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Genome-wide Identification and *in silico* Analysis of PHT1 Family Genes and Proteins in *Setaria viridis*: The Best Model to Study Nutrient Transport in Millets

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ABSTRACT Millets are small-seeded cereals predominantly cultivated and consumed by millions of poor people living in developing countries in Asia and Africa. Limited availability of genomic resources hinders studies of nutrient transport in millets. Two *Setaria* species, foxtail millet [*Setaria italica* (L.) P. Beauv.] and its wild relative green foxtail [*Setaria viridis* (L.) P. Beauv.], are considered to be suitable models to study the genomics of other millets. Understanding the nutrient mobilization of millets is essential for improving nutrient use efficiency and biofortification in millets and other cereal crops. Millets are adapted for low-input agriculture, so understanding and improving the phosphate use efficiency of these plants is important because (i) subsistence farmers cannot afford to buy expensive phosphate fertilizers and (ii) the phosphate rock used for phosphate fertilizer production is depleting quickly. In this minireview, I discuss various studies on nutrient transport in millets and highlight phosphate transport studies. I report the identification and phylogenetic and multiple sequence analyses of 12 PHosphate Transporter1 (PHT1) family genes and proteins of green foxtail for the first time. With the exception of *Sv*PHT1;5, all other green foxtail PHT1 transporters are closely clustered with foxtail millet PHT1 transporters. The multiple sequence analysis of SvPHT1s revealed that the key residues involved in phosphate and H-binding and transport are well conserved, as in other PHT1 transporters. Efforts need to be undertaken to understand and improve phosphate uptake and utilization in millets to strengthen food security in the developing world.

Abbreviations: AMF, arbuscular mycorrhizal fungus; *Dof, DNA binding with one finger*; *PBF Dof, prolamin-binding factor DNA binding with one finger only*; PHT1, Phosphate Transporter1; Pi, inorganic P; *Piriformospora indica* phosphate transporter, PiPT.

CORE IDEAS

- Millets are nutrient-rich cereals widely grown and consumed by people in less developed countries.
- *Setaria* species are closely related to millets and have advance release of whole-genome sequences.
- *Setaria* species provide insights for studying the nutrient transporters of other millets.
- *PHosphate Transporter1* genes and proteins of green foxtail have been identified.

Millets are nutrient-rich and drought-hardy cereal crops widely cultivated and consumed by people in less developed countries in Asia and Africa. Millets include finger millet [*Eleusine coracana* (L.) Gaertn.], pearl millet [*Cenchrus americanus* (L.) Morrone], foxtail millet, kodo millet (*Paspalum scrobiculatum* L.), proso millet (*Panicum miliaceum* L.), barnyard millet (*Echinochloa frumentacea* Link), and little millet (*Panicum sumatrense* Roth). Millets are rich sources of mineral nutrients and high fiber that feed millions of poor people in Asia and Africa (Goron and Raizada, 2015). Millet grains contain high amounts of vitamins, Fe, carbohydrates, Ca, K, Zn, P, Mg, and essential amino acids (Saleh et al., 2013). For example, finger millet has 10-fold higher Ca in seeds than any other major cereal (Kumar et al.,

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2016). As a result, this millet has also been proposed as a panacea for Ca biofortification (Puranik et al., 2017). The seeds of finger millet can also be stored for several years without any insect damage, which is a major risk avoidance strategy in drought-prone areas of Africa (Ceasar and Ignacimuthu, 2011). Pearl millet contains many health-promoting nutrients such as Fe, Zn, and lysine (Hadimani et al., 2001). Even other millets like barnyard millet, kodo millet, and foxtail millet also possess similar nutrients with health benefits (Saleh et al., 2013).

Despite these features, millets have been paid less attention until recent years for modern genetics and genomics studies because millets are cultivated in less developed countries in Asia and Africa (Ceasar and Ignacimuthu 2009). Complexities of genome size and the multiploid nature of millets have also further hindered progress in whole-genome sequencing. Apart from these complications with millets, both foxtail millet and its wild relative, green foxtail, have relatively small genomes (~500 mb), are diploid in nature, and serve as model for C_4 photosynthesis crops (Huang et al., 2016). The genome sequences of two different varieties of foxtail millet were released in 2012 (Bennetzen et al., 2012; Zhang et al., 2012) and an early version of the genome is also available for green foxtail (http://phytozome.jgi.doe.gov, accessed 2 Nov. 2018). However, the whole genome sequence is not yet available for many other millets. The genome assemblies have been recently released for finger millet (Hittalmani et al., 2017) and pearl millet (Varshney et al., 2017) only. As a result, both *Setaria* species (*S. viridi*s and *S. italica*) have been considered the best models for functional genomic studies in other millets with complex genomes. Foxtail millet is also considered to be a model for bioenergy crops for biofuel production (Li and Brutnell, 2011; Muthamilarasan and Prasad, 2015). Millets are generally considered to be recalcitrant for tissue culture studies; as a result, the transformation protocols are also lagging behind compared with other major cereals like rice (*Oryza sativa* L.) (Ceasar and Ignacimuthu, 2009). However, several transformation protocols have been reported for both foxtail millet and green foxtail, including a floral dip method for green foxtail, which is more efficiently used in other model plants like *Arabidopsis thaliana* (L.) Heynh. (reviewed in Huang et al., 2016). An efficient *Agrobacterium*-mediated transformation protocol has also been reported recently for foxtail millet (Ceasar et al., 2017). Therefore, both foxtail millet and green foxtail have been considered as the best model systems for genomic studies in other millets (Huang et al., 2016).

Although many genetic and genomics studies have been conducted in millets, only a few studies have focused on nutrient transporters in these millets. In particular, only a few reports are available on P transport studies in millets. However, P deficiency has been considered to be a major problem affecting global agriculture and the reserves for P fertilizer are expected to be exhausted in next 20–30 yr (Cordell et al., 2009; Baker et al., 2015). The growth and yield of millets like foxtail millet and finger millet have been shown to be affected by P deficiency (Maharajan et al., 2017; Ceasar et al., 2014). Efforts have been made to identify and characterize the P transporters of foxtail millet. In this minireview, I present past reports on nutrient transport studies in millets and focus on P transport studies. I also report the identification and a bioinformatics analysis of PHT1 family proteins of green foxtail for the first time. The genomes of foxtail millet and green foxtail may serve as the best models for the characterization of phosphate transporters in other millets, which would help to improve P use efficiency in nutrient-rich millets.

NUTRIENT TRANSPORTERS OF MILLETS

Membrane-bound transport proteins have been considered to be key targets for improving the uptake and use efficiency of water and nutrients. In particular, membrane transporters play pivotal roles in transporting mineral nutrients. Thus understanding the mechanism of plant membrane transporters has been considered a key contributor to global food security (Schroeder et al., 2013). Despite millet being the major source of nutrients and energy for poor people in less developed countries, only a limited number of studies have been conducted on the nutrient transporters of millets compared with similar studies on other major cereals like rice. Among millets, finger millet and foxtail millet have been investigated previously to some extent. The details are discussed below.

Studies on Ca Transporters

Calcium is one of the important macronutrients for growth and development in plants. As finger millet contains the richest source of Ca among the cereals, this millet has been investigated for Ca transport. A few molecular marker-based studies have been conducted to identify the candidate genes for high Ca traits in finger millet (Panwar et al., 2010; Nirgude et al., 2014; Yadav et al., 2014; Kumar et al., 2015). These studies have been considered as the foundation for improving the Ca content in finger and other millets (Puranik et al., 2017; Sharma et al., 2017). Recently, a few genomics studies have also been conducted to understand Ca transport in finger millet. The expression levels of key genes involved in Ca^{2+} transport such as *Ca^{2+}/H^+ antiporter* (*CAX1*), *two pore channel* (*TPC1*), *CaM-stimulated type IIB Ca^{2+} ATPase* and two *calmodulin-dependent protein kinase* genes (*CaMK1* and *CaMK2*) have been analyzed in two finger millet genotypes with contrasting Ca traits (Mirza et al., 2014). Following this, same group also reported the presence of 82 Ca sensor genes in developing spikes of finger millet (Singh et al., 2014). Whole-genome transcriptome profiling was also performed in the developing spikes of finger millet to find the key genes involved in Ca^{2+} transport (Singh et al., 2015). Expression of another key gene, *CBL interacting protein kinase24* (*CIPK24*) was also analyzed in two genotypes of finger millet (Chinchole et al., 2017). *CBL interacting protein kinase24* was

induced in root, shoot, leaf, and developing spike tissues of genotype GP-45 compared with GP1. It has been predicted that by activating the *Ec*Ca^{2+}/H+ antiporter b protein, *Ec*CIPK24 can play an important role in high seed Ca accumulation in finger millet (Chinchole et al., 2017).

Studies on N Transporters

Only a few reports are available that analyze the key genes involved in N transport in finger millet. A transcription factor, *prolamin-binding factor DNA binding with one finger only* (*PBF Dof*) was analyzed in different tissues of three finger millet genotypes ('PRM-1', 'PRM-701', and 'PRM-801') with differing seed protein content and color (Gupta et al., 2011). This gene was involved in the regulation of seed protein storage and was found to be induced in developing spikes, whereas the grain protein content of these three genotypes was directly related to the expression levels of *PBF Dof* (Gupta et al., 2011). Following this, the same group also analyzed other key genes involved in N signaling in these genotypes (Gupta et al., 2012). The genes studied include *nitrate reductase*, *glutamine synthetase*, *glutamate synthase*, and *glutamate dehydrogenase*. Nitrogen uptake efficiency was negatively correlated with nitrate reductase, glutamine synthetase, and glutamate synthase activities in PRM-1 but it was positively correlated in PRM-701 and PRM-801 (Gupta et al., 2012). The expression profile of key genes involved in N uptake and assimilation were analyzed in two genotypes that had contrasting grain protein content (low = 'GE-1437'; high = 'GE-3885') (Gupta et al., 2013). The expression pattern of *DNA binding with one finger 1* (*EcDof1)* and *EcDof2* genes was also analyzed in the same genotypes (GE-1437 and GE-3885) (Gupta et al., 2014). The *EcDof1*/*EcDof2* ratio was found to be higher in the roots of GE-3885 than in GE-1437, confirming the induction of these genes for enhanced uptake and assimilation of N in the high-protein genotype (GE-3885) (Gupta et al., 2014).

These are the preliminary reports available so far on the Ca and N transporters of finger millet. However, high-resolution studies such as development of Ca- or N-transporter defective mutants and further characterization of such transporters still need to be done in finger millet for better understanding of nutrient transport, especially on Ca transport and grain accumulation mechanisms. The recent release of a whole-genome assembly for finger millet (Hittalmani et al., 2017) combined with versatile genome editing techniques like clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein9 may aid in further progress of these studies.

Studies on Inorganic P Transporters

Understanding P transport in millets is also lagging far behind compared with those on other model crops and major cereals. The genes and proteins involved in P acquisition, transport and signaling mechanisms have been well studied in *A. thaliana* and other crop plants (reviewed in Rouached et al., 2010; Nussaume et al., 2011; Gu et al., 2016; Młodzińska and Zboińska, 2016; Wang et al., 2017). As foxtail millet is the first millet to have its whole genome sequenced, its phosphate transporter genes have been studied. Twelve of the PHT1 family of P transporters (*Si*PHT1;1–*Si*PHT11;12), which are involved in the acquisition and redistribution P, have been identified and characterized in foxtail millet (Ceasar et al., 2014). The PHT1 family transporters play key roles in the acquisition of P as inorganic P (Pi) from soil solutions (Nussaume et al., 2011). The expression patterns of 12 *PHT1* genes analyzed in response to Pi concentration and the influence of arbuscular mycorrhizal fungus (AMF) *Funneliformis mosseae* colonization. Most of these transporters also displayed tissue-specific expression patterns: *SiPHT1;2* was found to be expressed in all tissues and under all growth conditions tested. The expression of *SiPHT1;4* was induced under low Pi concentration. Expression of *SiPHT1;8* and *SiPHT1;9* in roots was selectively induced by colonization with *F. mosseae* (Ceasar et al., 2014). Homology modeling was also performed for all these transporters, along with other characterized plant PHT1s (Ceasar et al., 2016), with the crystal structure of the *Piriformospora indica* phosphate transporter (PiPT) as a template (Pedersen et al., 2013). The phosphate-binding site residues were found to be well conserved in SiPHT1 like in other plant PHT1 proteins such as *Saccharomyces cerevisiae* PHO84 phosphate transporter and PiPT. The important residues involved in Pi binding, H^+ binding, and transport were identified; such residues (aspartic acid 324, aspartic acid 45, aspartic acid 149, and lysine 459) were shown to be well conserved in foxtail millet PHT1 proteins (Ceasar et al., 2016).

More recently, some of these foxtail millet PHT1 transporters were further characterized by complementation in PHO84-deficient *Saccharomyces cerevisiae* mutants and *in planta* through downregulation of 3 *SiPHT1* genes (*SiPHT1;1*,– *SiPHT1;3*) via the RNAi strategy (Ceasar et al., 2017). Yeast complementation experiments provided evidence that *Si*PHT1;1, *Si*PHT1;2, *Si*PHT1;3, *Si*PHT1;7, and *Si*PHT1;8 may function as Pi transporters in foxtail millet. Downregulation of three transporters individually caused significant reduction of total P and Pi contents in shoot and root tissues and increased the number of lateral roots. Among these three mutants, downregulation of *Si*PHT1;2 had the strongest effects on total P and Pi content in shoot and root tissues (Ceasar et al., 2017). In finger millet, four PHT1 genes were identified and their expression was characterized in response to low Pi and AMF colonization (Pudake et al., 2017). Among these 4 PHT1 genes, *EcPHT1;4* was found to be exclusively induced by the AMF *Glomus intraradices* in root tissues. Phylogenetic analysis revealed that four finger millet PHT1 transporters are closely clustered with rice and maize (*Zea mays* L.) transporters (Pudake et al., 2017). This study was also performed with a limited amount of expressed sequence tag information available on finger millet in the National Center for Biotechnology Information database, which helped to find only four *PHT1* genes for this millet. It is the only report available so far on the phosphate transporters of

Table 1. Details of the PHosphate Transporter1 (PHT1) family of phosphate transporter genes and proteins of green foxtail (*Setaria viridis*).

Name	Chromosome location	Transcript (primary) name (Phytozome Id)	Location and orientation	Length of amino acids	Number of *trans*-membrane helices
SvPHT1;1	9	Sevir.9G542900.1	Chr_09:53,339,059–53,340,943 reverse	543	12
SvPHT1;2	9	Sevir.9G238300.1	Chr_09:18,322,931–18,324,826 forward	541	12
SvPHT1;3	7	Sevir.7G019300.1	Chr_07:6,749,849–6,753,197 reverse	536	12
SvPHT1;4	9	Sevir.9G542800.1	Chr_09:53,335,847–53,337,900 forward	527	12
SvPHT1;5	9	Sevir.9G238400.1	Chr_09:18,333,324–18,337,009 reverse	526	12
SvPHT1;6	9	Sevir.9G553200.1	Chr_09:54,029,056–54,030,675 forward	539	12
SvPHT1;7	4	Sevir.4G288300.1	Chr_04:38,488,786–38,490,420 reverse	515	12
SvPHT1;8	2	Sevir.2G118700.1	Chr_02:11,665,868–11,667,493 reverse	541	12
SvPHT1;9	5	Sevir.5G258200.1	Chr_05:30,597,670–30,600,241 reverse	564	12
SvPHT1;10	7	Sevir.7G019800.1	Chr_07:6,910,152–6,911,910 forward	538	12
SvPHT1;11	7	Sevir.7G020000.1	Chr_07:7,019,262–7,021,189 reverse	509	12
SvPHT1;12	6	Sevir.6G257900.1	Chr_06:35,243,655–35,245,280 forward	512	12

finger millet. A recent molecular marker study also identified novel quantitative trait loci for the seedling-stage low Pi stress response of finger millet (Ramakrishnan et al., 2017). It should be noted that first draft of whole genome and transcriptome assemblies were released very recently for finger millet (Hittalmani et al., 2017) and pearl millet (Varshney et al., 2017) and are publicly available in the National Center for Biotechnology Information database. These resources will be helpful for genome-wide identification of more *PHT1* and other P transporter families in these two millets; for example, each cereal was found to possess 10 to 13 *PHT1* genes within their genomes.

Other types of transporters like PHT2, PHT3, and PHT4, which are localized on the membranes of different organelles, and PHO1, which is an exporter of Pi from roots to shoots, have also not been studied in any millet. Furthermore, the transcription factor PHOSPHATE STARVATION RESPONSE 1 (PHR1), which plays a key role in transcriptional regulation under low-Pi conditions, and the microRNAs involved in long distance Pi signaling also need to be studied in millets although these are well characterized in *A. thaliana* and rice (Rouached et al., 2010; Wang et al., 2017). Some advancements made with studies of the foxtail millet PHT1 transporter (Ceasar et al., 2014, 2016, 2017) will be helpful for unraveling the Pi transport mechanism in other millets. Millets like kodo millet, little millet, and barnyard millet do not have any genomic resources, so the sequence information on the P transporter genes of *Setaria* spp. may be utilized to find the expression patterns of such genes in these millets.

PHT1 FAMILY PHOSPHATE TRANSPORTER GENES AND PROTEINS OF GREEN FOXTAIL

The PHT1 transporters are membrane proteins of a major facilitator superfamily and are the primary means of entry of Pi from the soil to the plants (Nussaume et al., 2011). The PHT1 transporters are also involved in Pi translocation as well as Pi remobilization in the aerial parts of the plants (Nagarajan et al., 2011; Mudge et al., 2002). The PHT1 family members are also strongly expressed in root tissues in response to low Pi stress to improve Pi uptake (reviewed by Baker et al., 2015). Even though two *Setaria* species provided some advancement with the early release of whole-genome sequences for these millets, Pi transporter studies have been lagging behind, apart from some initiatives with foxtail millet as discussed above. In particular, no information is available to date on Pi transporters of green foxtail although this species has been considered as a best model for such studies in other millets (Huang et al., 2016). Details of the PHT1 family phosphate transporters of green foxtail have been identified and analyzed with *in silico* tools. TBLASTN analysis using PHT1 protein sequences of foxtail millet, rice, and sorghum [Sorghum bicolor (L.) Moench.] identified 12 putative PHT1 proteins in the genome of green foxtail (www.phytozome.net, accessed 2 Nov. 2018), which were labeled as *SvPHT1;1* to *SvPHT1;12* based on the recent nomenclature of these transporters (Table 1). It is interesting to note that many of the *PHT1* genes of green foxtail are located on chromosome 9 (*SvPHT1;1, SvPHT1;2, SvPHT1;4, SvPHT1;5*, and *SvPHT1;6*), three genes are located on chromosome 7 (*SvPHT1;3, SvPHT1;10*, and *SvPHT1;11*) and another four genes are located on different chromosomes (*SvPHT1;7* on 4, *SvPHT1;8* on 2, *SvPHT1;9* on 5, and *SvPHT1;12* on 6). The amino acid lengths and the presence of 12 *trans*-membrane segments have confirmed the general features of plant PHT1s, which come under the Major Facilitator Superfamily of membrane proteins with 12 *trans*-membrane helices (Table 1). As green foxtail is a wild relative of foxtail millet, characterization of its PHT1 and other proteins would help to unravel any specific low Pi tolerance mechanisms in wild species that may have been lost during domestication.

PHYLOGENETIC RELATIONSHIP OF *S. VIRIDIS* PHT1 PROTEINS WITH THOSE OF OTHER MONOCOTS

A phylogenetic analysis of green foxtail PHT1 proteins with those of other monocots revealed their phylogenetic relationships (Fig. 1). As expected, most of the green foxtail PHT1 proteins are closely clustered with those of foxtail millet. With the exception of *SvPHT1;5*, all other

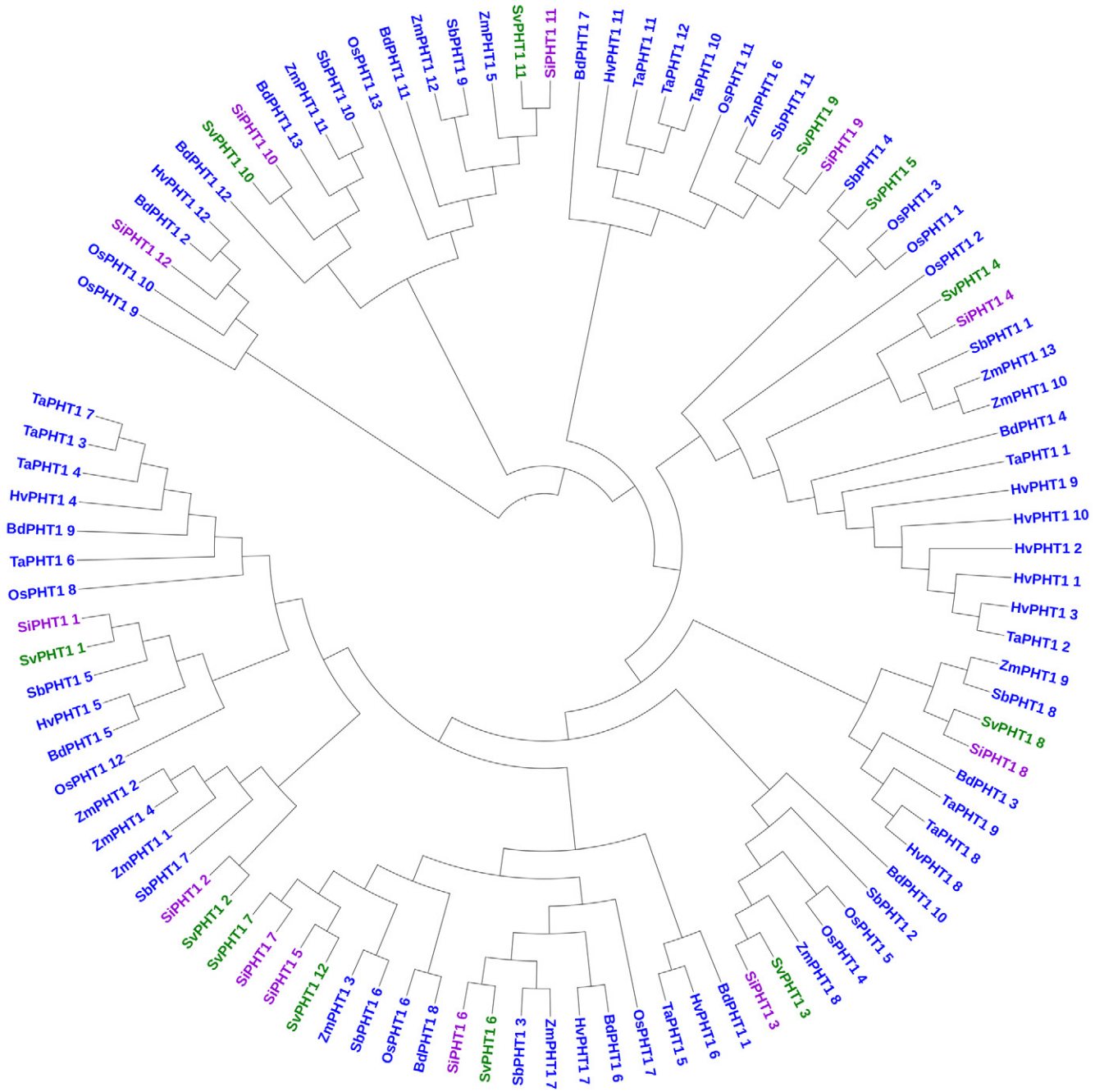

Fig. 1. Phylogenetic analysis of PHosphate Transporter1 (PHT1) family members of foxtail millet (*Setaria italica*) (purple), green foxtail (*Setaria viridis*) (green), and other monocots. The PHT1 family members of green foxtail were identified at the Phytozome website (www.phytozome.net, accessed 5 Nov. 2018) via a TBLASTN search with PHT1 protein sequences of foxtail millet (Ceasar et al., 2014), sorghum (Walder et al., 2015), and maize (Liu et al., 2016). Phylogenetic analysis was performed with the sequences of other monocots and by following methods as described previously (Ceasar et al., 2014). Phylogeny was constructed via the maximum likelihood method based on the Jones–Taylor–Thornton matrix-based model (Jones et al., 1992) with MEGA version 6 (Tamura et al., 2013). Bootstrap values are from 1000 replications. The phylogeny tree was deposited at iTOL for free access by members (http://itol.embl.de/shared/Ceasar, accessed 5 Nov. 2018).

green foxtail PHT1s were closely clustered with foxtail millet PHT1s. *Sv*PHT1;2 was clustered with *Si*PHT1;2, which is expressed in all tissues and at all stages of growth tested under both low and high Pi conditions (Ceasar et al., 2014). Downregulation of *SiPHT1;2* in foxtail millet caused severe reduction in growth and Pi content *in planta*. Heterologous expression of *SiPHT1;2* rescued the growth of PHO84 (a high affinity transporter) deficient *S. cerevisiae* mutant (Ceasar et al., 2017). Expression analysis and functional characterization of *Sv*PHT1;2 would help us to understand any further relationships between these two homologs. Both *Sv*PHT1;8 and *Sv*PHT1;9 of green foxtail have been closely clustered with *Si*PHT1;8 and *Si*PHT1;9 of foxtail millet respectively (Fig. 1). In

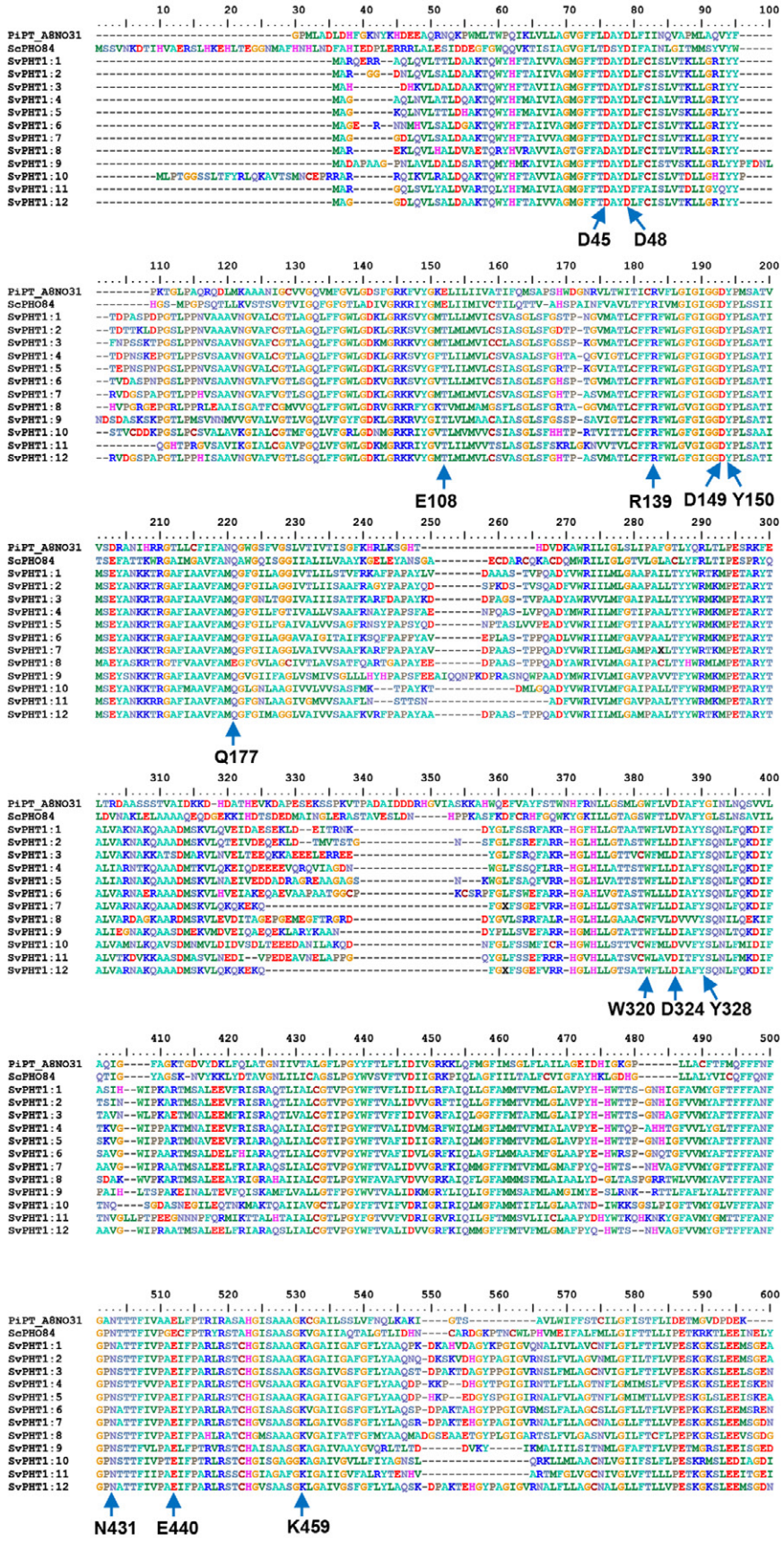

Fig. 2. Multiple sequence alignment of 12 PHosphate Transporter1 (PHT1s) of green foxtail (*Setaria viridis*), the *Saccharomyces cerevisiae* PHO84 phosphate transporter, and *Piriformospora indica* phosphate transporter (PiPT) sequences. Multiple sequence alignment was performed with the ClustalW tool in MEGA software (version 6). The key conserved residues involved in inorganic P and H^+ binding and transport are labeled with their positions relative to PiPT. The residue positions are based on the PiPT transporter.

foxtail millet, both these two isoforms have been shown to be induced by AMF colonization (Ceasar et al., 2014) and *Si*PHT1;8 was able to complement the growth of the *PHO84* mutant (Ceasar et al., 2017). It will be really interesting to see the expression pattern under AMF colonization and the functions of these two isoforms of green foxtail. It is interesting to see that *Sv*PHT1;7 and *Sv*PHT11;12 have been closely clustered with *Si*PHT1;5 and *Si*PHT1;7; both these two isoforms of foxtail millet are 98% identical and they did not show any expression under low Pi conditions (Ceasar et al., 2014). However, the expression profile and functions of these two green foxtail proteins remain to be analyzed. *Sv*PHT1;5 has been closely clustered with sorghum *Sb*PHT1;4, which has been found to be expressed in root tissues alone and also lacks the important *cis*-regulatory element P1BS in the promoter (Walder et al., 2015). As many PHT1s of monocots have already been characterized for their expression profile in response to Pi level, AMF colonization, and other functions, the functional characterization of green foxtail PHT1 transporters would help us to understand the mechanisms and relationships of these genes and proteins.

MULTIPLE SEQUENCE ANALYSIS OF PHT1 PROTEINS OF *S. VIRIDIS*

A multiple sequence alignment was performed for all 12 *Sv*PHT1s, yeast PHO84, and PiPT transporters in MEGA version 6.0 (Tamura et al., 2013) to analyze the functional residues involved in Pi binding and transport (Fig. 2). In a previous study, it was reported that all 12 foxtail millet PHT1s have the same conserved amino acids for Pi binding and H^+ and Pi transport as found in PiPT (Ceasar et al., 2016). The key resides involved in Pi binding for PiPT are tyrosine 150, glutamine 177, tryphtophan 320, aspartic acid 324, tyrosine 328, and asparagine 431 (Pedersen et al., 2013). All these resides are well conserved in all 12 green foxtail isoforms, with the exception of glutamic acid 177 instead of glutamine 177 in *Sv*PHT1;8 (Fig. 2). In a previous study, it was also shown that the closely related isoform of foxtail millet (*Si*PHT1;8) had the same change of residue at 177 when compared with the PHT1s of several other key plants (Ceasar et al., 2016). This reveals that both these two species might have evolved from the common ancestor and this residue remained the same even after the domestication of foxtail millet. This change is not seen in any non-millet cereal (Ceasar et al., 2016) so it is something that might have happened in a recently shared ancestor of *S. italica* and *S. viridis*. It will be really interesting to see further functions of these transporters in green foxtail. The proton transfers in PiPT and PHO84 have been shown to be mediated by four negatively charged residues such as aspartic acid 45, aspartic acid 48, glutamic acid 108, and aspartic acid 149 (Forrest et al., 2011; Pedersen et al., 2013). All these residues are conserved in *Sv*PHT1 isoforms, as shown in the alignment (Fig. 2). Lysine 459 is believed to increase the affinity for Pi in PiPT (Pedersen et al., 2013) and is also conserved in all *Sv*PHT1s. The amino acid resides did not show any variation in Pi binding and transport. However, many functions, such as the affinities of PHT1 proteins for Pi binding and transport may be affected by post translational modifications. As no study has been performed on the PHT1 transporters of green foxtail, expression analysis and functional characterization will help us to understand the functions of these transporters and their relationship with similar transporters of foxtail millet.

CONCLUSION

Millets are the important cereal crops supplying a major source of nutrients and energy to poor people in Asia and Africa. Only a limited number of genomic resources are available to date for most of the millets, which prevents improvement in millets. Both foxtail millet and its progenitor, green foxtail, serve as advance models for genomic studies in millets. However, only a limited number of studies have been conducted on the nutrient transporters of millets. More attention need to be paid to the phosphate transporters of millets because phosphate fertilizer reserves are depleting quickly and millets are also susceptible to low phosphate deficiency stress. Efforts were made to characterize the PHT1 family phosphate transporters of foxtail millet only. Both green foxtail and foxtail millet, with the early release of their whole genomes among the millets, are expected to serve as the best models to understand the phosphate transport mechanism of other millets, which will help to strengthen the food security among resource-poor farmers in Asia and Africa.

Conflict of Interest Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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