Article

Phosphorus transporter proteins from the PHT1 family and their potential use in modern agriculture

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Abstract

Agriculture has been globalized by its modern advances aimed at producing more and better food under a model of environmental protection. This practice is carried out in soils with different amounts of available nutrients and is based on the use of external mineral sources to satisfy the demand of the crop. Phosphorus (P) is a macroelement that participates in vital functions of plants such as the production of adenosine triphosphate (ATP), biomembrane formation and signaling reactions, among others. The plants use physio-morphological strategies in the face of a deficiency of P that manifest themselves in characteristic symptoms such as deficient development, root elongation, early maturation and reduction of crop productivity as a consequence. To maintain cellular homeostasis, plants induce overproduction of membrane proteins with phosphate transporting function in different organs. These proteins belong to the PHT1 family, they present a simport type transport that facilitates the introduction of inorganic phosphate (Pi) from the rhizosphere and allows to satisfy the biological demand during the signaling and energy processes. Structurally these proteins are highly conserved in plants (monocotyledonous and dicotyledonous) and are characterized by having 12 transmembrane domains, a conserved 2A0109 domain and an approximate size of 520 aa. The objective of this review is to put in perspective the current knowledge of PHT1 phosphate transport proteins, taking as a basis the advances in biological models to improve the productive processes and the techniques of nutritional management in crops.

Keywords: agriculture, fertilizers, homeostasis, improvement, phosphorus, PHT1.

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Introduction

Some mineral elements are essential for the growth and development of plants, which can be classified by their concentration in the plant in macronutrients and micronutrients (Agrios, 2004). The deficiency of these elements alters the metabolism and the physiology of the plants, with characteristic symptoms according to the limiting element. The visual symptoms of nutritional imbalances are considered late markers of deficiency; however, they are preceded by molecular processes of early signaling or intermediate biological response that can serve as early markers to identify the deficiency, generate management strategies and avoid productive losses.

The magnitude of spatio-temporal response of nutritional imbalances varies depending on the mineral, for example; nitrogen, P and potassium can be rapidly translocated and supply the metabolic-structural demand while elements such as boron, iron and calcium, are less mobile so the impact of their deficiency differs from the rest (Taiz and Zeiger, 2002). Plants can naturally be found in deficient conditions, but thanks to their genetic variability they have been able to modify their physiology and metabolism in order to adapt and survive these conditions (Ciereszko and Barbachowska, 2000).

Among the main mechanisms are the overexpression of genes related to the PHT1 transport proteins, which are regulated in a differential manner according to the organ of the plant, the time of exposure to deficiency and nutrition levels in a complex way (Nagy *et al.*, 2006). These proteins have a phosphate transport affinity that differs between species, for which it is necessary to deepen the knowledge of the functioning and regulation of these proteins in different organisms and experimental models, in order to have more tools available to face the coming challenges.

Phosphorus fertilizers

The intensive cultivation of plants necessarily implies the fertilization with P at levels that satisfy the specific requirements of each phenological stage of the productive cycle. The demand for phosphate fertilizers has increased in recent years and is projected to reach 46.6 million tons in 2018 (FAO, 2015). The estimated annual increase is 4% in the Americas and 2.4% worldwide, putting the internal supply at risk (FAO, 2015). One of the main limitations of modern agriculture is the availability of phosphate rock, a non-renewable source from which phosphorus fertilizers are produced (Zapata and Roy, 2007). Therefore, it is necessary to consider their availability in the context of present and future agriculture as well as to evaluate new strategies to satisfy a growing demand, and that is environmentally responsible.

The P and its importance in the plant

The P is assimilated by the plants as phosphate, being the monovalent orthophosphate (H_2PO_4) which is in greater quantity. Once incorporated it is part of the union between nucleosides and ribonucleosides, it is also present in membrane phospholipids bound to molecules of amino choline and phosphatidylcholine. In the cell most of the P remains stored in its inorganic form (Pi), whose release in the intracellular medium can trigger the respiratory explosion during maturation (Hawkesford *et al.*, 2012).

The Pi can also be used in the synthesis of high-energy phosphate ((P)-(P)) or in the form of ester (C-(P)), in both forms it is used in metabolic processes of biosynthesis and degradation where ATP is mainly required (eg. biosynthesis and phosphorylative degradation of starch). In signaling, it intervenes in phosphorylation reactions by adenosine diphosphate (ADP), adenosine triphosphate (ATP) or guanidine triphosphate (GTP) where it is released as inorganic pyrophosphate (PPi), which is subsequently used by various enzymes (Hawkesford *et al.*, 2012).

The variety of symptoms that occur in a deficient Pi plant are diverse due to their importance in vital biological processes (Figure 1). Leaves show necrotic spots and less growth of the plant. Some species stain purple (synthesis of anthocyanins) in several organs and in severe deficiency the leaves become grayish blue and the veins darken (Berry, 2010).

In corn (*Zea mays*) and beans (*Phaseolus vulgaris*), the symptoms include a lower fresh weight, a decrease in the content of radicular P, lower general development of the plant and increase of lateral roots (Ciereszko *et al.*, 1996; Hernández *et al.*, 2007; Qui-Lun *et al.*, 2007) in beans is also accompanied by a color change in young leaves (Hernández-Domínguez *et al*., 2012). However, these phenotypic alterations occur later and later as a consequence of a series of genetic and biochemical regulations.

Figure 1. Tomato plants (*Solanum lycopersicum* cv Micro-tom) 60 days of treatment under conditions of ideal nutrition (control) and deficient phosphorus (-P). Germinated seeds of tomato Micro-tom in nutritious medium Murashige-Skoog (MS) at photoperiod of 16 h light/8 h dark (27 °C and ~ 70% RH), after three weeks the seedlings were acclimated in hydroponic medium with nutrition Steiner (control) for three weeks. Later the plants were transferred to differential nutritional conditions of P [low P (-P:1.2 ppm) and control (P; 9 ppm)].

At the biochemical level, changes in the carbohydrate metabolism are manifested for a rapid adaptation and recovery to the levels of Pi in the plant, among them, the accumulation of sugars (glucose and starch in leaves), sucrose (in leaves and root) and the enzymes phosphate synthase, sucrose synthase and invertases (Ciereszko and Barbachowska, 2000).

In corn (*Zea mays*) there is also an increase in the enzymes acid phosphatase, peroxidase and superoxide dismutase, possibly as a regulation strategy of the redox environment, which contributes to the acquisition of Pi and adaptation to stress (Qui-Lun *et al.*, 2007). During this

adaptive process, anthocyanin accumulation has also been observed in grape plants (*Vitis vinifera*), attributed to the use of phenylalanine in the anthocyanin synthesis pathway and the reduction of its conversion in protein synthesis (Yin *et al.*, 2012).

These biochemical changes come to affect the phenotypic characteristics of plants. In tomato, known adaptive strategies include increased acid phosphatase activity associated with an increase in biomass at root to improve the release capacity of the enzyme (Goldstein *et al.*, 1988a), also of RNAase-like enzymes, with phosphotransferase activity (Nurnberger *et al.*, 1990), the release of both enzymes is a strategy of the plant to assimilate Pi from the environment by means of the degradation of substrates present in the area surrounding the rhizosphere for its subsequent absorption.

It is important to note that acid phosphatase in tomato has two variants or isoforms, probably as a mechanism of regulation since these are composed of two subunits that in addition to maintaining the catalytic activity independently, have the ability to aggregate, maintaining dual function as acid phosphatase and alkaline phosphatase (Goldstein *et al.*, 1988b).

Another type of adaptation modifications are those of a metabolic type, through which alternative routes are activated to keep the glycolytic cycle active.

In *Brassica nigra* before a deficiency of phosphorus, the levels of Pi (inorganic phosphate), ATP, ADP, Fru-2,6-P2 (Fructose 2,6-bisphosphate) and PPi (inorganic pyrophosphate) decrease, while the amount of Free amino acids is increased. In addition, the activity of the enzymes PFP (PPi: D-fructose 6-phosphate 1-phosphotransferase) non-phosphorylating NAPD-G3PDH, PEP (phosphoenolpyruvate) phosphatase and PEP (phosphoenolpyruvate) carboxylase, to create alternative routes to glycolysis (Duff *et al.*, 1989). These changes in metabolism allow the plant to adapt and survive the deficient conditions of Pi.

Molecular aspects of P deficiency

The molecular response to Pi deficiency occurs at the level of organs and organelles differently. Among the physiological factors, the expression of transporters and transcription factors stand out (Rubio *et al.*, 2001; Nilsson *et al.*, 2007), the former with the function of activating capture strategies and translocation of the mineral towards the interior of the cell, while the seconds activate or repress cascades of signaling and gene expression.

The spatiotemporal response is determined by the gene potential of the plant; in grape it manifests in the first 24 h and is oriented to the expression of genes for the synthesis of anthocyanins, including chalcone synthase, chalcone isomerase, flavonone-3-hydroxylase, dihydroflavonol-4-reductase, anthocyanin synthetase, UDP-glucose, UDP-glucose: flavonoid 3-*O* glucosyltransferase (UFGT) and a transcription factor VvmybA1 (Yin *et al.*, 2012). On the other hand, in bean approximately 13% of the expressed genes correspond to transporter proteins, although there were also changes in genes related to secondary metabolism, signal transduction, stress defense and cell cycle, among others (Hernández *et al*., 2007).

The overall response to a deficiency of this element in other species such as *Arabidopsis thaliana* involves the differential expression of 1835 genes during the first 72 h of exposure to mineral stress by Pi (Wu *et al.*, 2003). In rice (*Oryza sativa*), Pi deficiency also produced a negative gene regulation with 12 990 repressed genes (21% of the evaluated genes), while the total of activated genes was 8043 (13%) (Park *et al.*, 2012).

In addition, the proteins most produced by a P deficiency stress are the phosphorus transporter PHT1;4 and the acid phosphatase ACP5 (Lan *et al.*, 2012) which corroborates the participation of these proteins in the plant adaptation process to deficiency of P. In rice (*Oryza sativa*), 7 days of P deficiency modulated the expression of genes in stem and root, activating the expression of ~450 genes in root and ~250 in stem. On the other hand, they repressed the expression of ~400 genes in the root and ~180 in the stem.

It is important to mention that during the 7 days the activation of genes that code for transcription factors was observed in root, while in stem it happens until 24 h after the stimulus. Another fact of relevance is that at root the expression of some gene's members of the PHT1 family constant during the presence of the stimulus (Cai *et al.*, 2012). This indicates that the process occurs in a spatio-temporal coordination before a Pi deficiency, where the first absorption event triggers the earliest capture response of the root mineral and subsequently the translocation and mobilization of the Pi by other members of the same family of genes.

At the proteomic level in maize (*Zea mays*) genotypes 99038 (tolerant to low concentrations of P) and Qi-319 (wild) were evaluated, with the production of 140 proteins differentially accumulated to a deficiency of Pi, among the most regulated are found those related to carbon metabolism and cell proliferation (Li *et al.*, 2008). These processes are vital in the development of the plant and require a constant supply of Pi, where the process of reasimilation of this element in the face of a deficiency is largely related to the production of Pi transport proteins of the PHT1 family.

PHT1 proteins

The P transporter proteins of the PHT1 family are important in the process of recovery of homeostasis in plants. These proteins are dependent on the transcription factor PHR1 (phosphate starvation response) that regulates the phosphate starvation induced (PSI) genes, including the genes of the PHT1 family (Bari *et al.*, 2006).

These proteins contain 12 transmembrane domains with an approximate length of 520 aa (Poirier and Bucher, 2002), present a unique sequence conserved GGDYPLSATIxSE among all its members (Karandashov and Bucher, 2005) and its transcriptional and posttranscriptional regulation is a complex process that involves to the ubiquinase PHO2, the microRNA miR399 and the transcription factor PHR1.

The production of PHT1 proteins is regulated by ubiquinase PHO2, which under normal conditions degrades PHT1 proteins in an active manner; however, the PHO2 transcripts contain in the 5'-UTR region a sequence that is recognized by miR399 that when interacting with this region leads to the degradation of the transcript, preventing the production of the protein, in

turn, miR399 is regulated positively by the transcription factor PHR1 which is related to the deficiencies of Pi (Bari *et al.*, 2006), thus generating a regulation cycle dependent on intracellular Pi levels.

PHT1 family in monocots

Nagy *et al*. (2006) evaluated the expression of 5 members of the PHT1 family in corn (*Zea mays*) under conditions of Pi deficiency. The PHT1, 1 and PHT1, 4 genes are expressed in all organs of the plant with higher expression levels in the leaves and root. While PHT1, 2 is expressed mainly in root, PHT1, 3 in reproductive organs in the anthers and PHT1, 6 in root (Nagy *et al.*, 2006). In *Serratia italica*, an overexpression of the SiPHT1: 8, SiPHT1: 9 and SiPHT1: 10 genes was observed in the stem, without apparent change in leaves and root, after 15 days of a P deficiency (10 μM); however, after 31 days of the deficiency, a greater expression of SiPHT1, 4, SiPHT1, 6, SiPHT1, 11 and SiPHT1 was observed in the leaf, 12 all of the above when compared with plants kept under control conditions (Ceasar *et al.*, 2014).

The expression patterns of the aforementioned genes can be interpreted as a coordinated action to avoid functional redundancy between the proteins and act in a coordinated manner to translocate P from the root to various organs of the plant (Table 1).

Table 1. Phosphorus transporter genes of the PHT1 family.

Species	Gen(es)	Reference
Arabidopsis thaliana	ARAth; PHT1;1, PHT1;2, PHT1;3, PHT1;4, PHT1;5, PHT1;6, PHT1;7, PHT1;8; PHT1;9	Muchhal and Raghothama (1999)
Capsicum frutescens	CfPT1, CfPT2, CfPT3, CfPT4, CfPT5	(Chen *et al.*, 2007)
Glycine max	GmPT1, GmPT2, GmPT3, GmPT4, GmPT5, GmPT6, GmPT7, GmPT8, GmPT9, GmPT10, GmPT11, GmPT12, GmPT13, GmPT14	(Wu *et al.*, 2011; Qin *et al.*, 2012)
Hordeum vulgare	Horvu: PHT1:1, PHT1;2, PHT1;3, PHT1:4, PHT1:6	(Rae *et al.*, 2003; Zvobgo *et al.*, 2018)
Lotus japonicus	LjPT3	(Maeda *et al.*, 2006)
Lycium barbarum	LbPT1, LbPT2, LbPT3, LbPT4, LbPT5, LbPT7	(Hu *et al.*, 2017)
Medicago truncatula	MtPT1, MtPT2, MtPT3, MtPT5	(Kai *et al.*, 2002; Liu *et al.*, 2008)
Nicotiana tabacum	NtPT1, NtPT2, NtPT3, NtPT4, NtPT5	(Chen *et al.*, 2007)
Oryza sativa	OsPT1, OsPT 2, OsPT 3, OsPT4, OsPT5, OsPT6, OsPT7, OsPT8, OsPT9, OsPT10, OsPT11, OsPT12	(Paszkowski *et al.*, 2002; Yan *et al.*, 2014; Ye *et al.*, 2017)

Species	Gen(es)	Reference
Poncirus trifoliata	Pt1PT1, Pt1PT2, Pt1PT3, Pt1PT4, Pt1PT5, Pt1PT6, Pt1PT7	(Shu *et al.*, 2012)
Populus simonii	PtPHT1:1: PtPHT1:2: PtPHT1:3: PtPHT1:4: PtPHT1:5: PtPHT1:6: PtPHT1:7: PtPHT1:8: PtPHT1:9 PtPHT1:10 PtPHT1:11 PtPHT1:12: PtPHT1:13, PtPHT1:14	(Zhang *et al.*, 2016)
Populus trichocarpa	PtPT1, PtPT2, PtPT3, PtPT4, PtPT5, PtPT6, PtPT7, PtPT8, PtPT9, PtPT10, PtPT11, PtPT12	(Loth-Pereda *et al.*, 2011)
Pteris vittat	PvPHT1	(Ditusa *et al.*, 2016)
Setaria italica	StPHT1;1, StPHT1;2, StPHT1;3, StPHT1;4, StPHT1;5, StPHT1;6, StPHT1;7, StPHT1;8 StPHT1;9, StPHT1;10, StPHT1;11, StPHT1;12	(Ceasar *et al.*, 2014)
Solanum lycopersicum	LePT1, LePT2, LePT3, LePT4, LePT5, LePT6, LePT7, LePT8	(Liu *et al.*, 1998; Nagy *et al.*, 2005; Chen *et al.*, 2014)
Solanum melongena	SmPT1, SmPT2, SmPT3, SmPT4, SmPT5	(Chen *et al.*, 2007)
Solanum tuberosum	StPHT1:1, StPHT1:2, StPHT1:3, StPHT1:4, StPHT1:5, StPHT1:6, StPHT1:7, StPHT1:8	(Leggewie *et al.*, 1997; Nagy *et al.*, 2005; Liu *et al.*, 2017)
Sorghum bicolor	SbPHT1:1, SbPHT1:2, SbPHT1:3, SbPHT1:4, SbPHT1:5, SbPHT1:6, SbPHT1:7, SbPHT1:8, SbPHT1:9	(Tavares De Oliveira Melo, 2016)
Triticum aestivum	TaPHT1.1, TaPHT1.2, TaPHT1.6, TaPHT1.8, TaPHT1.9, TaPHT1.10	(Teng *et al.*, 2017)
Zea mays	ZEAma: PHT1;1, PHT1;2, PHT1;3, PHT1;4, PHT1;5, PHT1;6, PHT1;7, PHT1;8, PHT1;9, PHT1;10, PHT1;11, PHT1;12, PHT1;13	(Nagy *et al.*, 2006; Liu *et al.*, 2016)

PHT1 family in dicotyledons

The PHT1 family has been identified in several species and in *Arabidopsis thaliana* it is composed of nine genes with nomenclature from PHT1, 1 to PHT1, 9 and its expression is spatio-temporal specific so its physiological role can be highly regulated (Mudge *et al.*, 2002). In *Arabidopsis*, the repression of the PHT1, 1 and PHT1, 4 genes limit the absorption of the mineral; its regulation is at the transcriptional level and are an adaptation mechanism for the production of P transporter membrane proteins (Shin *et al.*, 2004). The tissue distribution of the PHT1, 4 gene is varied, and its expression depends on the organs with availability of P (Misson *et al.*, 2004).

In tomato, the PHT1 family is made up of 8 members, LePT1 to LePT8, in this culture the presence of LePT1 was identified with an early induction by P deficiency (in the first 24 h) (Muchhal and Raghothama, 1999). Chen *et al*. (2014) confirmed that genes of the PHT1 family are associated with P deficiency, LePT1 was expressed in root, stem, young leaves, flowers and fruits in the mature and green maturity stages, but the highest expression occurred in the root. LePT2 was only detected in the root with an induction ~15 times more in plants with deficiency of Pi compared to the control, LePT3, LePT5 and LePT7 were expressed in all tissues, but their highest levels were observed in leaf, ripe fruit and root, respectively. LePT6 was expressed in root, stem and leaf, but to a greater extent in root.

This suggests that the transport and assimilation of P in tomato and other species is a highly regulated process and that there are variants of space and time that define it. Other members of the PHT1 family have also been characterized in other monocotyledonous and dicotyledonous species (Table 1). In the cultivation of soybean (*Glycine max*) the expression of 14 PHT1 genes was evaluated under low P conditions (5 μM). The highest levels of root expression were presented by GmPT1, GmPT2, GmPT3, GmPT4, GmPT7, GmPT8, GmPT9 and GmPT12, in GmPT13 leaf, in flower GmPT5 and in stem GmPT6, GmPT9, GmPT11 and GmPT14; GmPT10 did not present changes in its relative expression during P deficiency (Wu *et al.,* 2011).

Table 1 shows the advance on the knowledge of Pi transporter proteins PHT1; however, in terms of advances in the knowledge of the regulation of P metabolism, some functional models have been generated based on studies carried out on mutant yeasts deficient in phosphorus assimilation; for example, MB192 yeasts (Qin *et al.*, 2012, Leggewie *et al.*, 1997), PAM2 (Nagy *et al.,* 2005) and EY917 (Nagy *et al.,* 2006) study the potential of genetic improvement of the proteins of interest in important agricultural crops, through the evaluation of parameters such as the transport kinetics of P and its affinity for this mineral (Qin *et al*., 2012), this information would allow to select genes with desirable characteristics for genetic engineering improvement, in order to produce material more resistant to mineral deficiency, considering the expected panoramas in view of the shortage foreseen in the coming years of phosphorus fertilizers.

Potential use as improvement tools

The characterization of Pi transporter proteins provides the basis for future applications. This knowledge could lead to the creation of plants more adaptable to adverse environmental conditions such as mineral deficiency through the use of molecular strategies that would allow obtaining more resilient plants during the first stages of development, as well as throughout their life cycle. Another alternative is the use of molecular techniques; for example, Yan *et al.* (2014), inserted the rice OsPT6 gene in soybean plants (NY-1001) and obtained higher plants, with a greater leaf coverage, longer roots, a higher P content, a more vigorous general growth and a general increase in biomass.

These phenotypic changes were related to an increase of at least 5 times in the expression of the OsPT6 gene in the transformed plants with respect to the control. Such phenotypic characteristics are desirable for intensive cultivation because favorable traits related to productivity and resistance to other types of biotic or abiotic stress are increased. In addition to

the coding sequences, the promoter regions of genes of interest could also be explored in order to increase the specificity of the expression in localized regions of the plant to meet a particular demand, for example, a greater uptake of P in poor soils in this element or in fructification.

In *Hordeum vulgare* it was observed that some specific promoter regions have an affinity for root expression, which has potential as an expression tool to be used with mineral transporter genes or coding for other proteins that are necessary in this organ of the plant (Schünmann *et al.*, 2004).

Conclusions

The application of joint strategies to improve the future landscape of agriculture is feasible, such as genetic improvement and the management of nutrition based on indicators of imbalances. Both require in-depth knowledge of the mechanisms of transcriptional, post-transcriptional and post-translational regulation of target proteins within the processes of growth and development. The strategy of molecular improvement is promising, in the case of phosphorus transporting PHT1 proteins; however, progress is insufficient and greater efforts are required to know its diversity and regulatory characteristics.

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