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ARTICLE

Biological Analysis and Response to Low Phosphate Stress of Phosphate Transporter Family 1 (*PHT1*) Genes in *Solanum tuberosum* L.

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ABSTRACT

Inorganic phosphate (Pi) is one of the main nutrients necessary for plant growth. Phosphate transporters mediate the acquisition, transport and recycling of phosphate, which is essential for plant growth and development. Although *PHT1* has been reported in many plants at home and abroad, it is rarely studied in potato. Therefore, it is of great significance to study the *PHT1* family members in order to understand the molecular response mechanism of potato in low phosphate state. In this study, a total of 6 potato *PHT1* genes were identified and isolated. It was found that after the expression of different members of potato *PHT1* gene, there were certain differences in amino acids and proteins, and the transmembrane domains ranged from 6 to 12. The difference in the secondary and tertiary protein structure of potato PHT1 also led to a difference in protein morphology. In addition, the expression of the *PHT1* gene in potato increased obviously during 3~9 h of Pi deficiency stress. Overall, the expression levels of different genes in roots, stems and leaves are distinctly different, but the expression levels of the *StPHT1;6* and *StPHT1;10* genes are very high in roots, stems and leaves, indicating that these two genes may participate in the absorption of Pi in potato and play a role in Pi translocation. These two genes play a major role in the regulation of expression under short-term Pi deficiency stress. Our results provide an important reference for further understanding the evolution and function of potato phosphate transporters, and have important significance for improving the ability of potato to tolerate low Pi.

KEYWORDS

Potato; phosphate transporter gene; short-term phosphate-deficiency stress; biological analysis

1 Introduction

Plant growth requires a large amount of P, which is not only an important component of nucleic acids, phospholipids, ATP, ADP and sugars, but also participates in various metabolic and material transportation pathways [1–3]. Phosphorus, as one of the most limiting nutrient elements for plant growth and crop production in the world [4–7], plays an irreplaceable role in plant growth. Although the total amount of P in the soil is very high, most of it exists in the form of an organic state that cannot be absorbed by plants [8], and the mobility of P in the soil is low, the effective utilization rate is poor, and it is easy to be

adsorbed and fixed by cations or organic compounds in the soil that convert P into insoluble and organic forms that cannot be absorbed and utilized by plants [9–17]. Phosphate transporters are the main transporters involved in the uptake of Pi (refers to $H_2PO_4^-$ in this paper) from the soil and its reuse and distribution in plants [18,19], they can also enable plants to resist the damage caused by various stresses by regulating the absorption and transportation of Pi under a variety of abiotic stress conditions to maintain the normal growth and development of plants [20].

The phosphate transporter family contains many members. According to the different gene protein structures, subcellular localizations and functions in plants, they can be divided into four subfamilies, named *PHT1*, *PHT2*, *PHT3*, *PHT4* [15,21–26]. Different families of phosphate transporter genes play different roles in the process of plant growth and development [1,26,27]. *PHT1* is the family with the most research results at present. It plays an important role in the process of Pi absorption, transport, distribution and reuse in plants [9,28–31]. PHT1 subfamily proteins belong to the 9th branch of the major facility superfamily (MFS) in structure. They usually have 12 transmembrane-spanning domains [8,15,16,32,33]. They are located on the plasma membrane and use the H^+-gradient at the plasma membrane to provide energy to drive the absorption of Pi by plants [15,16]. In general, the absorption and transport of one Pi by *PHT1* family genes requires 2~4 protons to enter the plasma membrane together with the change in membrane potential [16,34].

The first plant phosphate transporter gene (*PHT1*) was cloned from *Arabidopsis* [35]. It is a high-affinity phosphate transporter gene located on the cytoplasmic membrane and has a structure and function similar to those of *PHO84* cloned from *Saccharomyces cerevisiae* [8,23,33,36]. With increasing attention given to phosphate transporter genes, many related studies have been conducted, and this type of phosphate transporter gene has been found in rice, maize, tomato, sorghum, soybean, millet, poplar, wheat, pepper, tobacco, potato and other plants [3,19,21,23,26,37–44]. Among them, 13 *PHT1* genes were identified in rice (*Oryza sativa*) and maize (*Zea mays*) [40,45,46], 8 *PHT1* genes in tomato (*Solanum lycopersicum*) [43], 11 *PHT1* genes in sorghum (*Sorghum bicolor*) [42], 14 *PHT1* genes in soybean (*Glycine max*) [44], 12 *PHT1* genes in millet (*Setaria italica*) [3,47], 14 *PHT1* genes in poplar (*Populus trichocarpa*) [23], 35 *PHT1* genes in wheat (*Triticum aestivum*) [21]and 49 *PHT1* genes in oilseed rape (*Brassica napus*) [48], and 8 *PHT1* genes in potato (*Solanum tuberosum*) [19,49]. Different phosphate transporter genes involve different functions. At present, the molecular mechanism of the *PHT1* genes in *Arabidopsis* and rice is relatively clear. *AtPHT1;1*, *AtPHT1;2 AtPHT1;3* and *AtPHT1;4* genes in *Arabidopsis*, are mainly involved in the uptake of Pi; *AtPHT1;5* gene is responsible for the mobilization of Pi from the source to sink organs; *AtPHT1;8* and *AtPHT1;9* genes are likely to act sequentially in the interior of the plant during the root-to-shoot translocation of Pi [8,22,25,50–53]. In rice, *OsPHT1; 4* is mainly expressed in roots and leaves, *OsPHT1; 8* is mainly expressed in roots and stems, among which *OsPHT1;1*, *OsPHT1;4* and *OsPHT1;8* all plays in rice Pi uptake and translocation [25,26,37]. At the same time, *OsPHT1; 8* also plays an important role in redistribution of Pi from source to sink organs [38].

The potato (*Solanum tuberosum* L.) is an annual herb of Solanaceae. It is the fourth largest food crop in the world after rice, wheat and maize. Because of its high yield, wide distribution and strong adaptability, it has become the main food crop in many countries and regions. Phosphorus plays a key role in potato root development, tuber formation, nutritional quality and resistance to some diseases [54,55]. The lack of available phosphorus in the soil will inhibit the growth and development of potatoes and reduce the yield and quality of potatoes. However, at present, there are few studies on the function of potato *PHT1* gene. Therefore, the study of potato *PHT1* gene is of great significance to explore the molecular response mechanism of Pi stress and improve the utilization efficiency of Pi in potato. In this study, potato hydroponic seedlings were used as experimental materials to clone potato phosphate transporter *PHTI* family member *StPHT1;1*/*1;6*/*1;9*/*1;10*/*1;12*/*1;13* and analyzed the expression pattern of potato *PHTI* family gene in different tissues and organs under Pi stress. The purpose of this study was to elucidate the

function of potato phosphate transporter, explore the molecular response mechanism of potato under Pi stress, screen efficient Pi germplasm resources and provide basis for molecular breeding.

2 Materials and Methods

2.1 Test Materials and Treatment Methods

The plant material selected in this study was potato (*Solanum tuberosum* L.), and the variety is Atlantic (DXY); Prepare 60 potato tissue culture seedlings with neat and consistent growth and put them in an illuminated culture room (temperature: 25°C light/22°C dark, light cycle: 12 h light/12 h dark, light intensity: 240 μmol·m^{-2}·s^{-1}, relative humidity: 75%~80%). Full nutrient solution (1/2 Hoagland nutrient solution) (Pi = 1 mM) and nutrient solution lacking Pi (Pi = 0 mM) were prepared (Table S1), and the tissue culture seedlings were transplanted into full-nutrient solution (Pi = 1 mM), and the nutrient solution was changed every 5 days. After 20 days, half of the materials were transferred to nutrient solution lacking Pi, and Pi treatment was initiated (Pi = 1 mM and Pi = 0 mM). The roots, stems and leaves of 0, 3, 6, 9, 12, 24, 48 and 72 h were frozen in liquid nitrogen and stored at −80°C for later use, with 3 biological replicates per treatment.

2.2 RNA Extraction and Reverse Transcription

After grinding the tissue sample in liquid nitrogen, extract the total RNA according to the Easypure® Plant RNA Kit and store it at −80°C for standby; Refer to the instructions of the reverse transcription kit of Beijing TransGen Biotech Co., Ltd. (China), and use 20 μL Reverse Transcription 2 μg total RNA, and reverse transcribed cDNA were detected and stored at −20°C for standby.

2.3 Gene Cloning and Entry Vector Construction

According to the screening results of the NCBI gene library, and referring to the CDS sequence of the *Arabidopsis PHT1* gene, the homologous CDS sequence of the corresponding potato was found from the potato genome database (http://solanaceae.plantbiology.msu.edu/integrated_searches.shtml) using the homologous conservative sequence, and the cloning primer design (Table S2) was performed using Primer Premier 5.0 software. According to the predicted sequence, there are 12 *PHT1* members in potato genome, which are named *StPHT1;1*, *StPHT1;2*, *StPHT1;3*, *StPHT1;4.5*, *StPHT1;6*, *StPHT1;7*, *StPHT1;8*, *StPHT1;9*, *StPHT1;10*, *StPHT1;11*, *StPHT1;12* and *StPHT1;13*, respectively. The 12 *PHT1* gene family members are obtained by PCR. The target gene is connected to the entry vector (pGWCm-T) that has been enzyme-digested (enzyme digestion site: *Bam*HI) by in-fusion method, which is named *pGWCPHT1;1*, *pGWCPHT1;6*, *pGWCPHT1;9*, *pGWPHT1;10*, *pGWCPHT1;12* and *pGWCPHT1;13*, respectively. The PCR reaction procedure is: pre-denatured at 95°C for 2 min; Denatured at 94°C for 30 s; Anneal at 55°C for 30 s, extend at 72°C for 2 min, and cycle for 25 times, extend 72°C for 5 min. The total system of In-fusion connection reaction is: The PCR amplification product (1.5 μL), the enzyme digestion vector pGWC-T (2.5 μL), and 5 × Ligation-Free cioning MasterMix (1 μL) were mixed to undergo ice bath for 1 h, and then transformed into *E. coli* DH5α. The samples were smears on LB solid screening plates containing 34 μM chloramphenicol, and cultured inverted at 37°C for 12 to 16 h. Single colonies were selected for PCR identification. See the appendix for the corresponding primers (Table S3).

2.4 Bioinformatics Analysis of Target Genes

First, the ORF Finder program (http://www.ncbi.nlm.nih.gov/gorf/gorf.htlm) of NCBI was used to find the ORF. The GenBank BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and ClustalW software (http://www.ebi.ac.uk/clustalw) were used for sequence homology analysis. In EXPASY, the isoelectronic points and molecular weight of the encoded protein were predicted by using the Protparem program (http://www.expasy.org/tools/pi_tool.html). The hydrophobicity of the encoded protein was predicted and analyzed with the ProtScale program (http://www.expasy.ch/tools/protscale). Using the online transmembrane-spanning

domains prediction software TMHMM 2.0 provided by ExPASY (http://www.cbs.dtu.dk/services/TMHMM/), the transmembrane structure of the encoded protein was analyzed. CFSSP online software was used to predict the secondary structure of protein (http://www.biogem.org/tool/chou-fasman/). Phyre 2 protein tertiary structure model construction software was used to predict protein tertiary structure (http://www.sbg.bio.ic.ac.uk/phyre2/phyre2_output/87fecb0d043f5d4d/summary.html).

2.5 Gene Expression Analysis

According to the obtained potato *PHT1* phosphate transporter gene (*StPHT1;1*, *StPHT1;6*, *StPHT1;9*, *StPHT1;10*, *StPHT1;12* and *StPHT1;13*) Specific primers were designed (Table S4), and the expression characteristics of genes in potato roots, stems and leaves at different times were detected by RT-qPCR. The RT-qPCR reaction system was based on the TransScript® All-in-One kit instructions, and the reaction was performed on QuantStudio 7 Flex qPCR instrument. The total reaction system was 20 μL, including 0.5 μL cDNA template, 10 μL EvaGreen 2XqPCR Mastermix, Forwared Primer (10 μM) and Reverse Primer (10 μM) 0.4 μL, respectively. 8.3 μL Nuclease-free H_2O. RT-qPCR procedure: pre-denaturation 94°C for 30 s, Denatured 94°C for 5 s, annealing 55°C for 35 s, 45 cycles. All test samples were repeated for 3 times. $2^{-\Delta\Delta Ct}$ was used to calculate the relative gene expression.

2.6 Data Analysis

RT-qPCR data analysis was performed using Excel 2013, LinRegPCR and SPSS 25 to statistically analyze the results and calculate the average expression amount and standard error of genes. GraphPad Prism 5, Adobe Illustrator CS6 and Origin 2021 software were used for drawing.

3 Results

3.1 Cloning and Vector Construction of PHT1 Gene

The *PHT1* genes were cloned (Fig. S1). The results showed that 10 members of the predicted 12 *PHT1* gene family could be cloned by designed primer PCR. They were *StPHT1;1*, *StPHT1;3*, *StPHT1;4*, *StPHT1;6*, *StPHT1;7*, *StPHT1;8*, *StPHT1;9*, *StPHT1;10*, *StPHT1;12*, and *StPHT1;13*. By comparison with the marker, we can roughly see that the sequence length of these 10 members is approximately 1500 to 2000 bp. The construction of the entry vector for the *PHT1* gene (Fig. S2) showed that the base fragment sizes of *pGWCPHT1;1*, *pGWCPHT1;6*, *pGWCPHT1;9*, *pGWCPHT1;10*, *pGWCPHT1;12* and *pGWCPHT1;13* were 1901, 1944, 1789, 1787, 1645 and 987 bp, respectively (Table S5).

3.2 Bioinformatics Analysis of the PHT1 Gene

The transmembrane-spanning domains of PHT1 protein were analyzed (Fig. 1). The results showed that the potato PHT1 protein was a transmembrane protein, where StPHT1;1 and StPHT1;6 have 12 transmembrane-spanning domains; StPHT1;9 has 10 transmembrane-spanning domains; StPHT1;10 has 11 transmembrane-spanning domains; and StPHT1;12 and StPHT1;13 have 9 and 6 transmembrane-spanning domains, respectively. The protein secondary structures of PHT1, such as α-helix β-sheet β-turns and random coils, were analyzed (Fig. 2, Table 1). The results showed that the protein secondary structure of potato PHT1 had certain differences among different members. Among the α-helix structures, the StPHT1;1 protein accounted for the greatest proportion, reaching 84.4%; the StPHT1;10 protein accounted for the smallest proportion, reaching 74.3%; the β-sheet in the StPHT1;6 protein accounted for the most, up to 72.3%; the StPHT1;13 protein accounted for the least, reaching 52.4 %; β-turn in the StPHT1;12 protein accounted for the most, reaching 9.7%; and the StPHT1;10 proportion was the smallest, at 8.4%. The protein tertiary structure of PHT1 was analyzed (Fig. 3, Table S6). The results showed that in the predicted protein 3D model, the number of amino acid residues of four PHT1 proteins, StPHT1;1, StPHT1;6, StPHT1;9 and StPHT1;10, is large in potato, and the protein tertiary structure is dense. Because the number of amino acid residues of StPHT1;12 and StPHT1;13 proteins is small, the protein tertiary structure of StPHT1;12 and StPHT1;13 proteins is relatively loose.

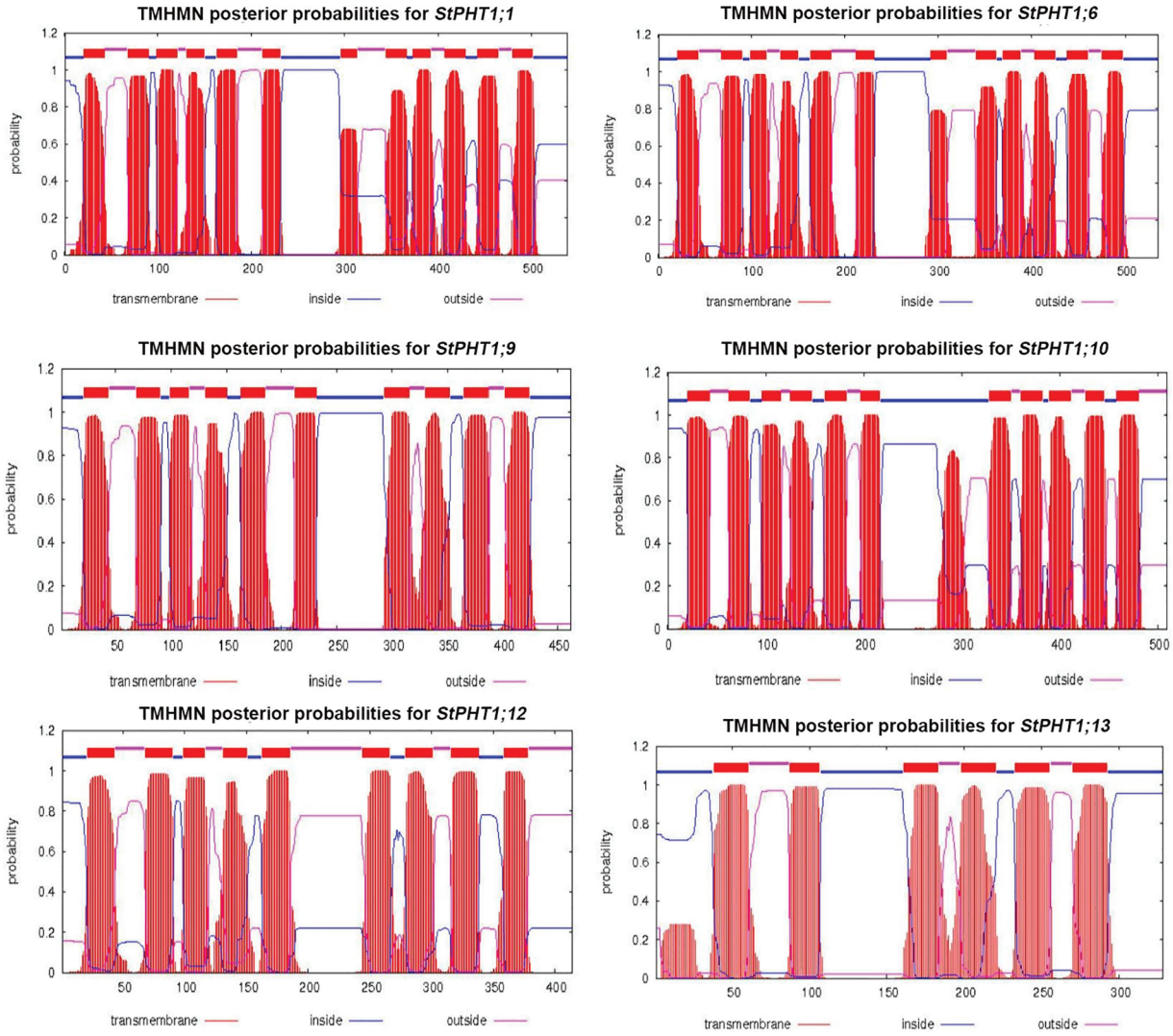

Figure 1: Transmembrane-spanning domain analysis of potato PHT1 protein

3.3 Expression Analysis of the PHT1 Gene in Potato under Short-Term Phosphate-Deficiency Stress

The expression of the *PHT1* gene in potato under short-term Pi deficiency stress was analyzed (Fig. 4). The results showed that under short-term Pi-deficiency stress, the expression of the *StPHT1;1*, *StPHT1;6*, *StPHT1;9*, *StPHT1;10*, and *StPHT1;12* genes in potato increased at 3~9 h, while the expression of the *StPHT1;13* gene increased at 48~72 h. Among them, the expression of the *StPHT1;1* gene reached a maximum in the leaves under Pi-deficiency stress for 3 h, which was 71 times more than that of the control group, 28 times and 31 times more than that of the control group in the stems under stress for 6 and 9 h, and 26 times more than that of the control group in the roots under stress for 6 h. The expression of the *StPHT1;6* gene reached the maximum value in the stems under Pi-deficiency stress for 9 h, which was 59 times that of the control group; the maximum value in the leaves under Pi-deficiency stress for 3 h, which was 48 times higher than that of the control group; and the maximum value in the roots under Pi-deficiency stress for 72 h, which was 25 times higher than that of the control group. In addition, there was also a high level of expression in stems under stress for 3 and 6 h, which was

40~45 times higher than that of the control group and 18 times higher than that of the control group in roots under stress for 6 and 9 h. The expression of the *StPHT1;9* gene in stems and leaves under Pi stress for 3 h reached a maximum, which was 39 and 20 times higher than that of the control group, respectively. The expression in roots under Pi stress for 24 h was the maximum, which was 25 times higher than that of the control group. The expression of the *StPHT1*;*10* gene reached a maximum value of 91 and 50 times higher than that of the control group in the stems and leaves under Pi stress for 6 h and more than 100 times that of the control group in the roots at 72 h. In addition, there was also high expression in the roots at 6 and 9 h, which was approximately 60 times higher than that of the control group. The expression of the *StPHT1;12* gene was the largest in stems and leaves under Pi stress for 3 h, approximately 80 times that of the control group, but there was little change in the expression of the *StPHT1;12* gene in roots. The expression of the *StPHT1;13* gene was higher in the leaves and stems under Pi stress for 3 and 72 h, but its peak appeared in the roots and leaves under stress for 48 h, which was about 60 times higher than that of the control group.

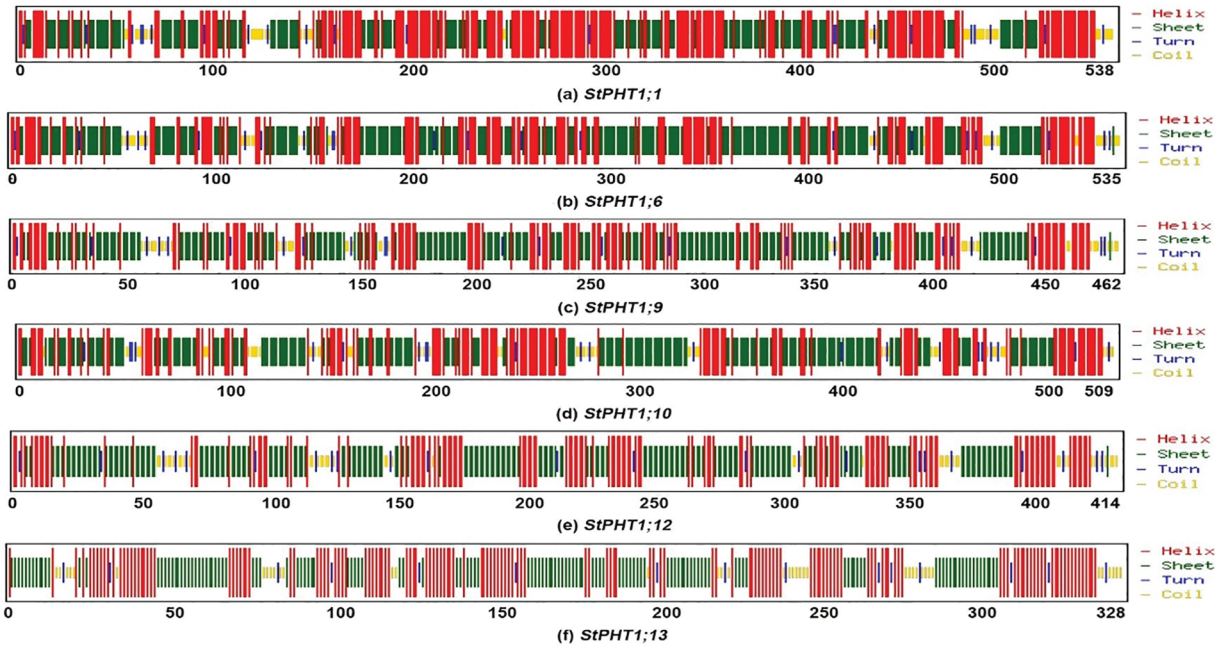

Figure 2: Potato PHT1 protein secondary structure prediction diagram

Table 1: Secondary structure analysis of PHT1 protein

	Helix residues	Helix percent (%)	Sheet residues	Sheet percent (%)	Turn residues	Turn percent (%)
StPHT1;1	454	84.4	286	53.2	47	8.7
StPHT1;6	417	77.9	387	72.3	46	8.6
StPHT1;9	356	77.1	319	69.0	42	9.1
StPHT1;10	378	74.3	316	62.1	43	8.4
StPHT1;12	315	76.1	272	65.7	40	9.7
StPHT1;13	263	80.2	172	52.4	29	8.8

Note: Helix: α-helix; Sheet: β-sheet; Turn: β-turn.

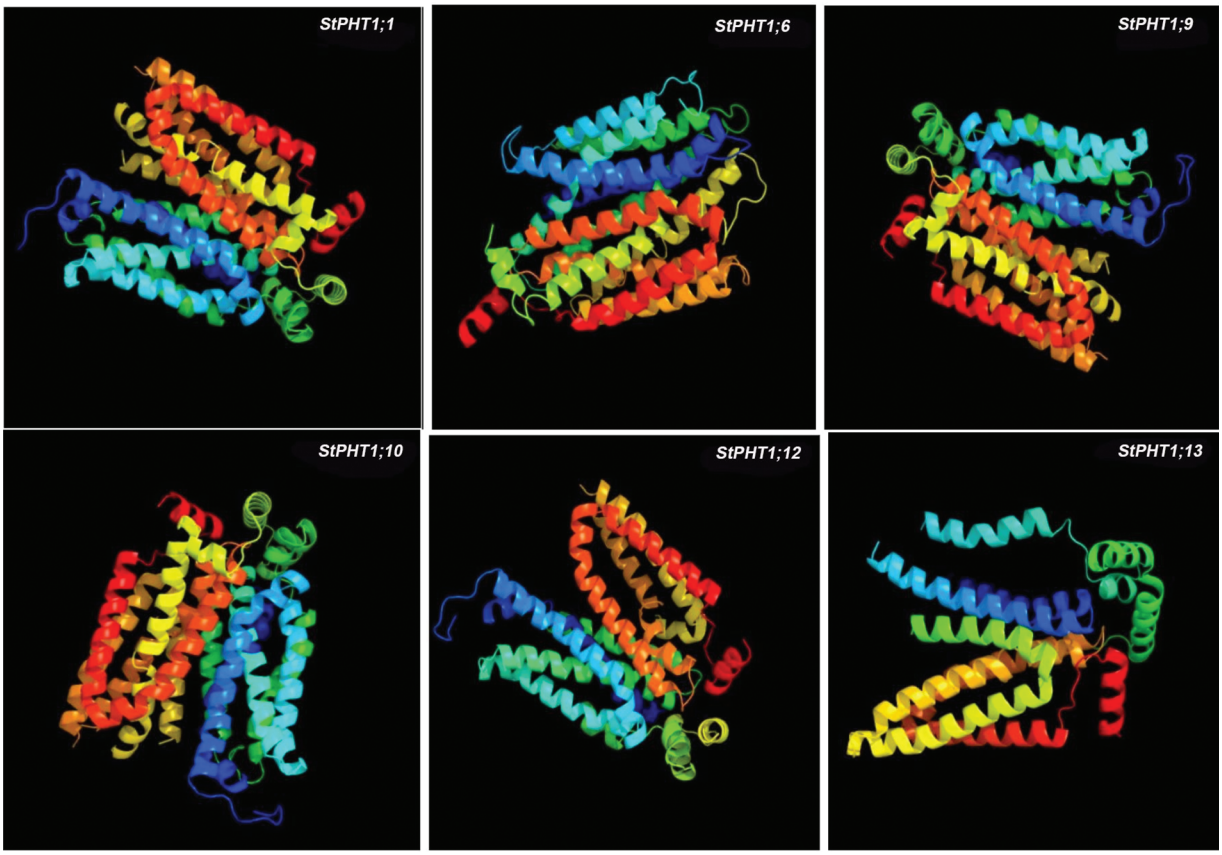

Figure 3: Prediction of the tertiary structure of the PHT1 protein

4 Discussion

Phosphorus plays an important role in plant growth, and the Pi concentration in soil solution is usually less than 2 μM. However, the concentration of Pi in plant tissue can be greater than 10 mM [10,12,50]. It is precisely because of the gradient difference in the concentration of Pi in soil and plant tissue that the phosphate transporter is indispensable. The *PHT1* subfamily is the most widely studied family in the current research on phosphate transporter family. However, the functions of many other members of potato *PHT1* family are still unclear. Therefore, the isolation and cloning of potato phosphate transporter genes is of great significance to explore the molecular response mechanism of potato under Pi stress. In this study, we cloned 6 members of potato *PHT1* gene family (*StPHT1;1*, *StPHT1;6*, *StPHT1;9*, *StPHT1;10*, *StPHT1;12*, *StPHT1;13*), Bioinformatics analysis results showed that potato PHT1 protein contain 9~11 transmembrane-spanning domains (Fig. 1), which is consistent with the results of previous studies which demonstrated that the 8 members of the potato *PHT1* gene contain 9~11 transmembrane-spanning domains [19,49]. As a phosphate transporter, the multi-transmembrane region means the improvement of transport efficiency. At the same time, the number of transmembrane-spanning domains of the potato *PHT1* gene is similar to that of maize [56], wheat [57], rice [45,58,59] and indicating that the protein is highly conserved in the number of transmembrane-spanning domains of different plants. In the analysis of protein secondary structure of PHT1 (Fig. 2, Table 1), Our results show that α-helix accounts for most of the amino acid residues of the protein, which is consistent with previous research results [49,60], which is key to the folding of the protein to form the domain. In the analysis of protein tertiary structure of PHT1 (Fig. 3), we can also clearly see that all proteins except StPHT1;13 have a

large number of helical structures. The coiled coil of proteins makes the tertiary structure of proteins compact and orderly. while StPHT1;13 protein looks looser than other proteins, which may be due to StPHT1;13 proteins have less secondary structure due to containing fewer amino acid residues, which results in less interaction between various forces. The resulting protein tertiary structure is relatively loose. Overall, the morphology of the PHT1 protein members is relatively similar, which reflects the conservation of the PHT1 protein structure.

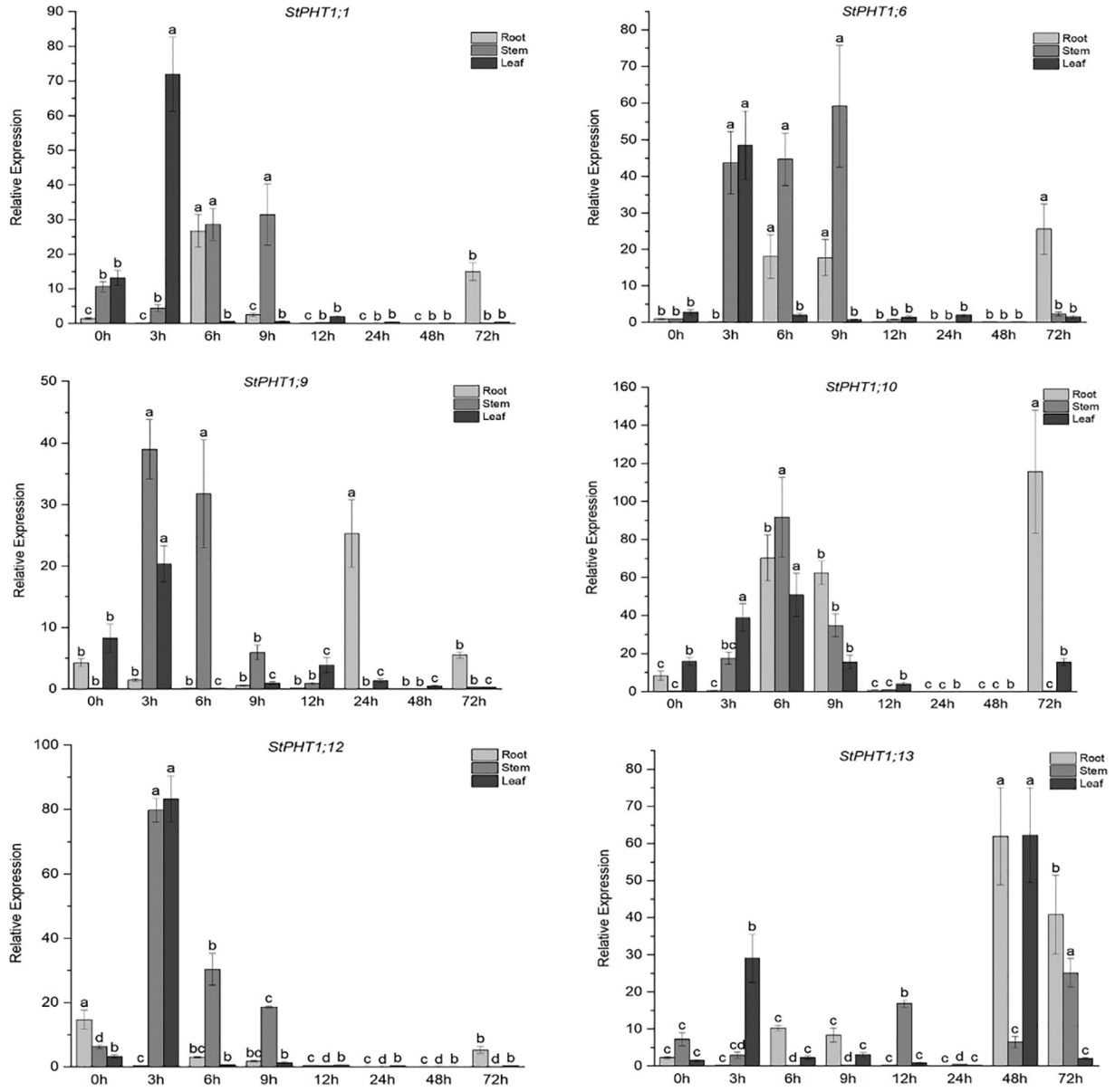

Figure 4: Transient expression of *PHT1* under a lack of phosphate

Most *PHT1* genes are transcribed through Pi starvation induction, and are regulated by Pi-starvation signals, and the expression level will be significantly up-regulated under Pi deficiency conditions [61–67]. In *Brassica napus*, Pi deficiency can significantly upregulate the expression of approximately 80% of *BnaPHT1* gene in roots [48]. In potato, Pi deficiency can also increase the expression of most *PHT1* genes in leaves and roots, including *StPHT1;4*, *StPHT1;7* and *StPHT1;8* genes were upregulated in potato roots, *StPHT1;1*, *StPHT1;4* and *StPHT1;5* genes were upregulated in potato leaves [19]. In *Arabidopsis*, *PHT1;9* gene was highly expressed in Pi deficiency roots [33]. In rice, the transcript level of *OsPHT1;4* increased significantly both in shoots and roots with a long time Pi deprivation, the expression of *OsPHT1;8* was significantly upregulated only in roots [25,26]. In millet, Pi deficiency will lead to *PHT1;2*, *PHT1;3*, *PHT1;4* and *PHT1;9* genes were overexpressed in roots [47]. In barley, *HvPHT1;1* and *HvPHT1;2* under the condition of Pi deficiency, expression was induced by up to 5-fold [65]. In this study, we found that the relative expression of potato phosphate transporter gene under short-term Pi stress changed with time, which may be because the concentration of Pi in the culture medium changed with the growth of the plant, thus affecting the gene expression. On the whole, *StPHT1;1*, *StPHT1;6*, *StPHT1;9*, *StPHT1;10*, *StPHT1;12* and *StPHT1;13* genes were up-regulated under the condition of short Pi deficiency, indicating that these *PHT1* genes were positive regulatory inducible genes in response to low Pi stress, with the characteristics of *PHT1* family gene expression, consistent with the results of *Arabidopsis* [22] and *Brassica napus* [48]. Secondly, under the condition of low Pi, the expression of *StPHT1;1* and *StPHT1;12* genes decreased in the underground part, but increased in the aboveground part, and the expression gradually decreased after reaching the maximum at 3 h. It is consistent with previous studies that the expression of *OsPT1* and *OsPT12* genes in rice *PHT1* family members decreased in the underground part and increased in the aboveground part of *OsPT1* under low Pi treatment [68].

PHT1 genes, as high-affinity phosphate transporters [21,28,69], are strongly expressed in roots, especially in root epidermal and outer cortical cells, and are related to the uptake of Pi, while the expression in other parts may also be related to the transfer of Pi [16]. In this study, we found that *PHT1* gene was detected in roots, stems and leaves from 0 to 72 h, but the time of maximum expression of different genes was different, and the expression of most genes increased to a great extent from 3 to 9 h, it has also been reported in the relevant literature that 6 and 9 h are key points for the expression of some genes in plants. This phenomenon may indicate that the expression of the *PHT1* gene was improved in a short time under Pi deficiency. Among them, the up-regulated expression of *StPHT1;9* and *StPHT1;13* genes in the roots were higher than that in the shoots after low Pi stress, which indicates that *StPHT1;9* and *StPHT1;13* was mainly expressed in the roots. It is speculated that these genes may participate in the uptake of Pi in potato, which is consistent with the result that *ZmPHT1;9* gene was mainly expressed in the root under low Pi stress found in previous studies in maize [56]; After low Pi stress, the up-regulated expression of *StPHT1;1* and *StPHT1;12* genes in the shoots were higher than that in the roots, indicating that *StPHT1;1* and *StPHT1;12* genes were mainly expressed in the shoots. It is speculated that these genes may participate in the transport of Pi in potato; While *StPHT1;6* and *StPHT1;10* genes were expressed in roots, stems and leaves, and the expression level was very high, which indicates that *StPHT1;6* and *StPHT1;10* genes not only play an important role in the process of Pi absorption from roots, but also may participate in the balance regulation of Pi transport between roots and shoots, and play a major role in the expression regulation under short-term Pi deficiency stress. It is consistent with the results that the expression of *OsPT2*, *OsPT6*, *OsPT9* and *OsPT10* genes in roots and shoots of *PHT1* genes induced by Pi deficiency increased exponentially in previous studies in rice [70], and these results also indicate that *PHT1* family genes may play a similar role in Pi uptake and transport of different crops. The situation that multiple genes cooperated to improve the expression level can show the interaction and synergistic expression of phosphate transporter genes. It is the synergy of phosphate transporters that can

ensure the distribution of Pi in specific tissues, cells and organelles and maintain the growth and development of plants even under stress conditions. Therefore, different phosphate transporters provide an opportunity for genetic engineering to improve Pi utilization to optimize the absorption and distribution of Pi in plants.

In addition, under short-term Pi deficiency stress, the change in gene expression in the stems and leaves of potato plants was the most obvious. This situation is caused by the fact that in the short-term Pi deficiency state, plants must first maintain their own homeostasis and regulate their own Pi transport in the body. Therefore, the gene expression level in the stems and leaves increased sharply. However, with the passage of time, at 72 h of Pi-deficiency stress, the expression level of the gene in the root was greatly improved, which indicates that over time, the plant itself begins to absorb Pi from the external environment to maintain the demand for Pi in the body, so *PHT1* gene expression in the plant root increases. However, the expression of the *PHT1* gene after more than 72 h and the effect of long-term Pi deficiency on the expression of the *PHT1* gene in potato need to be further verified by experiments.

Although people have some knowledge of potato *PHT1* gene, it is still in the preliminary stage. Its molecular regulation mechanism and the maintenance of Pi dynamic balance need further research, in order to provide theoretical and technical reference for potato production and research.

5 Conclusion

Six *PHT1* genes were isolated and identified in the potato genome, all of which were induced by low Pi stress. Among them, *StPHT1;6*, *StPHT1;9*, *StPHT1;10* and *StPHT1;13* genes were strongly expressed in the roots under low Pi conditions, *StPHT1;6*, *StPHT1;9*, *StPHT1;10* and *StPHT1;12* genes were strongly expressed in the stems under low Pi conditions, *StPHT1;1*, *StPHT1;6*, *StPHT1;10*, *StPHT1;12* and *StPHT1;13* genes were strongly expressed in the leaves under low Pi conditions, and *StPHT1;6* and *StPHT1;10* genes might be potential candidate genes for producing transgenic potatoes with improved Pi efficiency. All these results indicate that *PHT1* genes play an important role in the uptake and transport of plant Pi, and phosphate transporters may play a role in complex combinations in the uptake and transport of Pi. The identification of *PHT1* gene is helpful to improve the Pi efficiency of potato and obtain Pi efficient germplasm resources.

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Supplementary Materials

Table S1: Formula of full nutrient solution (1/2 Hoagland)

Classification	Chemical compound	Molecular weight	Concentration (mmol/L)	Concentrate 1000 × Dosage (g/L)
Liquid A	KNO_3	101.10	2.50	252.76
	$Ca(NO_3)_2{\cdot}4H_2O$	236.15	2.50	590.38
	Fe-EDTA(Na)	367.10	0.082	30.00
	$MnCl_2{\cdot}4H_2O$	197.91	4.57×10^{-3}	0.905
Liquid B	K_2SO_4	174.26	0.25	43.565
	$MgSO_4{\cdot}7H_2O$	246.47	1.00	246.47
	$ZnSO_4{\cdot}7H_2O$	287.56	0.38×10^{-3}	0.1099
	$CuSO_4{\cdot}5H_2O$	249.68	1.57×10^{-3}	0.0393
	$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$	1235.86	0.09×10^{-3}	0.01
	H_3BO_3	61.83	23.13×10^{-3}	1.43
Liquid C	KH_2PO_4	136.09	1.00 (+P)	136.09

Table S2: Primers for *PHT1* gene cloning

Primer name	Sequence (5′→3′)
*StPHT1;1*F	agcaggctttgactttATGGCGAACGATTTGCAAGTGCTA
*StPHT1;1*R	tgggtctagagactttccAACAGGAACTGTCCTTCCACTTGT
*StPHT1;6*F	agcaggctttgactttATGGGATTTTTTACTGATGCCTAC
*StPHT1;6*R	tgggtctagagactttccTACAGGAACGGTCCTGTTATCGGA
*StPHT1;9*F	agcaggctttgactttATGGCTGGGGATATGAAGGTTTTG
*StPHT1;9*R	tgggtctagagactttccTACAGGAACGGTCCTGTTATCGGA
*StPHT1;10*F	agcaggctttgactttATGGCACTCAAAGTCCTTACTGAC
*StPHT1;10*R	tgggtctagagactttccAGCCCTACCACCAACACTTTGATG
*StPHT1;12*F	agcaggctttgactttATGGCTGGGAATATGAAGGTTTTG
*StPHT1;12*R	tgggtctagagactttccCACAGGAACTGTCCTATTATCGGA
*StPHT1;13*F	agcaggctttgactttATGGCCACCATCTGCTTCTTTCGC
*StPHT1;13*R	tgggtctagagactttccAACAGGAACTGTCCTGTTATGATT

Table S3: Primers for vector validation

Primer name	Sequence (5′→3′)
M13F	GTTTTCCCAGTCACGACGTTG
M13R	CAGGAAACAGCTATGACCATG

Table S4: Primers for RT-qPCR

Primer name	Sequence (5′→3′)
*StPHT1;1*F	GCAGTTTGAGTTAATTTGTG
*StPHT1;1*R	GCAGAACTAAGGTAATAATGT
*StPHT1;6*F	GCAAAGGTTCTATCTAGTAGCA
*StPHT1;6*R	GCAATTACACAGAATCACAGA
*StPHT1;9*F	GCAAAGGTTCTATCTAGTAGCA
*StPHT1;9*R	GCAATTACACAGAATCACAGA
*StPHT1;10*F	GCAAGAACACAATACTAC
*StPHT1;10*R	ATGATTGGAGGAATACAA
*StPHT1;12*F	GCGTAGTCGGCAATTATTC
*StPHT1;12*R	GCAGAGGATGTTGATATTGAAT
*StPHT1;13*F	GTGTAGAGATACTAAGTTTCATCC
*StPHT1;13*R	GCTCCCAAAAGTAGTTCAAT
18s rRNAF	GGGCATTCGTATTTCATAGTCAGAG
18s rRNAR	CGGTTCTTGATTAATGAAAACATCCT

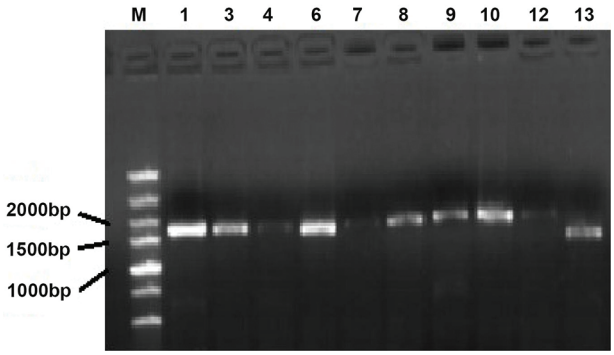

Figure S1: PCR electrophoresis of potato *PHT1* gene family members. M: DNA Marker; electrophoresis bands from 1 to 13 followed by *StPHT1;1*, *StPHT1;3*, *StPHT1;4*, *StPHT1;6*, *StPHT1;7*, *StPHT1;8*, *StPHT1;9*, *StPHT1;10*, *StPHT1;12*, *StPHT1;13*

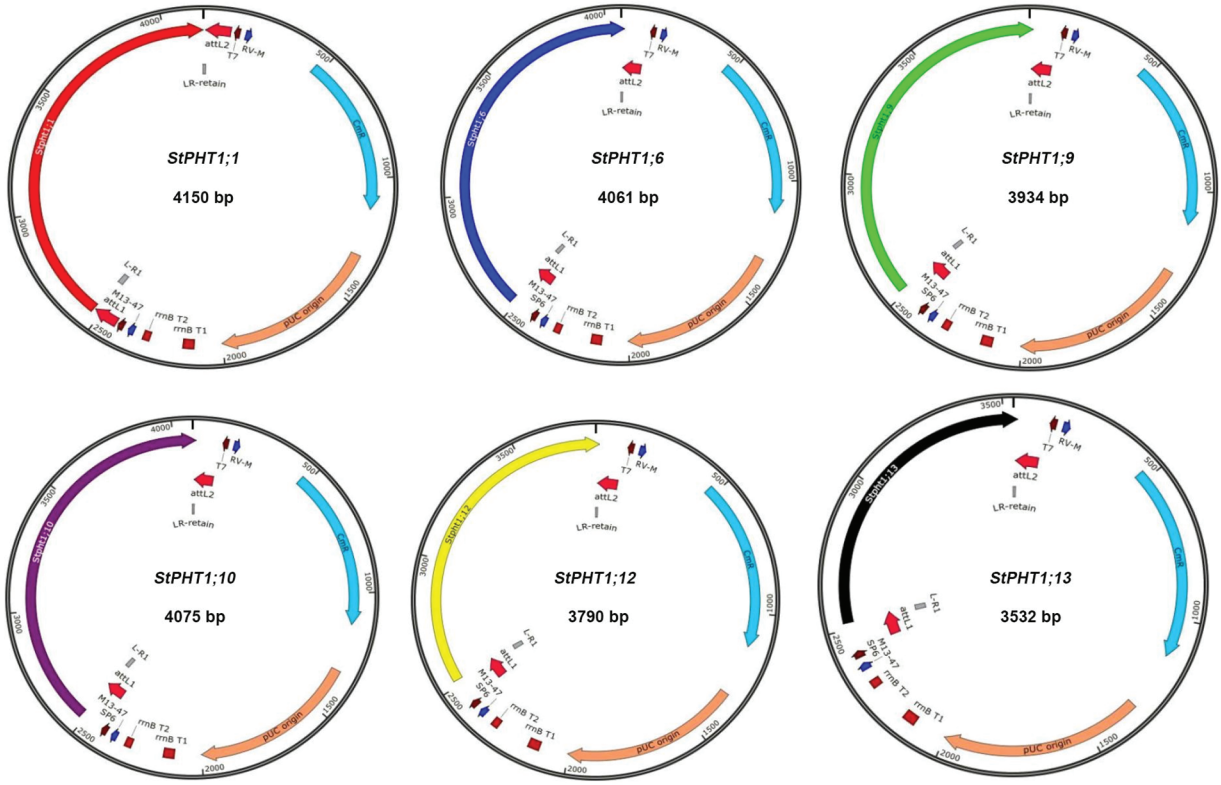

Figure S2: Schematic diagram of the entry vector construction

Table S5: The cloned sequence (CDS) of *PHT1* gene in potato is as follows

Genes	The cloned sequence (CDS) of *PHT1* gene
>*StPHT1;1* (GenBank accession number, OQ079612)	GTTGAATTCTTATAATTTACACCCTCTTGTTGTGTTTTTGCAGG AAGTTTAGTGATGGCGAACGATTTGCAAGTGCTAAATGCACTA GATGTGGCAAAGACACAACTGTATCACTTCACAGCAATTGTGA TTGCTGGGATGGGGTTTTTTACTGATGCTTATGACCTTTTCTGC ATTTCTATGGTCACTAAATTGCTTGGTCGTATTTACTACCATCAT GACAATGCATTGAAACCTGGCTCTCTGCCCCCTAATGTTTCAG CAGCTGTTAATGGAGTCGCCTTCTGTGGCACCCTTGCTGGACA GTTGTTCTTCGGGTGGCTTGGAGATAAAATGGGAAGGAAGAA AGTCTATGGAATGACCCTTATGATTATGGTCATTTGTTCAATTGC CTCGGGGCTTTCATTTGGTCATACACCAAAAAGTGTTATGACTA CGCTTTGTTTCTTCAGATTCTGGCTAGGATTTGGCATTGGTGGT GATTATCCCCTTTCTGCCACCATCATGTCTGAGTACGCTAACAA AAAGACTCGTGGAGCGTTCATTGCTGCTGTGTTTGCTATGCAA GGTTTCGGAATTCTGGCTGGTGGAATGGTGGCAATCATTGTTTC TTCAGCATTCAAGGGAGCATTCCCTGCACCAGCATATGAGGTTG ATGCTCTTGCTTCAACAGTTTCTCAGGCTGATTTCGTGTGGCGT ATAATTCTCATGTTCGGTGCAATCCCTGCTGGACTTACTTATTAC TGGCGTATGAAGATGCCTGAAACTGCTCGTTACACTGCCTTGGT CGCCAAGAACTTGAAACAGGCAGCTAACGACATGTCCAAGGTG TTGCAAGTCGAAATTGAAGCAGAGCCAGAGAAAGTTGCAGCTA TTTCTGTAGCAAATGGAGCCAATGAATTTGGTTTGTTCAGTAAG GAGTTCCTCCGTCGCCATGGACTTCACTTGCTTGGAACTGCTAG CACGTGGTTCTTGTTGGACATTGCTTTCTACAGTCAAAACCTTT TCCAGAAGGACATTTTCAGTGCAATTGGATGGATCCCACCAGCA CAAACCATGAACGCGTTGGAAGAAGTTTACAAGATTGCAAGGGC ACAAACTCTTATTGCTCTTTGTAGTACTGTTCCTGGTTACTGGTTC ACAGTTGCATTCATTGATAGGATTGGTCGATTCGCAATTCAGTTGA TGGGATTCTTCTTCATGACAGTCTTCATGTTTGCCTTAGCCCTTCC ATACCATCACTGGACTCTCAAGGATAACAGAATCGGCTTCGTGGT CATGTACTCACTCACCTTTTTCTTCGCCAACTTTGGTCCAAACGC CACAACATTCGTCGTCCCTGCTGAGATTTTCCCAGCCAGGCTTAG GTCCACATGCCATGGAATATCAGCAGCAGCAGGAAAAGCAGGAG CTATGGTTGGTGCATTCGGATTCTTGTACGCTGCTCAGCCCACAG ATCCAAAAAAAGACTGATGCTGGTTACCCTGCTGGCATTGGTGTGA GGAACTCGTTGATCGTCCTTGGTTGCGTTAACTTCCTTGGTATGT TGTTCACATTCTTGGTTCCAGAATCGAAAGGGAAGTCATTGGAA GAAATGTCGAGGGAAAACGAAGGGGAAGAGGAAACTGTAGCT GAAATGAGAGCAACAAGTGGAAGGACAGTTCCTGTTTAAGTTT TAGACAAGTTATCAATTAGTATATACTATGATGCAGTTTGAGTTA ATTTGTGGTATTTGGGATTAGAAAGAGATGTTTGTTTTGTTTGTT ATAAGATGGAATAAGCTCTTACCTTTTTTTTATATGTTTGTTTCTG ATGGTAATTAAACATTATTACCTTAGTTCTGCAAATCTCAGAAAT TCTGAGATTATATAAAGTAACCAAAGGAGGTTCTTTGGTTGTCCA

(Continued)

Table S5 (continued)

Genes	The cloned sequence (CDS) of *PHT1* gene
>*StPHT1;6* (GenBank accession number, OQ079613)	GTGGTTCATTTTTTGATTGTCGTCTAATTTTTTTTGTTTGTTATAATA AGTGTTTGCTGTTTTGAATTGATAATGCTGATTGAAAATTGGATTAG CTTACATGTTGAATATTGTTTTGATTTGCAGATAGAAGAAAATGGCT GGGGATATGAAGGTTTTGAATGCACTTGATTCAGCAAAAACACAAT GGTATCATTTTACAGCAATCATAATAGCTGGTATGGGATTTTTTACTG ATGCCTACGATCTCTTCTGCATATCTCTAGTCACTAAGTTGCTTGGAC GTATTTACTACCATGTCGATGGCTCTTCAAAGCCTGGTTCACTACCT CCCAATGTCTCCGCTGCTGTTAATGGTGTCGCCTTTTGTGGTACCCT TGCGGGACAACTCTTCTTTGGATGGCTTGGTGACAAAATGGGACGA AAGAAAGTCTATGGCATGACCCTTATGCTCATGTGTCTCTGTTCCA TTGCTTCTGGCCTTTCTTTCAGTAGGGAGCCTAAGACCGTCATGGC CACCCTTTGCTTCTTTCGCTTTTGGCTTGGTTTTGGAATTGGTGGC GATTACCCCCTTTCTGCTACTATTATGTCTGAATATTCCAACAAAAA GACTAGGGGTGCCTTCATTGCTGCTGTTTTCGCTATGCAAGGATTT GGTATCCTCGGAGGTGGTATCTTTGCCATCATTATCTCTGCTGTATT CCAGGCTTGTTTCAAGGCGCCAGCCTATCAAGTGGATCCCCTTGG TTCAACCGTTCCTCAAGCTGATTACGTGTGGAGGATCATATTGATG GCTGGGTCACTTCCTGCTCTTCTCTCTTACTACTGGAGGATGAAGA TGCCTGAAACGGCTCGTTACACTGCTCTTGTAGCCAAGAATGTGA AGCAGGCCACTGCAGATATGGTAAAGGTTATGCAGGTTAACATTGG GACAGAGCAAAAAGAGCCTGTTGTAAAGTCCGGCAAAGAATTTGG TTTGTTCACGAAAAAATTTCTTAATCGACATGGACTCCACTTGCTT GGCACAACCAGTACGTGGTTTCTTCTTGACATTGCATACTACAGCC AAAACTTGTTCCAAAAGGACATCTTCAGTGCTATTGGATGGATTCC AGCTGCCAAGACTATGAATGCAATTGAAGAGGTCCAAAAAATTGC ACGAGCCCAAACCCTTATCGCTCTCTGCAGTACAGTGCCTGGCTAC TGGTTCACAGTGTTCCTCATTGACAGGATTGGCAGGTTTACCATTC AAGTGATTGGTTTCACAATGATGACAGTGTTCATGTTTGCTCTGGC CATTCCTTACCACCACTGGACCCTCCCTGGACACCATATTGGGTTT GTGGTCCTCTATTCACTCACCTTCTTCTTTGCCAACTTTGGACCCA ACGCCACTACATTTGTCGTGCCTGCTGAGATTTTCCCTGCTAGATT GCGGTCAACTTGCCATGGAATATCAGCTGCATGTGGAAAGCTTGG GGCAATGGTTGGTGCATTTGGATTCCTGTATTTGGCTCAACCACAA GACAAGAGCAAGGCTGATGCAGGGTACCCTGCTGGAATTGGGGTT CGGAATTCACTCATTGTCCTTGGCGTAGTCAACCTTCTTGGATTATT TTTCACTTTCTTGGTTCCAGAATCCAAGGGGAAGTCACTGGAGGA GATGTCAAGGGAAAACGAAAACTCGGAGGAAGGAACGGAAGTG GAGAATCATTCATCCGATAACAGGACCGTTCCTGTATAAAAGCAG TTATACTAGTTTCATTTCTTTCCATTTTATCCTTCATGTGTTCTGCA AAGGTTCTATCTAGTAGCACTTTAAAGTTCAAGCTATTAGTGTAGA CATTCTATGTATCTGTGATTCTGTGTAATTGCAGCAACCATATATTG TATGTTTTGTGTCATATATATATTGGATGCAGTATGAAAAAACTCTT CTCTTAT

(Continued)

Table S5 (continued)

Genes	The cloned sequence (CDS) of *PHT1* gene
>*StPHT1;9* (GenBank accession number, OQ079614)	GTCACATACTATTGAATTTCTTATTAATATATATGCATGATCTTGCGT TTTATCGTTTTGTTTTGTGGTTCATTTTTTGATTGTCGTCTAATTTTT TTTGTTTGTTATAATAAGTGTTTGCTGTTTTGAATTGATAATGCTGA TTGAAAATTGGATTAGCTTACATGTTGAATATTGTTTTGATTTGCAG ATAGAAGAAAATGGCTGGGGATATGAAGGTTTTGAATGCACTTGAT TCAGCAAAAACACAATGGTATCATTTTACAGCAATCATAATAGCTGG TATGGGATTTTTTACTGATGCCTACGATCTCTTCTGCATATCTCTAGT CACTAAGTTGCTTGGACGTATTTACTACCATGTCGATGGCTCTTCAA AGCCTGGTTCACTACCTCCCAATGTCTCCGCTGCTGTTAATGGTGT CGCCTTTTGTGGTACCCTTGCGGGACAACTCTTCTTTGGATGGCTT GGTGACAAAATGGGACGAAAGAAAGTCTATGGCATGACCCTTATG CTCATGTGTCTCTGTTCCATTGCTTCTGGCCTTTCTTTCAGTAGGGA GCCTAAGACCGTCATGGCCACCCTTTGCTTCTTTCGCTTTTGGCTTG GTTTTGGAATTGGTGGCGATTACCCCCTTTCTGCTACTATTATGTCTG AATATTCCAACAAAAAGACTAGGGGTGCCTTCATTGCTGCTGTTTTC GCTATGCAAGGATTTGGTATCCTCGGAGGTGGTATCTTTGCCATCATT ATCTCTGCTGTATTCCAGGCTTGTTTCAAGGCGCCAGCCTATCAAGT GGATCCCCTTGGTTCAACCGTTCCTCAAGCTGATTACGTGTGGAGG ATCATATTGATGGCTGGGTCACTTCCTGCTCTTCTCTCTTACTACTGG AGGATGAAGATGCCTGAAACGGCTCGTTACACTGCTCTTGTAGCCA AGAATGTGAAGCAGGCCACTGCAGATATGGTAAAGGTTATGCAGGT TAACATTGGGACAGAGCAAAAAGAGCCTGTTGTAAAGTCCGGCAA AGAATTTGTGCCTGGCTACTGGTTCACAGTGTTCCTCATTGACAGG ATTGGCAGGTTTACCATTCAAGTGATTGGTTTCACAATGATGACAG TGTTCATGTTTGCTCTGGCCATTCCTTACCACCACTGGACCCTCCC TGGACACCATATTGGGTTTGTGGTCCTCTATTCACTCACCTTCTTCT TTGCCAACTTTGGACCCAACGCCACTACATTTGTCGTGCCTGCTGA GATTTTCCCTGCTAGATTGCGGTCAACTTGCCATGGAATATCAGCTG CATGTGGAAAGCTTGGGGCAATGGTTGGTGCATTTGGATTCCTGTA TTTGGCTCAACCACAAGACAAGAGCAAGGCTGATGCAGGGTACCC TGCTGGAATTGGGGTTCGGAATTCACTCATTGTCCTTGGCGTAGTC AACCTTCTTGGATTATTTTTCACTTTCTTGGTTCCAGAATCCAAGGG GAAGTCACTGGAGGAGATGTCAAGGGAAAACGAAAACTCGGAGG AAGGAACGGAAGTGGAGAATCATTCATCCGATAACAGGACCGTTC CTGTATAAAAGCAGTTATACTAGTTTCATTTCTTTCCATTTTATCCTT CATGTGTTCTGCAAAGGTTCTATCTAGTAGCACTTTAAAGTTCAAG CTATTAGTGTAGACATTCTATGTATCTGTGATTCTGTGTAATTGCAGC AACCATATATTGTATGTTTTGTGTCATATATATATTGGATGCAGTATGA AAAAACTCTTCTCTTAT

(Continued)

Table S5 (continued)

Genes	The cloned sequence (CDS) of *PHT1* gene
>*StPHT1;10* (GenBank accession number, OQ079615)	CACGAAATTGCCTTCAAAAATCCATCAATATCCAACTCAAAAC AAACAAATCATTCTGTGTATATTCATTCCTCTTAAACCAAGAAA AAAATGGCACTCAAAGTCCTTACTGACCTTGACTCCGCAAGAA CACAATACTACCACTTTAAGGCTATTATCATAGCCGGCATGGGCT TATTCACCGATGCGTACGACCTCTTTTGTATTCCTCCAATCATGA AACTCATCGGTCGAATCTACTACGCGGACTCTAACAACCTTTAC GAGGTCCCTAGAGCTGTCACGTCTGCTATGGTCGTGACAGCCC TTCTAGGGACCGTTATAGGTCAGTTAGTTTTCGGCCGATTAGGT GACCTAATCGGCCGACGCAAAGTCTACGGCTTTGCTCTAATGAT AATGGTGTTGAGCTCATTTGGATGTGGACACTCAATATGTACGT CTAGGACTTGTGTTTTACTAAGTCTTGGATTTTTTAGGTTTTTAT TGGGAATTGGGATTGGTGGAGATTACCCTTTATCAGCCACAATT ATGTCTGAATTTGCTAATAGAAAAACTAGGGGTGCTTTTATTGC TGGGGTTTTTTCAATGCAAGGATTTGGTATACTTGCTAGTTCAA CTGTCACAATGATTGTTTGTTCAATTTTTAATCGTGCTAATGGAG GAAGTCATGGTGCTGATTTGGCTTGGAGATTAATACTTATGATTG GTGCTATTCCTGCTGGACTCACTTATTATTGGCGCATGATGATGC CCGAGACCGCCAGGTATACGGCTTTAGTCGAAAGAGATGTGCC ACAAGCAGCCAGGGACATGGAAAAAGTACTTGATATTTCAGCA AGTCAAATCGTTGAGGAATTTTCTACATACTTACCAAATTCACC GAGCTCTAATTATTCACTCTTCTCCAAAACTTTCATTCATAACCA TGGCATTGATCTTTTTGCTTGTTCAATTTCATGGTTCCTAGTTGA TATTGTTTTCTATAGCAGCAATCTGTTTCAATCCCAAATATACAA AAGATATTTAAGTAATAACCACAATGTCAATGCATTTCAAGAAG CTTTTGAAGTTGCAAGAATTCAGGCTATTATTGCAATTTGTTCAA CAATTCCTGGCTATTTTGCAACAATGTACTTCATTGACCGAATTG GGAGAGTCAAAATTCAAATAATGGGATTTTTCTTCATGGCAATTA GCTTATTGGCAATTGGAATTCCATATTACTCCTATTGGAATAATAA CACCAATATAGGGTTCATGTTTTTGTATGGACTAACATTCTTCTTC TCCAATTTCGGTCCGAACACGACTACCTTCATCGTGCCCGCGGA GCTTTTCCCTGCTCGTTTCAGAACAACATGTCATGGAATTTCTG GAGCTGTAGGAAAATTGGGTGCGATAATCGGATCGATAGGATTT TTATGGGCTTCTCAGAATAAAAAAGATGGATATAATGAAGGAAT TGGAATGACAGCCTCTTTAATATTACTAGCTGGAGTTTGTGTAG TGGGAATGATCACTACATATTTTTTCACTAGTGAGACTATGGGA AAATCACTTGAGGAAAATGAAAATATTGTTATCAATGAAGATCA TCAAAGTGTTGGTGGTAGGGCTTAATGATTATTAATTTTTCCAAA TCTTGTGTATGTTAAAGTGAAATTATATTTTTCAACTTTTCTTGTA TGAGTTTTCATATTTGTACATATTAGTGAGTTGACTTAATTAGGTA AGTGTACCTCATGACCATATATGTGGTAGAGAATGAAGTTGATTT TGATACCAAT

(Continued)

Table S5 (continued)

Genes	The cloned sequence (CDS) of *PHT1* gene
>*StPHT1;12* (GenBank accession number, OQ079617)	ATTTTAAATTGTAAATTAATATATTTATGGTAGTTAGGTTAAAA TCCTATTTGGACAGAGAAAAGGAAAGCCAAGCGTAGTCGGC AATTATTCTAAACCAATTCGGACAGGGAAATCAAATCTAAGC TTATAAAAGGAGTTGATATTGTCAATATTCAATATCAACATCC TCTGCATCTTTATTTTTAACAGTTGTGAAAATGGCTGGGAAT ATGAAGGTTTTGAATGCGCTTGATTCCGCAAAAACACAATG GTATCACTTCACAGCAATCATAATAACTGGTATGGGATTTTTT ACTGATGCCTACGATCTCTTCTGCATATCTCTAGTCACTAAGT TGCTTGGACGTATTTACTACCATGTCGATGGCTCTTCAAAGCC TGGTTCACTACCTCCTAATGTTTCCGCTGCTATCAATGGTGTC GCCTTCTGTGGTACCATTGCGGGACAACTCTTCTTTGGATGG ATTGGTGACAAAATAGGAAGGAAGAAAGTCTATGGCATAAC CCTTATGATTATGTCTATCTGTTCCATTGCTTCTGGCCTTTCTT TTGGTAGGGATCCTAAGACCGTCATGGCCACCCTCTGCTTCT TTCGCTTCTGGCTTGGTTTTGGTTTTGGTGGCGATTACCCCCT TTCTGCTACTATTATGTCTGAATATGCCAACAAAAAGACTAGG GGTGCCTTCATTGCTGCTGTTTTCGCTATGCAAGGATTTGGTA TCCTCGGAGGTGGTATCTTTGCCATCATTATCTCTGCTGTATTC CAGGCTTGTTTCAAGGCGCCAGCCTATCAAGTGGATCCCCTTG GTTCAACCGTTCCTCAAGCCACTGCAGATATGGAAAACGTTAT GCAGGTTGACATTGGGACAGACCAAAAAGAGCCTGCTGTAAA GTCCGGCAATGAATTTGTATTCCTCATTGACAGGATTGGCAGG TTTACCATTCAATTGATTGGTTTCACAATGATGACAGTGTTCAT GTTTGCTCTAGCCATTCCTTACCACCACTGGACTCTCCCTGGC CACCATATCGGGTTTGTGGTCCTCTATTCACTGACCTTCTTTTT TGCCAACTTTGGACCCAACGCCACTACATTTGTCGTGCCTGCT GAGATTTTCCCTGCTAGATTGCGGTCGACTTGCCATGGAATATC AGCTGCATGTGGAAAGTTAGGGGCAATGGTTGGTGCATTTGGA TTCCTGTATTTGGCTCAACCACAAGACAAGACCAAGGCTGATG CAGGGTATCCTGCTGGAATTGGGGTGAGGAATTCACTCATTGT CCTTGGCGTAGTCAACCTTCTCGGATTATTTTTCACTTTCTTGG TTCCAGAATCGAAGGGGAAGTCACTTGAAGAGATGTCAAGGG AAAACGAAGACTCAACTGAGGAAGGAGCAGAAGTGGAGAAT CATTCATCCGATAATAGGACAGTTCCTGTGTAAAAGCAGTTACT AGTTTCATCTCTTTCCATTCTATCCTTCATATGTTCTGCAAAGGT TCTATGAAGCTATTAGTGTAGACATTCTGTTTAATTGCAGCAACT AGAGTTCCATATGTTTTATGTTTTGTGTCATATATTGGATGCAGTA TGTAAAAACTCTTCTCTTATTAACCTTTCTTCATTACTCCCTTAT CTATAATA

(Continued)

Table S5 (continued)

Genes	The cloned sequence (CDS) of *PHT1* gene
>*StPHT1;13* (GenBank accession number, OQ079618)	ATGGCCACCATCTGCTTCTTTCGCTTCTGGCTTGGTTTTGGTTTT GGTGGCGATTACCCCCTTTCTGCTACTATTATGTCTGAATATGCC AACAAAAAGACTAGGGGTGCCTTCATTGCTGCTGTTTTCGCTAT GCAAGGATTTGGTATCCTCGGAGGTGGTATCTTTGCCATCATTAT CTCTGCTGTATTCCAGGCTTGTTTCAAGGCGCCAGCCTATCAAG TGGATCCCCTTGGTTCAACCATTCCTCAAGCAGATTATGCGTGG AGGATCATATTGATGGCTGGGTCACTTCCTGCTCTTCTCACTTAC TACTGGAGGATGAAGATGCCTGAAACGGCTCGTTACACTGCTCT TGTAGCCAAGAATGTGAAGCAGGCCACTGCAGATATGGAAAAC GTTATGCAGGTTGACATTGGGACAGACCAAAAAGAGCCTGCTG TAAAGTCCGGCAATGAATTTGTGTTCCTCATTGACAGGATTGGC AGGTTTACCATTCAAGTTATTGGTTTCACAATGATGACAGTGTT CATGTTTGCTCTGGCCATTCCTTACCACCACTGGACTCTCCCTG GCCACCATATCGGGTTTGTGGTCCTCTATTCACTAACCTTCTTCT TTGCCAACTTTGGACCCAACGCCACTACATTTGTCGTGCCTGCT GAGATTTTCCCTGCTAGATTGCGGTCAACTTGCCATGGAATATC AGCTGCATGTGGAAAGTTAGGAGCAATGGTGGGTGCATTTGGA TTCCTGTATTTGGCTCAACCACAAGACAAGAGCAAGGCTGATG CAGGGTACCCTGCTGGAATTGGGGTTCGGAATTCACTCATTGTC CTTGGCGTAGTCAACCTTCTCGGATTATTTTTCACTTTCTTGGTT CCAGAATCCAAGGGGAAGTCACTGGAGGAGATGTCAAGGGAC GGCGAAGACTCGGCAGAGGATGGAGCAGAAGTGGAGAATCAT AACAGGACAGTTCCTGTTTAA

Table S6: The ORF amino acid sequence of potato *PHT1* gene is as follows

Genes	The ORF amino acid sequence of potato *PHT1* gene
>*StPHT1;1*	MANDLQVLNALDVAKTQLYHFTAIVIAGMGFFTDAYDLFCISMVTKLLGR IYYHHDNALKPGSLPPNVSAAVNGVAFCGTLAGQLFFGWLGDKMGRKKV YGMTLMIMVICSIASGLSFGHTPKSVMTTLCFFRFWLGFGIGGDYPLSATIM SEYANKKTRGAFIAAVFAMQGFGILAGGMVAIIVSSAFKGAFPAPAYEVDAL ASTVSQADFVWRIILMFGAIPAGLTYYWRMKMPETAR YTALVAKNLKQAANDMSKVLQVEIEAEPEKVAAISVANGANEFGLFSKEFL RRHGLHLLGTASTWFLLDIAFYSQNLFQKDIFSAIGWIPPAQTMNALEEVYK IARAQTLIALCSTVPGYWFTVAFIDRIGRFAIQLMGFFFMTVFMFALALPYH HWTLKDNRIGFVVMYSLTFFFANFGPNATTFVVPAEIFPARLRSTCHGISAA AGKAGAMVGAFGFLYAAQPTDPKKTDAGYPAGIGVRNSLIVLGCVNFLGM LFTFLVPESKGKSLEEMSRENEGEEETVAEMRATSGRTVPV
>*StPHT1;6*	MAGDMKVLNALDSAKTQWYHFTAIIIAGMGFFTDAYDLFCISLVTKLLGR IYYHVDGSSKPGSLPPNVSAAVNGVAFCGTLAGQLFFGWLGDKMGRKKV YGMTLMLMCLCSIASGLSFSREPKTVMATLCFFRFWLGFGIGGDYPLSATI

(Continued)

Table S6 (continued)

Genes	The ORF amino acid sequence of potato *PHT1* gene
	MSEYSNKKTRGAFIAAVFAMQGFGILGGGIFAIIISAVFQACFKAPAYQVDP LGSTVPQADYVWRIILMAGSLPALLSYYWRMKMPETARYTALVAKNVKQ ATADMVKVMQVNIGTEQKEPVVKSGKEFGLFTKKFLNRHGLHLLGTTST WFLLDIAYYSQNLFQKDIFSAIGWIPAAKTMNAIEEVQKIARAQTLIALCST VPGYWFTVFLIDRIGRFTIQVIGFTMMTVFMFALAIPYHHWTLPGHHIGFV VLYSLTFFFANFGPNATTFVVPAEIFPARLRSTCHGISAACGKLGAMVGAF GFLYLAQPQDKSKADAGYPAGIGVRNSLIVLGVVNLLGLFFTFLVPESKG KSLEEMSRENENSEEGTEVENHSSDNRTVPV
>StPHT1;9	MAGDMKVLNALDSAKTQWYHFTAIIIAGMGFFTDAYDLFCISLVTKLLGR IYYHVDGSSKPGSLPPNVSAAVNGVAFCGTLAGQLFFGWLGDKMGRKKV YGMTLMLMCLCSIASGLSFSREPKTVMATLCFFRFWLGFGIGGDYPLSATI MSEYSNKKTRGAFIAAVFAMQGFGILGGGIFAIIISAVFQACFKAPAYQVDP LGSTVPQADYVWRIILMAGSLPALLSYYWRMKMPETARYTALVAKNVKQ ATADMVKVMQVNIGTEQKEPVVKSGKEFVPGYWFTVFLIDRIGRFTIQVI GFTMMTVFMFALAIPYHHWTLPGHHIGFVVLYSLTFFFANFGPNATTFVV PAEIFPARLRSTCHGISAACGKLGAMVGAFGFLYLAQPQDKSKADAGYPA GIGVRNSLIVLGVVNLLGLFFTFLVPESKGKSLEEMSRENENSEEGTEVEN HSSDNRTVPV
>StPHT1;10	MALKVLTDLDSARTQYYHFKAIIIAGMGLFTDAYDLFCIPPIMKLIGRIYYA DSNNLYEVPRAVTSAMVVTALLGTVIGQLVFGRLGDLIGRRKVYGFALMI MVLSSFGCGHSICTSRTCVLLSLGFFRFLLGIGIGGDYPLSATIMSEFANRK TRGAFIAGVFSMQGFGILASSTVTMIVCSIFNRANGGSHGADLAWRLILM IGAIPAGLTYYWRMMMPETARYTALVERDVPQAARDMEKVLDISASQIV EEFSTYLPNSPSSNYSLFSKTFIHNHGIDLFACSISWFLVDIVFYSSNLFQSQ IYKRYLSNNHNVNAFQEAFEVARIQAIIAICSTIPGYFATMYFIDRIGRVKIQ IMGFFFMAISLLAIGIPYYSYWNNNTNIGFMFLYGLTFFFSNFGPNTTTFIVP AELFPARFRTTCHGISGAVGKLGAIIGSIGFLWASQNKKDGYNEGIGMTAS LILLAGVCVVGMITTYFFTSETMGKSLEENENIVINEDHQSVGGRA
>StPHT1;12	MAGNMKVLNALDSAKTQWYHFTAIIITGMGFFTDAYDLFCISLVTKLLGRI YYHVDGSSKPGSLPPNVSAAINGVAFCGTIAGQLFFGWIGDKIGRKKVYGI TLMIMSICSIASGLSFGRDPKTVMATLCFFRFWLGFGFGGDYPLSATIMSEY ANKKTRGAFIAAVFAMQGFGILGGGIFAIIISAVFQACFKAPAYQVDPLGST VPQATADMENVMQVDIGTDQKEPAVKSGNEFVFLIDRIGRFTIQLIGFTMM TVFMFALAIPYHHWTLPGHHIGFVVLYSLTFFFANFGPNATTFVVPAEIFPA RLRSTCHGISAACGKLGAMVGAFGFLYLAQPQDKTKADAGYPAGIGVRN SLIVLGVVNLLGLFFTFLVPESKGKSLEEMSRENEDSTEEGAEVENHSSDN RTVPV
>StPHT1;13	MATICFFRFWLGFGFGGDYPLSATIMSEYANKKTRGAFIAAVFAMQGFGIL GGGIFAIIISAVFQACFKAPAYQVDPLGSTIPQADYAWRIILMAGSLPALLTY YWRMKMPETARYTALVAKNVKQATADMENVMQVDIGTDQKEPAVKSGN EFVFLIDRIGRFTIQVIGFTMMTVFMFALAIPYHHWTLPGHHIGFVVLYSLTF FFANFGPNATTFVVPAEIFPARLRSTCHGISAACGKLGAMVGAFGFLYLAQ PQDKSKADAGYPAGIGVRNSLIVLGVVNLLGLFFTFLVPESKGKSLEEMSR DGEDSAEDGAEVENHNRTVPV