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Short communication

Over-expression of *OsPT2* under a rice root specific promoter *Os03g01700*

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ABSTRACT

Identification of root-specific promoters is a good method to drive root-specific gene expression for nutrient uptake. Constitutive over-expression of *OsPT2* may have negative effects on the growth of rice seedlings under high Pi condition. Thus, characterization and utilization of root-specific promoters are critical for genetic breeding. Here, a root-specific promoter (*Os03g01700*) with a number of specific regulatory elements has been confirmed. Interestingly, *cis*-regulatory element S449 is significantly enriched in the −1475 ~ -2013 bp and −1077 ~ -1475 bp regions of *Os03g01700* promoter. The activities of several deletion derivatives of *Os03g01700* promoter were analyzed using both transient expression and genetic transformation system. The results showed that the root-specific *cis*-acting elements might be present in the −2013 bp ~ -1475 bp and −1077 bp ~ -561 bp regions of *Os03g01700* promoter. To determine the actual effect of root-specific expression of *OsPT2*, a construction consisting of *Os03g01700* promoter and *OsPT2* CDS was used to transform rice. Under Pi-sufficient condition, there were a series of symptoms of phosphorus toxicity in the shoots of *OsPT2* over-expressing (Ov-OsPT2) seedlings. Under Pi-deficient condition, more soluble Pi was accumulated in the shoots of Ov-OsPT2 seedlings than that in the wild type. Our data provide a candidate root-specific promoter in the breeding of rice with high phosphorus uptake variety.

1. Introduction

Plant-specific promoters play important roles in control of transgenic expression. Several constitutive-expressed promoters have been well-studied and used for genetic engineering (Cornejo et al., 1993; He et al., 2009). For example, the cauliflower mosaic virus (CaMV) 35S, ACTIN1 and UBI1 were widely applied in genetic transformation (Odell et al., 1985; McElroy et al., 1991; Christensen et al., 1992). Constitutive over-expression of target genes may have negative effects on the growth of plants. Thus, characterization and utilization of tissue-specific promoters plays an important role in genetic breeding (Li et al., 2013).

Recently, many studies on the isolation of tissue-specific promoters have been carried out in plants. For example, a root-specific gene, *salT*, was isolated from the roots of salt-sensitive rice cultivar (Claes et al., 1990). Two cDNAs, *RCc2* and *RCc3*, which are highly expressed in the root of rice, were identified as root-specific genes (Xu et al., 1995). *OsEXPA8* is another important root-specific gene that was involved in the establishment of root system architecture by regulating cell extension (Ma et al., 2013). A root-specific gene *Os04g24469* was selected from public microarray data (Zhao et al., 2014). The promoters of three root-specific expressed genes, *OsrRSP1*, *OsrRSP3*, and *OsrRSP5*, were reported to be used for the enhanced expression of target genes in root (Huang et al., 2015).

Phosphorus (P) is one of the most essential and limiting mineral nutrients involved in plant growth and development (Shen et al., 2014; Koyama et al., 2005). Soil inorganic phosphate (Pi) is only available at very low level, and thus, for crops, it is a challenge to maintain a constant and stable concentration of internal Pi in cells (Abel, 2011; Veneklaas et al., 2012). Many studies have revealed a complex and selective system consisting of membrane-spanning phosphate transporter (PT) proteins, which participate in Pi absorption and transport from soil into root cells (Liu et al., 2011).

OsPT2, a low-affinity Pi transporter in rice, is responsible for the OsPHR2-mediated accumulation of Pi in the shoots of rice (Wu and Xu, 2010). *OsPT2* is mainly expressed in the stele of primary and lateral roots and is responsible for the transport of Pi from roots to shoots (Ai et al., 2009). However, despite the importance, over-expression of *OsPT2* under constitutive 35S promoter results in the accumulation of excess Pi in the shoots under Pi sufficient condition (Liu et al., 2010). Constitutive over-expression of *OsPT2* may have negative effects on the growth of rice seedlings. Thus, characterization and utilization of root-specific promoters in rice is a good way to avoid the harmful effects of

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the constitutive expression of the genes involved in Pi transport (Li et al., 2014).

Our previous study has characterized two root-specific promoters, *Os03g01700* and *Os02g37190*, in rice (Li et al., 2013). However, the mechanism of their root-specific expressions was largely unknown. Here, a series of deletion derivatives of *Os03g01700* promoter in transgenic rice plants were conducted to reveal the roles of various *cis*-acting elements in the root-specific expression. Studies on the root-specific expression of *OsPT2* provide us a potential approach to breed rice with high phosphorus uptake variety.

2. Materials and methods

2.1. Plant materials and growth conditions

Wild-type rice *Nipponbare* (*Oryza Sativa* L. ssp *japonica*) was used in our study. Quantitative data was derived from two lines (three independent replications for each line). Rice seedlings were grown in a standard culture solution containing 1.425 mM NH_4NO_3, 0.323 mM NaH_2PO_4, 0.513 mM K_2SO_4, 0.998 mM $CaCl_2$, 1.643 mM $MgSO_4$, 0.009 mM $MnCl_2$, 0.075 mM $(NH_4)_6Mo_7O_{24}$, 0.019 mM H_3BO_3, 0.155 mM $CuSO_4$, 1 mM $FeCl_3$, 0.070 mM citric acid and 0.152 mM $ZnSO_4$, pH 5.8, in a greenhouse with a light/dark cycle of 12 h:12 h, at a day/night temperature of 30 °C:24 °C, and 70% humidity. For hydroponics, seedlings were transferred to a plastic net floating on the nutrient solution containing 0.1 mM NaH_2PO_4 for Pi-sufficient condition and 0 mM NaH_2PO_4 for Pi-deficient treatment for one month.

2.2. Analysis of cis-acting elements

A total of 3500 bp upstream sequence of *Os03g01700* promoter was obtained from the TIGRE database (http://rice.plantbiology.msu.edu/). The sequence was assigned to PlantCARE (http://www.bioinformatics.psb.ugent.be/webtools/plantcare/html/) and PLACE (http://www.dna.affrc.go.jp/PLACE/) to analyze the sites of *cis*-acting elements.

2.3. GUS staining

The GUS staining solution was prepared using 10 mM EDTA, 1 mM potassium ferrocyanide, 1 mM potassium ferricyanide, 20% [v/v] methyl alcohol, 0.5% [v/v] Triton X-100) and 0.2 mM 5-bromo-4-chloro-3-indoyl glucuronide. The histochemical analysis was performed as described previously (Li et al., 2013). In detail, different tissues, including roots, leaves and glumes, from rice seedlings were incubated at 37 °C for 20 min in GUS staining solution. To stop the action, the GUS solution was removed. The glume samples were indirectly observed with a Carl Zeiss LSM510 laser scanning system (http://www.zeiss.com/). The root samples were embedded in 5% agar and cut into 20–40 μm thick sections for photographing using a Leica MZ95 stereomicroscope (Leica Instrument, Nusslosh, Germany). The leave samples were dipped into 100% ethanol at room temperature to remove chlorophyll, and were observed using a Carl Zeiss laser scanning system LSM510.

2.4. Vector construction of the deletion derivatives of Os03g01700 promoter

The primers of *Os03g01700* promoter deletion vectors were designed according to the rice genome sequence on the TIGRE database. All primers were redesigned into In-Fusion primers with the restriction site *KpnI* using the Clontech online support tool (http://bioinfo.clontech.com/infusion/). The In-Fusion primers of *Os03g01700* promoter deletion vectors are listed in Table S1. A series of deletion derivatives of *Os03g01700* promoter, including 312 bp (P312), 516 bp (P516), 1077 bp (P1077), 1475 bp (P1475), and 2013 bp (P2013), were cloned into the GUS plus vector (pBI101.3 backbone) (Fig. S1).

2.5. Particle gun mediated transient expression and Agrobacterium mediated transformation

Calluses of rice mature embryo were put on hyper-osmotic medium containing 46.67 g/L sorbitol and 46.67 g/L mannitol for 4 h. For microprojectile preparation, 30 mg gold powder (Φ1.0 μm) was added with 1 mL of 70% ethanol in a 1.5 mL centrifuge tube. After 3 min vortex, the 70%ethanol was replaced with 1 mL of 100% ethanol for 15 min. The supernatant was abandoned by 5 s short and low speed centrifugation. The gold powder was suspended in 1 mL of 70% ethanol by 5 s vortex. Then, the gold powder was washed with ddH_2O three times and re-suspended in 500 μL of 50% glycerol until used. Particle gun mediated transient expression was performed, as previously described (Romyanon et al., 2003).

The correct plasmids were firstly introduced into *A. tumefaciens* EHA105. Embryogenic calluses were co-cultivated with positive *A. tumefaciens* cells to regenerate transgenic rice plants, as previously described (Supartana et al., 2005).

2.6. RNA isolation and qRT-PCR analysis

Total RNAs were isolated using Qiagen RNeasy plant mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. RNAs from roots and leaves were used for tissue-specific expression confirmation. The total RNAs were treated with RNase-free DNase I to remove contaminated DNA. After agarose gel electrophoresis, the concentration of RNA was determined by a ND-1000 NanoDrop (Thermo Fisher Scientific, Shanghai,China). Then, cDNA was generated using 4.0 μg of RNAs from each sample using a cDNA synthesis kit (Promega, Madison, WI, USA) (Yu et al., 2017). The qRT-PCR primer sequences were designed using the Roche Primer Designer and listed in Table S1. The qRT-PCR was performed, as previously described (Li et al., 2013).

2.7. Vector construction

For the POs03g01700:OsPT2 vector construction, a 3500-bp sequence of the Os03g01700 promoter was amplified using PCR and inserted into the pGOFS1-R vector with EcoR I and Sma I. The primer sequences are: F: 5′- CCGgaattcTGAAAAGAGCTTGAGAGAAT-3′ and R: 5′- TCCcccgggCTTCTTCTTCTTCGATCGAC-3'. Then, a 1825-bp sequence of *OsPT2* gene was amplified and into the pGOFS1-R-Pomoter vector with B*amH* I. The primer sequences are: F: 5′- ATCCCCCCGGG GATCCGCTTATAACTTTGCAGCTTGAGG-3′ and R: 5′- GCAGGTCGAC GGATCCGGGAAAGTTCACAAAATCTCACA-3'.

2.8. Identification of the $P_{Os03g01700}$:OsPT2 (Ov-OsPT2) seedlings

For positive transgenic material screening, a *Hygromycin Phosphotransferanse* gene (*Hpt*) was used as a marker (Hpt-F: 5′-AGAAG AAGATGTTGGCGACCT-3'/Hpt-R: 5′-GTCCTGCGGGTAAATAGCTG-3′). The PCR detection reaction was prepared including 0.2 μL DNA template, 10 mm DNA primer pairs and 2.0 μL PCR buffer 2.0 μL, 2 μM dNTP mixture and 1.5 μL of 25 mM $MgCl_2$. The reaction process was as follows: 1 cycle, 94 °C, 10 min, 30 cycles, 94 °C, 1 min, 58 °C, 1 min, 72 °C, 1 cycle, 72 °C, 5 min. The amplified products were detected using 1.0% agarose gel electrophoresis.

A series of transgenic seedlings were selected for RNA extraction. The root and shoot samples from four-week-old seedlings were separately harvested. The root-specific over-expression of *OsPT2* was checked by qRT-PCR (primers in Table S1).

For transgenic locus and insertion copy characterization, southern hybridization using DIG chemiluminescent technology was applied according to the standard protocol (Trijatmiko et al., 2016). DNAs were extracted and digested with *Hin*d III.

2.9. Measurement of Pi contents

Plant height and primary root length were surveyed using a ruler. Leaves and roots of the WT and Ov-OsPT2 seedlings from either Pi-sufficient or Pi-deficient treatments were harvested separately. The concentration of Pi was determined using the procedure described previously (Liu et al., 2010). Briefly, 100 mg of fresh tissue samples were homogenized with 6 mL extraction buffer containing 100 μL of 5 M H_2SO_4. Then, the homogenate was transferred to a new 15 mL tubes and centrifuged at 10,000 × *g* for 10 min at 4 °C. The supernatant in 10-fold dilution was mixed with a malachite green reagent in 3:1 ratio and kept for 20 min. The absorption values at 650 nm wavelength were determined using a Spectroquant NOVA60 spectrophotometer (Merck, Darmstadt, Germany). KH_2PO_4 was used as standard to generated a standard curve for Pi concentration calculation.

2.10. Statistical analyses

Statistical analyses were performed using SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA), and a one-way ANOVA was applied to compare the growth parameters and Pi contents between WT and Ov-OsPT2 seedlings. The expression analysis was performed for three biological replications, and the figures show the average values. A P value < 0.05 was considered to be statistically significant and indicated by "*"; $P < 0.01$ was indicated by "**".

3. Results

3.1. Analysis of cis-acting elements in the promoter of Os03g01700

In total, 3000 bp promoter sequence of *Os03g01700* was analyzed. Several important *cis*-acting elements, including TATA-box, CAAT-box, LTR element, MBS element, NON-box, and CAT-box, were identified in the promoter of *Os03g01700* (Fig. S2 and S3). The number of these specific regulatory elements in different promoter regions, including 312 bp, 516 bp, 1077 bp, 1475 bp, and 2013 bp, were counted (Table S2 and Fig. S4). Among these elements, the largest numbers came from CACTFTPPCA1 (S449, YACT(Y = T/C), 48 sites), a mesophyll-specific expressed *cis*-regulatory element, and ROOTMOTIFTAPOX1 (S98, ATATT, 34 sites), a root-specific expressed *cis*-regulatory element (Gowik et al., 2004; Elmayan and Tepfer, 1995). Interestingly, most S98 elements were identified in the −1475 ~ -2013 bp and −1077 ~ -1475 bp regions and most S449 elements were found in the 0 ~ -312 bp, −312 ~ -561 bp and −561 ~ -1077 bp regions (Fig. S5a).

3.2. Os03g01700 promoter deletion analysis using transient expression and genetic transformation systems

To determine the activities of the deletion derivatives of *Os03g01700* promoter, particle gun-mediated transient expression was performed. Our results showed that all five deletion derivatives of *Os03g01700* promoter could drive the expression of GUS reporter gene (Fig. S5b).

To investigate their spatial expression patterns, all deletion derivatives of *Os03g01700* promoter with GUS reporter gene were introduced into embryogenic calluses to regenerate transgenic rice plants. Three independent T3 transgenic lines of each promoter construct were analyzed in various organs, including root, leaf, glume and flower. Our data showed that the expressions of all deletion derivatives of *Os03g01700* promoter could be detected in at least one tissue.

Under the P2013 promoter, the expression of GUS gene was only detected in the roots, particularly in the inner epidermis and middle column rather than in the outer epidermis and cortex, and could not be detected in roots. Under the P1475 promoter, the expression of GUS gene could be detected in the outer epidermis, leaves, glumes, and flowers. Under the P1077 promoter, GUS gene was strongly expressed in the primary roots rather than lateral roots, leaves, glumes, and flowers, suggesting that P1077 is a root-specific promoter. Besides, the expressions the GUS gene under the P561 and P312 promoters could be detected in all the tested organs (Fig. 1).

3.3. Overexpression of OsPT2 under the root-specific promoter of Os03g01700

In total, 10 independent T_0 transgenic lines of Ov-OsPT2 were randomly selected for hygromycin screening. Nine of these lines were hygromycin resistance, suggesting a high positive rate (90%) of our transgenic experiment (Fig. S6).

In order to determine the root-specific expression, qRT-PCR was performed in seven randomly selected hygromycin-resistant lines (T_0). Our data showed that the root-specific expression was successfully achieved in four independent lines, including line 2, 4, 6, and 7

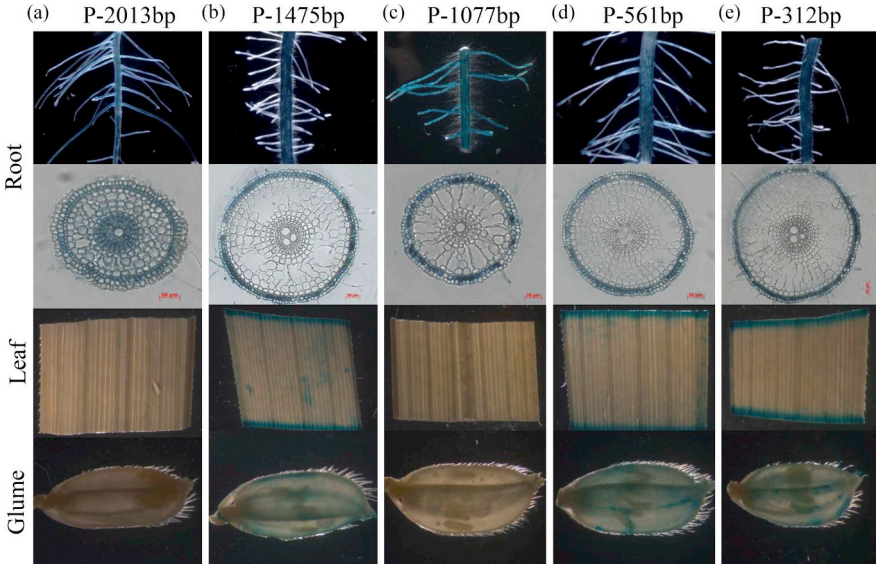

Fig. 1. *Os03g01700* promoter deletion analysis using genetic transformation system. The expression patterns of a series of deletion derivatives of *Os03g01700*, including P2013 (a), P1475 (b), P1077 (c), P516 (d) and P312 (e) were analyzed using genetic transformation.

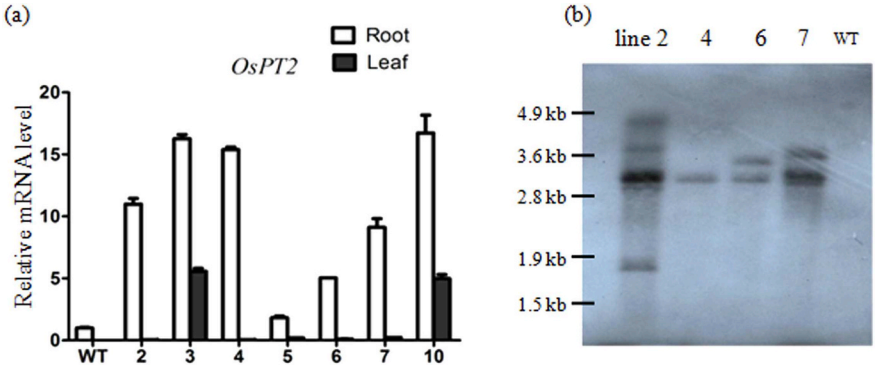

Fig. 2. Overexpression of *OsPT2* under the root-specific promoter of *Os03g01700*. (a) The expression level of *OsPT2* in the roots and leaves of different hygromycin positive lines. (b) Identification of the copy numbers of T-DNA insertion in four root specific over-expression lines. DIG-labeled DNA Molecular Weight Marker VII (Roche) was used as marker.

(Fig. 2a). In spite of the different expression levels among various lines, the average expression level of *OsPT2* in the roots of transgenic lines was 10-folds higher than that in WT.

The copy numbers of T-DNA insertion were detected in the four over-expression lines (line 2, 4, 6 and 7) using southern blot method. The PCR products of hygromycin gene were used as probes. The hybridization results showed that the Ov-OsPT2 line 4 was a single-copy line, and Ov-OsPT2 line 2, 6, and 7 were multi-copy lines (Fig. 2b). The Ov-OsPT2 line 4 (Ov-OsPT2-1) and line 6 (Ov-OsPT2-2) was used for further study.

3.4. Phenotype of the transgenic lines under different Pi conditions

Thirty-d-old T_3 seedlings (from Ov-OsPT2-1) were used for qualitative phenotyping under different Pi conditions (Fig. 3a). Under the Pi-sufficient condition, the heights of Ov-OsPT2-1 and Ov-OsPT2-2 seedlings were 84% and 85% of the WT, the tiller numbers of Ov-OsPT2-1 and Ov-OsPT2-2 seedlings were 55.4% and 60.0% of the WT, and the primary root length of Ov-OsPT2-1 and Ov-OsPT2-2 seedlings were 69.7% and 72.7% of the WT, respectively. Although the Ov-OsPT2 seedlings were smaller than WT, there was no obvious symptoms of phosphorus toxicity in Ov-OsPT2 seedlings. Under the Pi-deficient condition, the height and tiller number of the WT were significantly reduced and the root system of the WT was significantly induced. The heights of Ov-OsPT2-1 and Ov-OsPT2-2 seedlings were 70.5% and 75.7% of the WT, the tiller numbers of Ov-OsPT2-1 and Ov-OsPT2-2 seedlings were 97% and 93.1% of the WT, and the primary root lengths of Ov-OsPT2-1 and Ov-OsPT2-2 seedlings were 81.0% and 86.4% of the WT, respectively (Fig. 3b–d). Under Pi sufficient condition, the root/shoot ratios of the WT were larger than that of Ov-OsPT2-1 and Ov-OsPT2-2 seedlings. Under Pi deficient condition, the root/shoot ratios of WT were smaller than that of Ov-OsPT2-1 and Ov-OsPT2-2 seedlings (Fig. S7). The growth of Ov-OsPT2 under Pi-deficient condition was weaker to that of the WT.

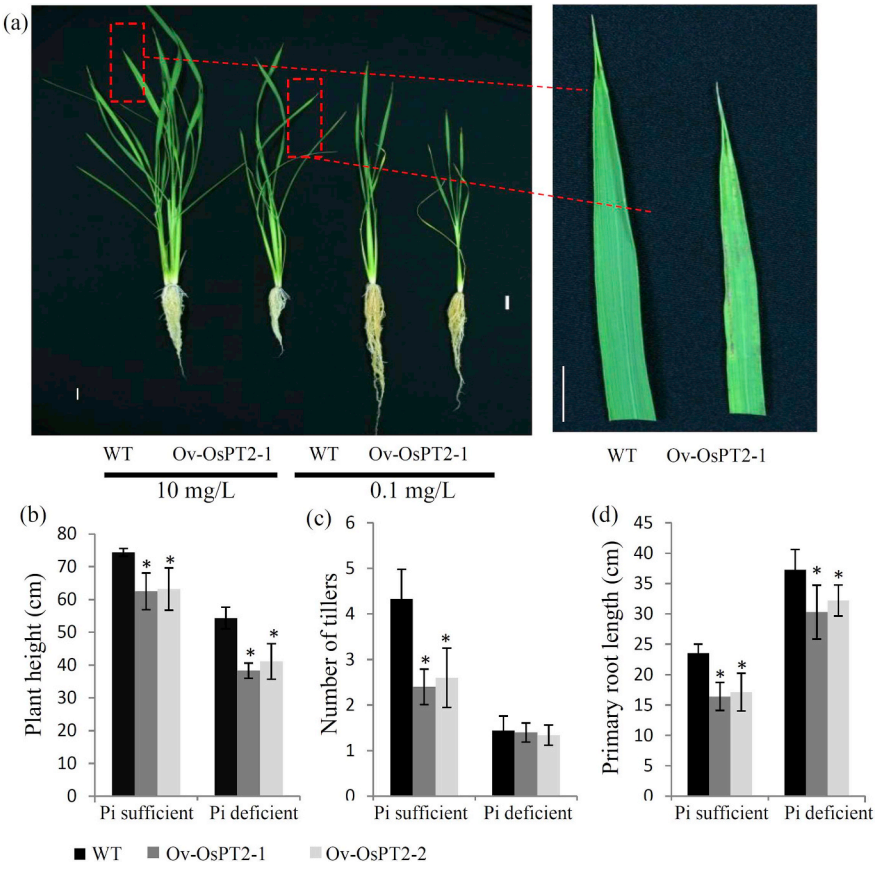

Fig. 3. Phenotype of the Ov-OsPT2 seedlings under different Pi conditions. (a) Phenotype of the Ov-OsPT2 and WT seedlings under Pi-sufficient and Pi-deficient conditions. Three important parameters, including plant height (b), number of tillers (c), and primary root length (d), were measured. Significant differences ($P < 0.01$) were indicated by "*". Quantitative data was derived from two lines ($N = 3$).

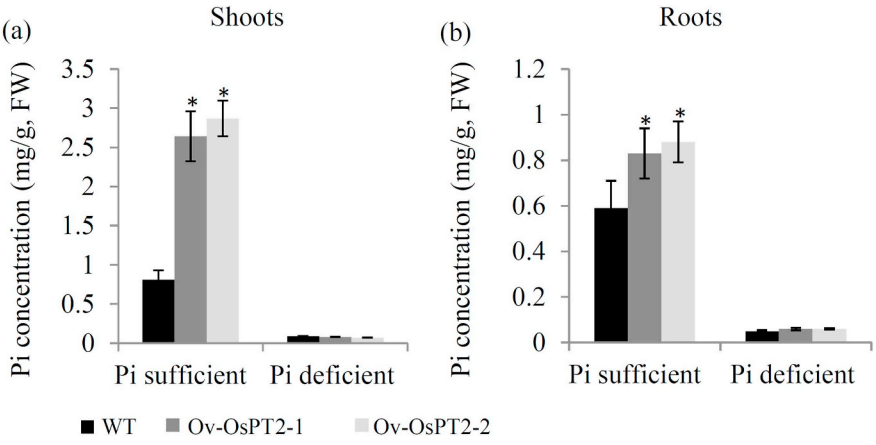

Fig. 4. Determination of the soluble Pi contents of the Ov-OsPT2 and WT seedlings. The soluble Pi contents were determined in the shoots (a) and roots (b) of Ov-OsPT2 and WT under Pi-sufficient and Pi-deficient conditions. Significant differences ($P < 0.01$) were indicated by "*". Quantitative data was derived from two lines ($N = 3$).

3.5. Determination of the soluble Pi contents of transgenic lines

To investigate the differences in the Pi uptake between Ov-OsPT2 and WT, the soluble Pi content was determined. Under the Pi-sufficient condition, the soluble Pi contents in the shoots of WT, Ov-OsPT2-1, and Ov-OsPT2-2 were 0.79, 2.59 and 2.87 mg/g, respectively, and in the roots of WT, Ov-OsPT2-1 and Ov-OsPT2-2 were 0.59, 0.84 and 0.88 mg/g, respectively. Under the Pi-deficient condition, the soluble Pi contents in the shoots of WT, Ov-OsPT2-1 and Ov-OsPT2-2 were 0.09, 0.08 and 0.07 mg/g, respectively, and in the roots of WT, Ov-OsPT2-1 and Ov-OsPT2-2 were 0.05, 0.06 and 0.06 mg/g, respectively (Fig. 4).

4. Discussion

Phosphorus is a nutrient element that is essential for the growth and development of plants. Intensive studies have identified a number of putative rice *PT* genes, which are responsive to Pi starvation (Wang et al., 2014). *OsPT2* is required for the long-distance transport of Pi from roots to shoots (Ai et al., 2009). In our previous study, two novel rice root-specific promoters (*Os03g01700* and *Os02g37190*) were isolated and characterized (Li et al., 2013). To explore the roles of *OsPT2* in the utilization, translocation, and distribution of Pi in rice, *Os03g01700* was taken as an introduction to investigate the regulation mechanism underlying the root-specific expression of *OsPT2* gene.

In the sequence of *Os03g01700* promoter, a variety of tissue-specific *cis*-elements were identified, such as leaf-specific expressed S449, endosperm-specific expressed S148, pollen-specific expressed S378 and S245, root-specific expressed S468 and S98, seed-specific expressed S246, and fruit-specific expressed S422 (Gowik et al., 2004; Elmayan and Tepfer, 1995; Shirsat et al., 1989; Ellerstrom et al., 1996; Filichkin et al., 2004; Yamagata et al., 2002). Sequence deletion analysis has been performed to reveal the roles of different elements in root-specific expression of *Os03g01700*. It is worth noting that S449 was significantly enriched in the −1475 ~ -2013 bp and −1077 ~ -1475 bp regions (Fig. S5a). The P2013 promoter still showed root-specific expression, indicating that there was no root-specific *cis*-regulatory element in the 2013–3500 bp region. Under the P2103 promoter, the expression of GUS reporter gene could not be detected in cortex, indicating that there were cortex specific elements in the 2013–3500 bp region. Under the P1077 promoter, the expression of GUS reporter gene also showed root-specific expression. The results showed that the root-specific *cis*-acting elements might be present in the −2013 bp ~ -1475 bp and −1077 bp ~ -561 bp regions of *Os03g01700* promoter. In addition, S449 was significantly enriched in the 0 ~ -312 bp and −561 ~ -1077 bp regions of *Os03g01700* promoter. Due to the existence of a large number of S449 elements, constitutive expression of GUS reporter gene was observed under the P561 and P312 promoters. Although the mechanisms of tissue-specific expression of *Os03g01700* are poorly elucidated, *Os03g01700* promoter can be used in the breeding of rice.

The expression patterns of Pht1 family genes were affected by the changing environmental factors (Fang Zhu et al., 2018). Apart from Pi-response elements, a large number of *cis*-elements involved in environmental and hormonal responses have been identified in the 2-kb upstream promoter regions of the *OsPht1* family genes (Liu et al., 2011). For example, several stress-related *cis*-regulatory elements, such as bHLH, WRKY, and ABRE elements, have been found in the promoters of the *OsPht1* family genes (Fang Zhu et al., 2018; Dai et al., 2016). In rice, 11 OsPht1 family genes, including *OsPT2*, are significantly regulated by various hormone treatments. Differential expression profiles showed that the expression of *OsPT2* was up-regulated by NAA, GA3, and cytokinin (Liu et al., 2011). Taking advantage of the *Os03g01700* promoter, plants with more stable expression of *OsPT2* under the changing environment are available now (Liu et al., 2011).

To confirm the actual effect of root-specific expression of *OsPT2*, a construction consisting of *Os03g01700* promoter and *OsPT2* CDS was used to transform rice. Under the Pi-sufficient condition, there were significant differences in the contents of soluble Pi in both roots and shoots between Ov-OsPT2 and WT. Although the significant decreases in the plant height, number of tillers, and primary root length were observed under the Pi-sufficient condition, there was no obvious symptoms of phosphorus toxicity in the shoots of Ov-OsPT2 seedlings. Overexpression of *OsPT2* enhanced the transport of Pi and caused the accumulation of soluble Pi in the shoots of Ov-OsPT2 seedlings (Liu et al., 2010). Most of PTs in rice were predicted to be high-affinity transporters, whereas OsPT2 is a low-affinity Pi transporter (Goff et al., 2002; Ye et al., 2015). It was suggested that OsPT2 functions as Pi transporter under both Pi sufficient and deficient conditions (Ghillebert et al., 2011). As an important low affinity Pi transporter in rice, over-expression of *OsPT2* under 35S promoter resulted in the accumulation of excess shoot Pi and even phosphorus toxicity. In the shoots, the Pi concentration was up-regulated by approximately four-fold in the plants expressing *OsPT2* under 35S promoter, and the Pi concentration was up-regulated by *2* three-fold in the plants expressing *OsPT2* under *Os03g01700* promoter. In the roots, the Pi concentration was up-regulated by approximately two-fold under 35S promoter, and the Pi concentration was up-regulated by only 1.3-fold under *Os03g01700* promoter (Liu et al., 2010).

In addition to *OsPT2*, most of the *OsPht1* family genes are potential candidates for generating transgenic rice with improved Pi use efficiency. The expressions of many other OsPht1 family genes could be detected in various organs and tissues (Liu et al., 2011). Constitutive over-expression of the *OsPht1* family genes may cause some unexpected growth and developmental defects. The development of root-specific expression promoter will bright prospects for application.

5. Conclusions

In our study, a root-specific promoter (*Os03g01700*) with a number of specific regulatory elements has been confirmed. A *cis*-regulatory element (S449) is significantly enriched in the −1475 ~ -2013 bp and −1077 ~ -1475 bp regions of *Os03g01700* promoter. Transient expression and genetic transformation showed that the root-specific *cis*-acting elements might be present in the −2013 bp ~ -1475 bp and −1077 bp ~ -561 bp regions of *Os03g01700* promoter. A construct consisting of *Os03g01700* promoter and *OsPT2* CDS was used to transform rice, indicating an effective role of *OsPT2* in the transport of Pi. Our data provided a potential approach to cultivate rice with high phosphorus uptake variety.

Conflicts of interest

The authors declare that they have no competing interests.

Authors' contributions

YL, CL, LC and SC carried out the molecular studies, participated in the analysis and drafted the manuscript. YP carried out the qRT-PCR analysis. SY performed the statistical analysis. YL conceived of the study, and participated in its design. YL acquired of funding. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.01.009.

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