Full Length Article

Expression of *OsPSTOL1* Improves the Phosphorus Utilization Efficiency of Transgeni*c Populus tomentosa*

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Abstract

OsPSTOL1 encodes a phosphorus (P) deficiency-tolerant protein expressed in the aus rice variety, Kasalash, which functions by enhancing the ability of the plant to obtain P and other nutrients; however, its role in woody plants remains unknown. In the present study, we isolated *OsPSTOL1* from Oryza sativa L. and subsequently generated *OsPSTOL1*-overexpressing transgenic *Populus tomentosa*. It was found that in the state of P deficiency, the transgenic *P. tomentosa* had a greater root length, the symptoms of P-deficiency appeared much later, and the total nitrogen (TN) and phosphorus (TP) content was higher as compared with that of wild type plants. Upregulation of P-tolerance genes in transgenic *P. tomentosa* under P-deficient conditions was analyzed by real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR), the results of which were consistent with the physiological traits. In summary, these findings show that *OsPSTOL1* plays a positive role in the regulation of P uptake and utilization in transgenic *P. tomentosa* trees under P-deficient conditions and provide evidence for the potential application of *OsPSTOL1* in woody plants to overcome soil P deficiency. © 2021 Friends Science Publishers

Introduction

Phosphorus (P) is an essential element for life in natural ecosystems, especially for plant growth. Inorganic phosphate (Pi) derivatives are also involved in almost all significant metabolic pathways, in addition to being present as a structural component of many biochemicals including nucleic acids, coenzymes, phosphoproteins, and phospholipids (Xiang *et al.* 2016; Milner *et al.* 2018). Moreover, P is also the key component of the cellular energy source, adenosine triphosphate (ATP), which plays an important role in protein and fat metabolism. The concentration of P in the vacuole of plant cells has a buffering effect on the cytoplasm and maintains normal photosynthesis of the leaves to a certain extent. P is mainly absorbed by plants from soil in the form of orthophosphate (hydrogen phosphate, Na_2HPO_4 or dihydrogen phosphate, NaH_2PO_4) (Irfan *et al.* 2020). P deficiency affects a series of cellular metabolic processes, such as carbon metabolism and photosynthesis (Rao and Pessarakli 1996), resulting in stagnant growth, short and thin

plants, small leaves, delayed maturity, small fruit or empty seeds, and reduced yield and quality (Yu *et al.* 2010).

According to statistical data, 40−60% of the world's arable land lacks sufficient available P, which results in a decline in crop yields (Cheng *et al.* 2011); globally, 5.7 billion hectares of land are deficient in P (Jewell *et al.* 2010). In addition, the price of Pi fertilizer is relatively expensive, and the annual global P consumption has increased by approximately 3% (Gaxiola *et al.* 2011), with roughly 40 million tons of Pi fertilizer currently being applied. Surprisingly, less than 20% of Pi is absorbed by crops and the rest is wasted, resulting in poor utilization of soil P and requiring increased Pi fertilizer application at additional costs. To cope with low-pressure stress, plants have evolved many mechanisms such as changing the anatomical structure and morphology of the roots, symbiosis, and biochemical changes in metabolites (Ceasar 2018; Maharajan *et al.* 2018). Many plant species are known to grow in low-P soils, such as certain tropical plants, legumes, and corn, achieving a moderately successful yield (Smith

1934; Tong *et al.* 2001); however, whether woody plants can tolerate P-deficiency remains unknown. A quantitative trait locus (QTL) for phosphate starvation tolerance 1 (*OsPSTOL1*) has been localized to chromosome 12 in rice and is known to be involved in P uptake (Ni and Wand 1998; Pariasca-Tanaka *et al.* 2014). *OsPSTOL1* is considered to have the potential to improve yield in P-deficient and/or drought-prone environments across diverse genetic backgrounds (Chin *et al.* 2010; Gamuyao *et al.* 2012). At present, studies on *OsPSTOL1* are limited to crops, and its role in woody plants remains to be elucidated.

Plants exhibit great developmental plasticity in response to adverse environments. A large number of studies have confirmed that the morphological distribution of roots directly affects the absorption of nutrients and water by plants, which in turn influences the growth and ecological functions of their above-ground parts (Liang *et al.* 2007). Therefore, under P-deficient conditions, the development of the root system improves plant nutrient absorption to a certain extent (Qizhen *et al.* 2008).

In the present study, we investigated the role of *OsPSTOL1* in P absorption and utilization in *P. tomentosa* and found that overexpression of *OsPSTOL1* increased the absorption of P under low-P conditions. Our experimental results provide a reference for the further exploration of *OsPSTOL1* gene function, molecular mechanism, and effect on plant growth and development, in addition to providing a basis for the adaptation of woody plants to poor growth with a view to achieving overall improvement in wood production.

Materials and Methods

Experimental materials

P. tomentosa seedlings were preserved by the Agricultural Bioengineering Research Institute, Guiyang Guizhou, China. The SYBR® Select Master Mix, Murashige and Skoog Medium, rifampicin, kanamycin, timentin, sucrose, agar, and GUS stain were obtained from Beijing huayueyang.biomart.cn.

Cloning of *OsPSTOL1* and construction of plant expression vectors

OsPSTOL1 cDNA was used as a template for PCR with the following primers: F: 5'-AGAGCTCTGATCTATGAGTACATGC-3'; R: 5'-CAGTCTATCCCAGCTCAGGG-3'. The PCR product was cloned into the T-vector (Beijing Biomed Biotechnology Co., Ltd.) and sequenced (Wuhan Jinkairui Biological Engineering Co., Ltd. China). The pSH737 plasmid was restriction digested with *EcoR*I and *Xba*I, and the *OsPSTOL1* sequence was cut with *Kpn*I and *BamH*I. The vector contained the *OsPSTOL1* expression element, with the 35S promoter driving the GUS-NPTII fusion protein as a screening marker and reporter. The PCR product size of *OsPSTOL1* is 119 bp (Fig. 1).

Plant transformation and transgenic plant detection

P. tomentosa was transformed with *OsPSTOL1* using the *Agrobacterium*-mediated leaf disc method. *Agrobacteria* expressing pSH737-35S-OsPSTOL1 were resuspended in Murashige and Skoog (MS) nutrient medium and the concentration was measured using a nucleic acid analyzer at OD_{600} (0.4). The *P. tomentosa* leaf disks were subjected to the *Agrobacterium* solution for 6−8 min and dried on sterile filter paper prior to being placed on co-cultivation medium (MS + 0.1 mg L^{-1} NAA) for 2−3 d at 26 ± 2°C in the dark. Next, the leaves were transferred sequentially onto callus induction medium (MS + 2.0 mg L^{-1} ZT + 1.0 mg L^{-1} NAA + 200 mg L^{-1} timentin + 50 mg L^{-1} kanamycin + 30 g L^{-1} sugar + 8 g L^{-1} agar powder pH 5.80); value-added medium (MS + 1.0 mg L^{-1} 6-BA + 200 mg L^{-1} timentin + 50 mg L^{-1} kanamycin + 30 g L^{-1} sugar + 8 g L^{-1} agar powder pH 5.80); and bud induction medium (MS + 2.0 mg L^{-1} ZT + 1.0 mg L^{-1} NAA + 200 mg L^{-1} timentin + 50 mg L^{-1} kanamycin + 30 g L^{-1} sugar + 8g L^{-1} agar powder pH 5.80) (Dai 2018; Yao *et al.* 2020). Following shoot development at 3−5 cm, the shoots were separated from the calli and transferred onto rooting medium (MS + 0.1 mg L^{-1} NAA + 200 mg L^{-1} timentin + 50 mg L^{-1} kanamycin + 30 g L^{-1} sugar + 6.5 g L^{-1} agar powder pH 5.80). The rooted shoots were subsequently transplanted into pots and allowed to grow. Two or three leaves from each plant were analyzed by β-glucuronidase (GUS) staining and PCR to verify *OsPSTOL1* gene integration.

Plant growth conditions

T_0 generation *OsPSTOL1*-overexpressing transgenic *P. tomentosa* strains were cultured to rooting in MS medium (under a 16 h light/8 h dark photoperiod with photosynthetically active radiation of 200 μmol $m^{-2} s^{-1}$) and then transplanted into pots containing medium-sized (2−4 mm diameter) vermiculite. The plants were cultured for 3−4 weeks prior to the experiment (MS full-nutrition solution).

Phosphorus deficiency experiment

P-deficient conditions were introduced by transferring the transgenic and wild type *P. tomentosa* seedlings to Hoagland's P-deficient culture medium (1/4 of the P content) and growing them in a greenhouse (under a 16 h light/8 h dark photoperiod, 25°C, and 50% relative humidity) until symptoms of P deficiency developed. Each experiment was repeated at least three times, the results of which were similar.

Quantitative reverse transcription PCR (RT-qPCR)

Synthesis of cDNA from total RNA (10 μL) (fresh leaves of

wild type and transgenic lines) was conducted using an M-MLV reverse transcriptase kit (28025013, Invitrogen™) according to the manufacturer's instructions. RT-qPCR was performed using the SYBR® Select Master Mix, CFX Manager (Bio-Rad), and CFX96TM RT PCR detection system (Bio-Rad). A three-step reaction procedure was performed in 96-well optical reaction plates. The cycling profile was 95°C for 15 s; 39 cycles of 95°C for 10 s, 55°C for 30 s, and 72°C for 30 s; and the solubility curve was 95°C for 15 s, 60°C for 60 s, 95°C for 15 s, and 60°C for 15 s. The total volume of the PCR reaction (25 μL) contained double-distilled H_2O (8.7 μL); forward (0.90 μL) and reverse (0.90 μL) primers; SYBR® Select Master Mix (12.5 μL); and cDNA (2.0 μL, 0.16 μg). The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative gene expression (Wu *et al.* 2016). The primer sequences of related genes are shown in Table 1.

Determination of total N, P and K content

Each plant sample (3−5 leaves from the base to the top) was pulverized, passed through a 0.2-mm sieve, and dried at 60−80°C.

Determination of N content: A mass of 0.10−0.20 g ground and dried plant sample (0.25−0.5-mm sieve) was weighed and placed in a 100-mL Erlenmeyer flask. The sample was moistened with a small amount of water, to which 5 mL concentrated sulfuric acid was added, covered, and shaken overnight. The neck of the flask was slowly heated, and as the temperature gradually increased, the concentrated sulfuric acid was decomposed, emitting a white smoke. The sample was digested until the solution was evenly brownish-black in color, to which 10 drops of H_2O_2 were added while shaking. The sample was reheated to almost boiling for 10−20 min and allowed to cool slightly, following which 5−10 drops of H_2O were added. This step was repeated 2−3 times until the liquid became colorless, which was then heated for 5−10 min (to drive off the H_2O_2). The sample was allowed to cool, the flask was rinsed with water, and the liquid was transferred to a volumetric flask containing a constant volume of water and subsequently filtered.

A 5−10-mL aliquot of the clear liquid was transferred to a distillation tube, which was placed in the nitrogen analyzer. A volume of 2 mL boric acid was placed in a 150-mL Erlenmeyer flask, to which 2 drops of indicator were added and shaken. The Erlenmeyer flask was placed in the nitrogen analyzer to condense. At the end of the tube, where the mouth of the tube is 3−4 cm above the liquid level of boric acid, approximately 5 mL 10 mol·L NaOH solution was added to the distillation tube and subjected to steam distillation. When the level of the distillate was roughly 50 cm, the results were calculated.

Determination of P content: A 10-mL aliquot of the test solution that had been digested and boiled at a constant volume (same as total N determination) was placed in a 50-mL volumetric flask, to which 2 drops of 2,6 dinitrophenol indicator were added, and the pH was adjusted with 6

mo1·L^{-1} NaOH to a yellow color. Subsequently, 10 mL ammonium vanadyl molybdate reagent was added and the volume made constant with water. The flask was shaken well and incubated for 5 min, following which the absorbance was measured using a spectrophotometer at 450 nm. The zero point of the instrument was adjusted with a blank solution. The standard curve was prepared using 0, 1.0, 2.5, 5, 7.5, 10.0, and 15.0 μg·mL^{-1} P. The results were subsequently calculated.

Determination of K content: A 5−10-mL aliquot of the test solution that had been digested and boiled at a constant volume (same as total N determination) was placed in a 50-mL volumetric flask and made up to a constant volume with water. The sample was measured directly using a flame photometer, and the galvanometer reading was recorded. The mass fraction (%) of quasi-plant potassium was calculated according to the calibration curve or linear regression equation.

Statistical analysis

Three independent biological replicates and three technical replicates were used to calculate the mean value and standard deviation (SD) of the metabolite concentrations. The SPSS 17.0 software was used to determine significant differences by Duncan's multiple-range test. Any difference relative to the control at *P < 0.05 or **P < 0.01 was considered statistically significant.

Results

### Generation of genetically modified *P. tomentosa*

The leaves and stems of sterile *P. tomentosa* seedlings were used as explants to genetically transform *P. tomentosa* via Agrobacterium-mediated leaf disc transformation. Kanamycin (Kan) was added to the medium for selection. Following refinement of resistant plants for approximately 7 days (during this period, the leaves should not lose too much water), the root medium was removed and the plants were planted in the greenhouse soil for cultivation. A few young leaves were taken for β-glucosidase (GUS) histochemical staining, and genomic DNA was removed from the plants for PCR verification. We obtained 12 transgenic *P. tomentosa* plants (Fig. 2), of which 3 overexpressing lines, along with 3 wild type strains, were selected for subsequent experiments (5, 8, 13).

Expression of *OsPSTOL1* in transgenic *P. tomentosa* regulates their growth and development

To verify whether *OsPSTOL1* influences the P uptake capacity of *P. tomentosa*, we subjected wild type and transgenic plants to P-deficient conditions and observed their growth status in real time. Prior to the introduction of P-deficient conditions, the above-ground development of

Table 1: Oligonucleotide primers used for confirmation of transgenic lines and expression analysis of various endogenous genes

Gene name	Gene ID	Annealing Tm (°C)	Product size (bp)	Primer (5'-3')
PHT1	Potri.010G072000.1	55	115	TGCTGGGCTTACATTCTATTGG
				TCTGACGCTGCTTGTATTGC
PHT2	Potri.010G046300.1	55	120	GAGGAACATCAAGAAACGGTAA
				CAAGCCCTGTCCCAAAGTC
PHR1	Potri.002G257800.6	55	203	CTGCACTAGAAGGAGGATCACA
				ATGGCTCAGAATATTCAGGAGT
PHO1	Potri.008G110800.1	55	175	GTGCTAGGCGATGGTTTGAC
				TCCTGCTGCTAACATTGCTGA
Actin	Potri.001G309500	55	156	AAACTGTAATGGTCCTCCCTCCGG
				CATCATCACAATCACTCTCCGA
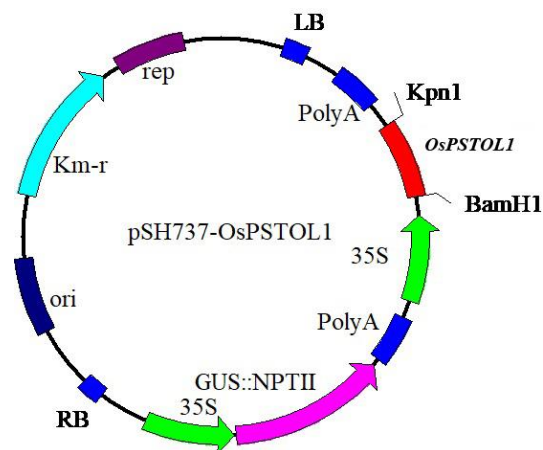
Fig. 1: pSH-35S-OsPSTOL1 expression plasmid map. RB: Right border of T-DNA; 35S: CaMV 35S poly A; LB: Left border of T-DNA; GUS: β-glucuronidase reporter gene
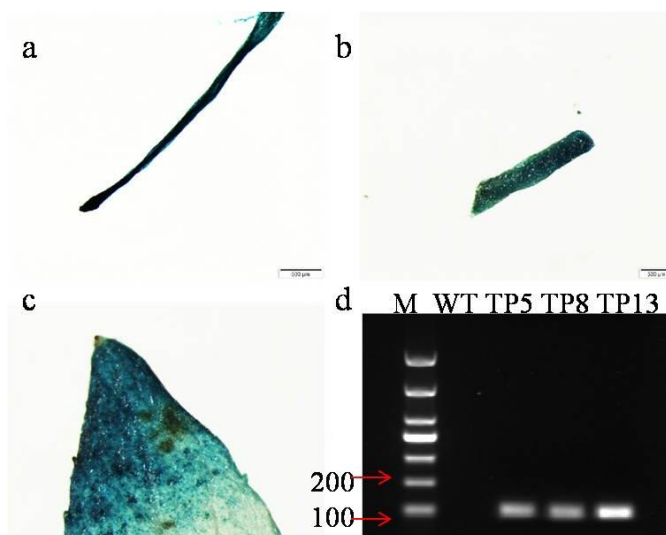
Fig. 2: β-Glucuronidase (GUS) staining and PCR identification. (**a**) GUS staining of the roots of transgenic *P. tomentosa.* (**b**) GUS staining of the stems of transgenic *P. tomentosa*. (**c**) GUS staining of the top leaves of transgenic *P. tomentosa*. (**d**) PCR identification of transgenic *P. tomentosa* (119 bp). Molecular identification of leaves extracted from the same part of wild type and transgenic *P. tomentosa*. M: DNA Marker; WT: wild type; TP: transgenic plant lines (TP5, TP8, TP13)

wild type and transgenic plants was almost identical (Fig. 3a). However, under P-deficient conditions, the leaves of wild type plants exhibited typical symptoms of P deficiency, such as chlorosis, yellowing, and tissue necrosis (Fig. 3c), while the leaves of transgenic plants were green (Fig. 3b). After being subjected to these conditions for 20 days, the root length and fresh weight of transgenic and wild type *P. tomentosa* were measured (Fig. 3b; Table 2).

Table 2: Root fresh weight and length of wild type and transgenic *P. tomentosa*

Type	Root fresh weight (g)	Root length (cm)
WT	3.21 ± 0.22	17.55 ± 1.11
TP	4.58 ± 0.163*	23.97 ± 2.97*

Note: Root length refers to the longest distance of the plant root. Data are the average of three wild type and three transgenic plants, respectively. Specific data for each strain are shown in Supplementary Materials. TP (mean value for transgenic plants); *statistically significant
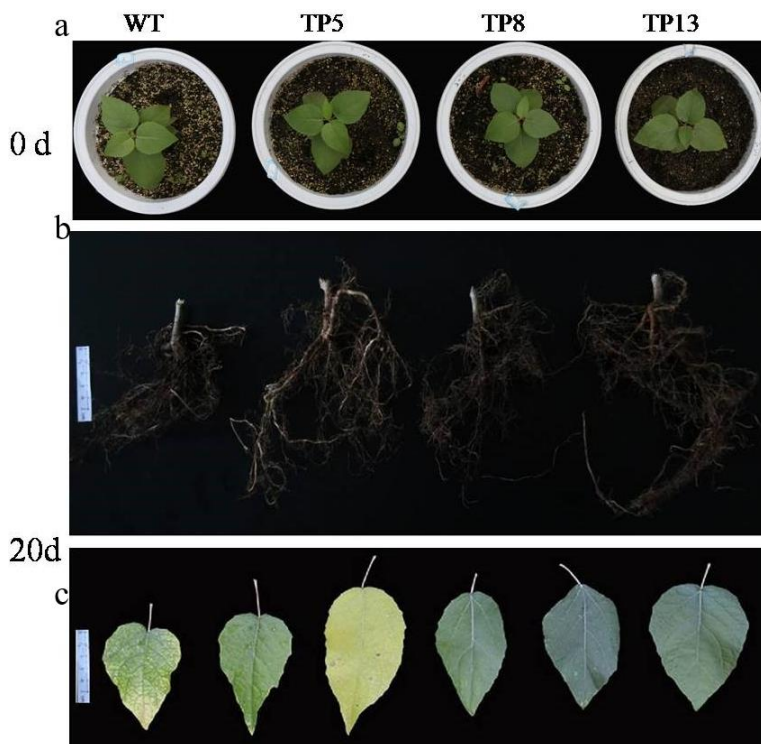

Fig. 3: Root growth and symptoms of transgenic and wild type (WT) *P. tomentosa* under P-deficient conditions. (**a**) Transgenic and WT *P. tomentosa* prior to the introduction of P-deficient conditions. (**b**) Roots of transgenic and WT *P. tomentosa* following the introduction of P-deficient conditions for 20 d. (**c**) Transgenic and WT *P. tomentosa* following the introduction of P-deficient conditions for 20 d. The order of the leaves from left to right is WT1, WT2, WT3, TP5, TP8, and TP13. The displayed leaves are fifth from the base

Effects of *OsPSTOL1* on the total N, P and K content of transgenic *P. tomentosa*

We investigated whether *OsPSTOL1* can regulate the total N, P, and K content of wild type and transgenic *P. tomentosa* under low-P conditions (Fig. 4a−c) and found that the N and P content of transgenic plants was 1.18- and 1.35-fold higher than that of wild type plants, respectively. However, there was no obvious difference in K content between wild type and transgenic plants.

***OsPSTOL1* influences the expression of related genes**

Plants have evolved to possess many mechanisms for adapting to low P concentrations, including the induction of high-affinity P transporters, acid phosphatase, and organic acid secretion (Wasaki *et al.* 2006). To investigate the mechanism of *OsPSTOL1* underlying tolerance to P deficiency, we examined several typical stress response genes, including Phosphorus 1 (*PHO1*), Phosphate

transporter 1 (*PHT1*), and Phosphate starvation response 1 (*PHR1*). The expression levels of these genes under P-deficient conditions were detected by RT-qPCR, and it was found that their expression levels were higher in transgenic plants as compared with those in wild type plants (Fig. 5). These results indicate that *OsPSTOL1* plays a significant role in regulating the expression of P transporter- and acid phosphatase-related genes in *P. tomentosa*.

Discussion

P is an essential macronutrient for plant growth and development. Pi is the major form of P that the plants absorb and assimilate, which is taken up by the roots and translocated between cells or tissues *via* the action of Pi transporters. When the surrounding environment is nutrient-deficient, the growth of the plant will be directly affected. In addition to the height of the plant, size of the leaves, and growth of the roots, the dry weight of the plant can also serve as a measure of the N, P and K content (Sheng 1999).
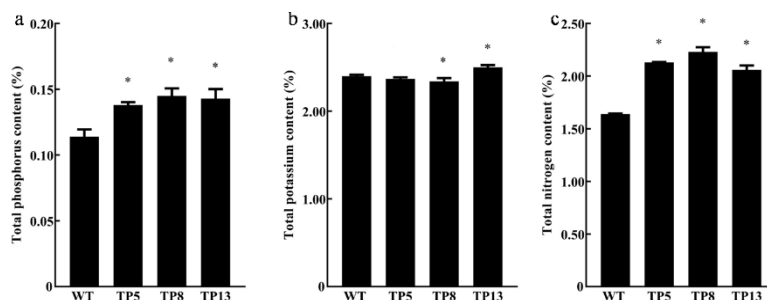
Fig. 4: Nitrogen (N), P, and potassium (K) content of transgenic and wild type (WT) *P. tomentosa* following the introduction of P-deficient conditions. (**a**) P content of transgenic and WT *P. tomentosa* following the introduction of P-deficient conditions. (**b**) K content of transgenic and WT *P. tomentosa* following the introduction of P-deficient conditions. (**c**) N content of transgenic and WT *P. tomentosa* following the introduction of P-deficient conditions. Three WT samples were mixed
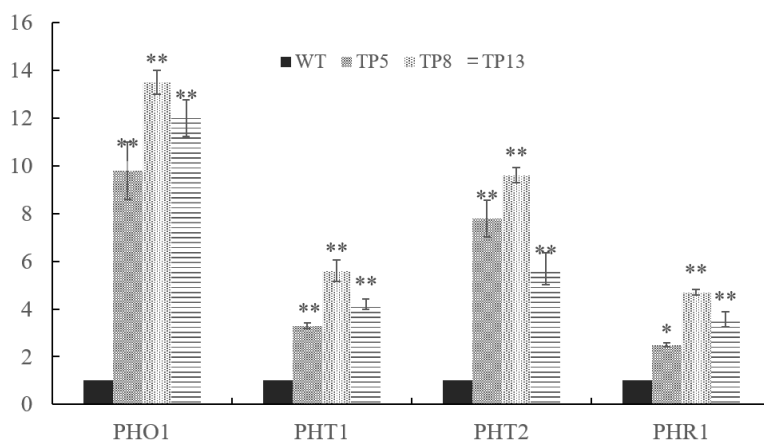

Fig. 5: Expression pattern of the typical stress response genes, *PHT1*, *PHT2*, *PHR1*, and *PHO1*, in *P. tomentosa* following the introduction of P-deficient conditions, as assessed by RT-qPCR. WT (three wild type samples mixed); TP (three transgenic samples mixed). Data are representative of at least three independent experiments

The state of P deficiency in the soil is not only important for crop yield but also for the wood yield of woody plants, since this is also limited in low-P soil (Jia *et al.* 2017).

OsPSTOL1 has previously been cloned and its function in rice verified (Heuer *et al.* 2009). This gene has been shown to be directly involved in the uptake and utilization of P in rice (Chin *et al.* 2011). In P-deficient soils, overexpression of *OsPSTOL1* in crops can encourage normal growth and increase yields, which is of great significance for increasing food production; however, *OsPSTOL1* remains functionally uncharacterized in woody plants. Here, to further investigate its function, we isolated *OsPSTOL1* from rice and demonstrated a positive effect on the uptake and utilization of P in transgenic *P. tomentosa*.

The present study found that under P-deficient conditions, the associated symptoms in transgenic *P. tomentosa* were significantly delayed as compared with those in the wild type plant (Fig. 3c). Malnutrition during the growth and development of plants is directly manifested as the health of the leaves, for example: fading; a color change from green to yellow, red, or purple; tissue necrosis, black heart, dead spots, atrophy or death of growth points; abnormal plant type;

organ deformities; and delayed growth or progression. Moreover, the N, P, and K content of the transgenic and wild type *P. tomentosa* was also measured under low-P conditions, and the results show that the N and P content of transgenic *P. tomentosa* was higher than that of the wild type plant; however, the K content was not significantly different between the two plants. Taken together, these data suggest that *OsPSTOL1* participates in and improves the regulation of P absorption and utilization in plants under P-deficient conditions and reduces the associated symptoms. Studies have shown that low P concentrations inhibit the elongation of primary roots and promote the differentiation of lateral roots (Sánchez-Calderón *et al.* 2005). Plant roots absorb P nutrients from the soil solution, and the basic process of regulating P transport to vascular tissues is regulated by related genes (Schünmann *et al.* 2004). In the present study, it was found that under P-deficient conditions, the main roots of transgenic *P. tomentosa* were longer than those of the wild type plant, and the lateral roots were well developed. At the same time, fresh weight of roots of transgenic *P. tomentosa* was higher than that of the wild type plant (Table 2).

To regulate the absorption rate of mineral nutrients and ensure the normal activities of plants under P-deficient conditions, the expression levels of P transporters and various other related genes are increased (Abel *et al.* 2010). For example, members of the P transporter family, *PHT1* and *PHT2* (Rae *et al.* 2003; Catarecha *et al.* 2007; Wu *et al.* 2011); the transcription factors, *PHR1* and *PHR2* (Jie *et al.* 2008; Ren *et al.* 2012); and PHO1 and *PHO2* involved in P transport (Franco-zorrilla *et al.* 2004; Bari *et al.* 2006; Stefanovic *et al.* 2011) are induced by low-P stress. The present study investigated the growth status and related gene expression of transgenic and wild type *P. tomentosa* under P-deficient conditions. Under the influence of *OsPSTOL1* overexpression, the levels of *PHT1*, *PHR1*, *PHR1*, and *PHO1* expression in transgenic plants were higher than those in wild type plants. The expression of these genes has been shown to increase the soil nutrient absorption of transgenic *P. tomentosa*. In summary, the present study provides a theoretical basis for improving the absorption and utilization of P by *P. tomentosa* and other woody plants.

Conclusion

GUS staining and observation of the root indicated that *OsPSTOL1* is expressed in the vegetative organs of the plants. In the case of poor nutritional status, transgenic plants had more developed roots to compensate for lower nutrient concentrations. Introduction of P-deficient conditions demonstrated that *OsPSTOL1* participates in tolerance to P deficiency in *P. tomentosa* and can alleviate the associated symptoms. *OsPSTOL1* can regulate the total content of N, P, and K and may be involved in the expression of genes related to the regulation of P uptake to alleviate the damage resulting from P deficiency stress in *P. tomentosa.*

Acknowledgments

Author Contribution

LLT conceived and designed the research, WL and YY conducted the experiments. WL contributed analytical tools and analyzed data. YXZ wrote the manuscript. YXZ and ZDG supervised the studies and revised the manuscript. All authors read and approved the manuscript.

References

Abel S, CA Ticconi, CA Delatorre (2010). Phosphate sensing in higher plants. *Physiol Plantarum* 115:1–8

Bari R, PB Datt, M Stitt, W Scheible (2006). PHO2, microRNA 399 and PHR1 define a phosphate-signaling pathway in plants. *Plant Physiol* 141:988–999

Catarecha P, MD Segura, JM Franco-Zorrilla, B García-Ponce, M Lanza, R Solano, J Paz-Ares, A Leyva (2007). A mutant of the *Arabidopsis* phosphate transporter PHT1;1 displays enhanced arsenic accumulation. *Plant Cell* 19:1123–1133

Ceasar SA (2018). 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. *Plant Genomics* 12; Article 180019

Cheng LY, B Bucciarelli, JB Shen (2011). Update on white lupin cluster root acclimation to phosphorus deficiency. *Plant Physiol* 156:1025–1032

Chin, JH, R Gamuyao, C Dalid, M Bustamam, J Prasetiyono, S Moeljopawiro, M Wissuwa, S Heuer (2011). Developing rice with high yield under phosphorus deficiency: Pup1 sequence to application. *Plant Physiol* 156:1202–1216

Chin JH, X Lu, SM Haefele, R Gamuyao, A Ismail, M Wissuwa, S Heuer (2010). Erratum to: Development and application of gene-based markers for the major rice QTL Phosphorus uptake 1. *Theor Appl Genet* 120:1087–1088

Dai TT (2018). Effects of overexpression of *AtBAS1* gene on the growth and development of *Populus tomentosa*. *Master Thesis*, p:33. Guizhou University, Guizhou, China

Franco-zorrilla JM, E Gonzalez, R Bustos, F Linhares, A Leyva, J Paz-Ares (2004). The transcriptional control of plant responses to phosphate limitation. *J Exp Bot* 55:285–293

Gamuyao R, JH Chin, J Pariascatanaka, P Pesaresi, S Catausan, C Dalid, I Slametloedin, EM Tecsonmendoza, M Wissuwa, S Heuer (2012). The protein kinase *Pstol1* from traditional rice confers tolerance of phosphorus deficiency. *Nature* 488:535–539

Gaxiola RA, M Edwards, JJ Elser (2011). A transgenic approach to enhance phosphorus use efficiency in crops as part of a comprehensive strategy for sustainable agriculture. *Chemosphere* 84:840–845

Heuer, X, X Lu, JH Chin, JP Tanaka, H Kanamori, T Matsumoto, TD Leon, VJ Ulat, AM Ismail, M Yano, M Wissuwa (2009). Comparative sequence analyses of the major quantitative trait locus phosphorus uptake 1 (*Pup1*) reveal a complex genetic structure. *Plant Biotechnol J* 7:456–471

Irfan M, M Abbas, JA Shah, MA Akram, MY Memon (2020). Categorization and identification of *Brassica* genotypes for phosphorus utilization efficiency. *Intl J Agric Biol* 23:227–234

Jewell MC, BC Campbell, ID Godwin (2010). Transgenic plants for abiotic stress resistance. *In: Transgenic Crop Plants*, pp:67–132. Springer, Berlin, Heidelberg

Jia XL, TX Wen, MX Gao, W Yang (2017). Leaf nitrogen and phosphorus concentration and the empirical regulations in dominant woody plants of shrublands across southern China. C*hin J Plant Ecol* 41:31–42

Jie Z, CJ Fang, CW Zhong, YL Yi, MW Xu, WH Xiao, QZ Wei, W Ping (2008). OsPHR2 is involved in phosphate-starvation signaling and excessive phosphate accumulation in shoots of plants. *Plant Physiol* 146:1673–1686

Liang Q, H Liao, X Yan (2007). Quantitative Analysis of Plant Root Architecture. *Chin Bull Bot* 24:695–702

Maharajan T, SA Ceasar, TPA krishna, M Ramakrishnan, V Duraipandiyan, AD Naif, A Bdulla, S Ignacimuthu (2018). Utilization of molecular markers for improving the phosphorus efficiency in crop plants. *Plant Breed* 137:10–26

Milner MJ, RM Howells, C Melanie, B Sarah, G Neil, EJ Wallington (2018). A PSTOL-like gene, TaPSTOL, controls a number of agronomically important traits in wheat. *BMC Plant Biol* 18; Article 15

Ni JP, DS Wand (1998). Mapping QTLs for phosphorus deficiency tolerance in rice (*Oryza sativa* L.). *Theor Appl Genet* 97:1361–1369

Pariasca-Tanaka J, JH Chin, ND Khady, C Dalid, S Heuer, M Wissuwa (2014). A novel allele of the P-starvation tolerance gene *OsPSTOL1* from African rice (*Oryza glaberrima* Steud) and its distribution in the genus *Oryza*. *Theor Appl Genet* 127:1387-1398

Qizhen ZM, TY Xi, M Lei (2008). The ecological roles and influencing factors of plant root architecture. *Henan Sci* 26:172–176

Rae AL，DH Cybinski，JM Jarmey (2003). Characterization of two phosphate transporters from barley; evidence for diverse function and kinetic properties among members of the Pht1 family. *Plant Molecular Biol* 53:27－36

Rao I, M Pessarakli (1996). The role of phosphorus in photosynthesis. *In: Handbook Photosynthesis*, pp:173–194. Marcel Dekker, Inc. New York, USA

Ren F, QQ Guo, LL Chang, L Chen, CZ Zhao, H Zhong, XB Li (2012). *Brassica napus PHR1Gene* encoding a MYB-Like protein functions in response to phosphate starvation. *PLoS One* 7; Article e44005

Sánchez-Calderón L, J López-Bucio, A Chacón-López, A Cruz-Ramírez, F Nieto-Jacobo, JG Dubrovsky, L Herrera-Estrella (2005). Phosphate starvation induces a determinate developmental program in the roots of *Arabidopsis thaliana*. *Plant Cell Physiol* 46:174–184

Schünmann PHD, AE Richardson, FW Smith, E Delhaize (2004). Characterization of promoter expression patterns derived from the Pht1 phosphate transporter genes of barley (*Hordeum vulgare* L.). *J Exp Bot* 398:855–865

Sheng XL (1999). The current state and prospect of plant nutrition and fertilizer science. *Plant Nutr Fert Sci* 5:193–205

Smith SN (1934). Response of inbred lines and crosses in maize to variations of nitrogen and phosphorus supplied as nutrients 1. *Agron J* 26:785-804

Stefanovic A, AB Arpat, R Bligny, E Gout, C Vidoudez, M Bensimon, Y Poirier (2011). Over-expression of *PHO1* in *Arabidopsis* leaves reveals its role in mediating phosphate efflux. *Plant J* 66:689–699

Tong AHY, M Evangelista, AB Parsons, H Xu, GD Bader, N Page, M Robinson, S Raghibizadeh, CWV Hogue, H Bussey, B Andrews, M Tyers, C Boone (2001). Systematic genetic analysis with ordered arrays of yeast deletion mutants. *Science* 294:2364–2368

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

Wu Z, H Ren, SP Mcgrath, P Wu, FJ Zhao (2011). Investigating the contribution of the phosphate transport pathway to arsenic accumulation in rice. *Plant Physiol* 157:498–508

Wu ZJ, C Tian, Q Jiang, XH Li, J Zhuang (2016). Selection of suitable reference genes for qRT-PCR normalization during leaf development and hormonal stimuli in tea plant (*Camellia sinensis*). *Sci Rep* 6; Article 19748

Xiang OY, H Xia, XQ Hong, X Zhao, W Zhang, X He, W Ma (2016). Knock out of the *phosphate* 2 gene *TaPHO2-A1* improves phosphorus uptake and grain yield under low phosphorus conditions in common wheat. *Sci Rep* 6; Article 29850

Yao X, M Chen, D Zhao, L Lv (2020). Overexpression of the Sorghum bicolor K^+/Na^+ transporter gene, *SbSKC1*, enhances salt tolerance in poplar (*Populus tomentosa*). *Intl J Agric Biol* 24:304–310

Yu LP, CL Zhang, N Ma, J Li, SJ Zhang (2010). Rapeseed physiological response to drought and phosphorus deficiency. I. Gas exchange and yield. *Chin J Oil Crop Sci* 32:506–511