intensification of maize (*Zea mays* L.) + legume intercropping systems; experiences; challenges and opportunities in India; a critical review. *J. Pure Appl. Microbiol*. 10: 725-740.

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.

Gahoonia TS and Nielsen NE (2004). Root traits as tools for creating phosphorus efficient crop varieties. *Plant Soil*. 260: 47-57.

Gao W, Lu L, Qiu W, Wang C, et al. (2017). OsPAP26 encodes a major purple acid phosphatase and regulates phosphate remobilization in rice. *Plant Cell Physiol*. 58: 885-892.

Gu M, Chen A, Sun S and Xu G (2016). Complex regulation of plant phosphate transporters and the gap between molecular mechanisms and practical application: what are missing? *Mol. Plant.* 7: 396-416.

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

Heuer S, Gaxiola R, Schilling, Herrera-Estrela L (2017). Improving phosphorus use efficiency: a complex trait with emerging opportunities. *Plant J*. 90: 868-885.

Jain A, Vasconcelos MJ, Sahi SV and Raghothama KG (2007). Molecular mechanisms of plant adaptation to phosphate deficiency. *Plant Breed. Rev*. 29: 359-419.

Jewel ZA, Ali J, Mahender A, Hernandez J, et al. (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.

Johri AK, Oelmüller R, Dua M, Yadav V, et al. (2015). Fungal association and utilization of phosphate by plants: success, limitations, and future prospects. *Front Microbiol*. 6: 984.

Lahner B, Gong J, Mahmoudian M, Smith EL, et al. (2003). Genomic scale profiling of nutrient and trace elements in *Arabidopsis thaliana*. *Nat. Biotechnol*. 21: 1215-1221.

Leiser WL, Rattunde H, Frederick W, Piepho HP, et al. (2015). Phosphorous efficiency and tolerance traits for selection of sorghum for performance in phosphorous-limited environments. *Crop Sci*. 55: 1152-1162.

Leiser WL, Rattunde HFW, Weltzien E, Cisse N, et al. (2014). Two in one sweep: aluminum tolerance and grain yield in P-limited soils are associated to the same genomic region in West African Sorghum. *BMC Plant Biol*. 14: 206.

Liu C, Su J, Stephen GK, Wang H, et al. (2018). Overexpression of phosphate transporter gene *CmPht1;2* facilitated Pi uptake and alternated the metabolic profiles of chrysanthemum under phosphate deficiency. *Front. Plant Sci*. 9: Article 686.

Malhotra H, Vandana, Sharma S and Pandey R (2018). Phosphorus nutrition: Plant growth in response to deficiency and excess. In: Plant Nutrients and Abiotic Stress Tolerance (Hasanuzzaman M, Fujita M, Oku H, Nahar K et al. eds). Springer, Singapore.

Marcante NC, Muroaka T, Bruno IP and Camacho MA (2016). Phosphorus uptake and use efficiency of different cotton cultivars in savannah soil (Acrisol). *Acta Sci. Agron*. 38: 239-247.

Marschner H and Rimmington G (1988). Mineral nutrition of higher plants. *Plant Cell Environ*. 11: 147-148.

Muchhal US and Raghothama KG (1999). Transcriptional regulation of plant phosphate transporters. *Proc. Natl. Acad. Sci. U. S. A*. 96: 5868-5872.

Nguyen GN, Steven R, German S and Surya K (2015). Role of microRNAs involved in plant response to nitrogen and phosphorous limiting conditions. *Front. Plant Sci*. 6: 629.

Pawlowski K, Kunze R, de Vries S and Bisseling T (1994). Isolation of total, poly(A) and polysomal RNA from plant tissues. In: Plant Molecular Biology Manual (Gelvin SB, Schilperoort RA, eds.). Springer, Dordrecht.

Plaxton WC and Tran HT (2011). Metabolic adaptations of phosphate-starved plants. *Plant Physiol*. 156: 1006-1015.

Rai V, Sanagala R, Sinilal B, Yadav S, et al. (2015). Iron availability affects phosphate deficiency-mediated responses, and evidence of cross-talk with auxin and zinc in Arabidopsis. *Plant Cell Physiol*. 56: 1107-1123.

Raghothama KG and Karthikeyan AS (2005). Phosphate acquisition. *Plant Soil*. 274: 37-49.

Raghothama KG (2000). Phosphate transport and signaling. *Curr. Opin. Plant Biol.* 3: 182-187.

Richardson AE, Hadobas PA and Hayes JE (2000). Acid phosphomonoesterase and phytase activities of wheat (*Triticum aestivum* L.) roots and utilization of organic phosphorus substrates by seedlings grown in sterile culture. *Plant Cell Environ.* 23: 397-405.

Schaffert RE, Alves VMC, Parentoni SN and Raghothama KG (1999) Genetic control of phosphorus uptake and utilization efficiency in maize and sorghum under marginal soil condition. In: Molecular approaches for the genetic improvement of cereals for stable production in water-limited environments (Ribaut JM, Poland D, eds.). CIMMYT, El Batan.

Schneider HM and Lynch JP (2018). Functional implications of root cortical senescence for soil resource capture. *Plant Soil*. 423(1-2): 13-26.

Sou HD, Masumori M, Kurokochi and Tange T (2019). Histological observation of primary and secondary aerenchyma formation in adventitious roots of *Syzygium kunstleri* (King) Bahadur and R.C. Gaur grown in hypoxic medium. *Forests*. 10(2): 137.

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.

Wang J, Yang Y, Liao L, Xu J, et al. (2019). Genome-Wide Identification and Functional Characterization of the Phosphate Transporter Gene Family in Sorghum. *Biomolecules*. 9(11): 670

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.

Yan X, Liao H, Trull MC and Beebe SE (2001). Induction of a major leaf acid phosphatase does not confer adaptation to low phosphorus availability in common bean. *Plant Physiol*. 125: 1901-1911.

Yang X, Liu Y, Wu F, Jiang X, et al. (2018). Quantitative trait loci analysis of root traits under phosphorus deficiency at the seedling stage in wheat. *Genome*. 61: 209-215.

Zhang XM, Hong K and Yi Y (2015). Effect of short-term phosphate starvation on acid phosphatase activity of *Carpinus pubescens* and *Eurycorymbus cavalerei*. *Russ. J. Plant Physiol*. 62: 57-64.