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RESEARCH ARTICLE

**Li GUO, Jiaojiao WANG, Kai XIAO**

# Isolation and characterization of a novel phytase gene (*Sphy1*) from soybean (*Glycine max* (L.) Merr.)

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**Abstract** A novel phytase gene *Sphy1* was isolated based on screening a cDNA library which was constructed from germinated soybean (*Glycine max* (L.) Merr. cv. Kefeng 6) cotyledon. The full-length cDNA of *Sphy1* was 1644 bp predicated to encode 547 amino acids including an N terminal signal peptide of 27 amino acids. Phylogenetic analysis indicated that *Sphy1* had high similarities with the phytase genes from *Medicago truncatula* and rice, and acid phosphatase genes from *M. truncatula* and *Arabidopsis*. Prokaryotic expression of *Sphy1* in *Escherichia coli* BL21 (DE3) showed that the induced protein had high phytase activities. The transcripts of *Sphy1* could be detected in various tissues, such as cotyledons, leaves, stems and roots of seedlings. The phytase activities in the above tissues were in accordance with their corresponding *Sphy1* transcripts. The transcripts of *Sphy1* in cotyledons showed an increasing trend from 5 to 30 days after germination, suggesting that *Sphy1* had involved the hydrolyses of the organic phosphorus compounds in seeds from the stages of early seed germination to young seedlings in soybean. Therefore, it was speculated that *Sphy1* played an important role during the seed germination and the growth of the seedlings by releasing inorganic phosphorus ($P_i$) from phosphorus reserve in seeds.

**Keywords** soybean (*Glycine max* (L.) Merr.), phytase gene, *Sphy1*, gene expression, phytase activity

## 1 Introduction

Phosphorus is one of the indispensable inorganic nutrients for crops. In plants, inorganic phosphorus ($P_i$) has various biological functions, including the key substrates in the energy metabolism and biosynthesis of nucleic acids and membranes. Thus, $P_i$ plays a fundamental role in photosynthesis, respiration and the regulation of a number of enzymes (Raghothama, 1999). In soybean, a lot of phosphorus produced during the stages of growth and development was transported into seeds at the seed filling stage. Meanwhile, most phosphorus stored in the vegetative organs, such as leaves, stems and roots, was also remobilized into the developing seeds.

The $P_i$ in soybean seeds, transported or remobilized from other parts of the plant, has been changed into the organic form by biochemical reactions during the seed filling stage (Lott et al., 1995; Raboy, 1997), but little phosphorus in the seeds at maturity stage is kept in $P_i$ form. The main form of the organic phosphorus in the maturated seeds is phytic acid and its derivatives (Loewus and Murthy, 2000). During the seed germination and the early growth stage of young seedlings, the phytase activated in seeds or re-synthesized in plants plays important roles in hydrolyzing of the phytic acid and its derivatives, the main category of organic phosphorus compounds in the seeds, into $P_i$, the available phosphorus for plant growth (Loewus and Murthy, 2000).

The biochemical properties of some phytases from various species have been extensively studied in the livestock area (Wodzinski and Ullah, 1996; Brinch-Peterson et al., 2002; Vohra and Satyanarayana, 2003), in which the phytase was used as food additives aiming at enhancing the phosphorus utilization efficiency of feeds for monogastric animals by promoting the degradation of organic phosphorus compounds in the feeds. Currently, phytase isolated from *Aspergillus niger* has been widely used in livestock husbandry (Vohra and Satyanarayana, 2003).

Up to now, there are few reports on phytase from plant species, though two phytase genes from maize (Maugenest et al., 1997; 1999) and one phyatse gene from *M. truncatula* (Xiao et al., 2005), the model legume plant, have been isolated. In this paper, a novel soybean phytase gene was cloned by screening a cDNA library which was constructed from germinated soybean (*Glycine max* (L.) Merr. cv. Kefeng 6) cotyledon. During the screening, a fragment of cDNA from *M. truncatula* phytase gene (GenBank accession No. AY878355) was used to be the probe. Here, we report the results of cloning and characterization of this novel soybean phytase gene.

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## 2 Materials and methods

The seeds of soybean (cv. Kefeng 6) were soaked into distilled water for 24 h at 25°C in a growth chamber, and then transferred into plastic pots holding vermiculite and growing in the growth room, under the condition of photoperiodic regime of 12 h (day) / 12 h (night) and at the temperatures of 25°C (day)/ 20°C (night). The germinated seeds and seedlings were irrigated with the Hoagland nutrient solution once every three days so as to maintain suitable supply of water and nutrients to the plants.

### 2.1 Isolation of *Sphy1*

A cDNA library of germinated soybean (cv. Kefeng 6) cotyledon was constructed according to the manufacturer's guidelines (Invitrogen). A cDNA fragment of *M. truncatula* phytase gene (GenBank accession No. AY878355) was PCR amplified using the cDNA clone plasmid and a specific primer pair. The forward primer was 5′-CAGTTGGTAGCATAGTTCAATATG-3′and reverse primer was 5′-TATATACACGGTTCGATCTCTCAT-3′. The length of polymerase chain reaction (PCR) product was 924 bp. The PCR products were labeled with $\alpha^{32}$-dATP following the protocol (Invitrogen) and used as a probe to screen the soybean cDNA library. A routine cDNA screening was performed according to Sambrook et al. (1989). After the screening, in total, three positive clones were identified under a stringent condition. Based on sequencing analysis, it was found that all of them were the same except the different lengthes of insertions. The one containing the whole open reading frame (ORF) was used in this study. For cloning the ORF of this gene, a forward primer containing the start codon ATG (5′-<u>ATG</u>GCGTCAATTACTTTTTCTC-3′) and a reverse primer containing the termination codon TAA (5′-<u>TTA</u>TATCGAAGCAATGCAAT-3′) were synthesized (Sangon, Shanghai, China). The PCR amplification condition was as follows: denaturation at 94°C for 3 min, then 35 cycles at 94°C for 1 min, 55°C for 1 min and 72°C for 2 min, with another extension at 72°C for 7 min.

### 2.2 Characterization of *Sphy1*

The encoded amino acids of *Sphy1* were predicted based on the ExPASy web server (www.expasy.org) online analysis. The molecular weight and isoelectric point of *Sphy1* were calculated using DNAStar software. The analysis of phylogenetic tree and ClustalW of *Sphy1* was also carried out by using DNAstar software. The subcellular location predication was performed by Target P (Emanuelsson et al., 2000).

### 2.3 Expression analysis of *Sphy1*

The various tissues including cotyledons, leaves, stems and roots were separately harvested 10, 20 and 30 days after seed germination. Except the above time points, another time point of five days after seed germination was also included for cotyledons. The harvested tissues at each time point were rapidly frozen in liquid nitrogen and then stored at −80°C for RNA isolation. The total RNA of the tissue samples were isolated using Trizol reagent (Gibco). The transcripts of *Sphy1* in various tissues at each time point were detected with the method of semi-quantitative reverse transcriptase PCR (RT-PCR) according to Xu et al. (2007), with a specific primer pair the same as the used in *Sphy1* ORF amplification mentioned above.

### 2.4 Expression of *Sphy1* in *E. coli* (*Escherichia coli*) BL21 (DE3) and phytase activity assay of the induced *Sphy1*

The ORF without the N terminal 27-aa signal peptide coding sequence of *Sphy1* was PCR amplified using a specific primer pair and a high-fidelity Taq polymerase (TaKaRa). The forward primer was 5′-T<u>GGATCC</u>TGC-CATATTCCGTCAACCCT-3′ and the reverse primer was 5′-T<u>GAATTC</u>TTATATCGAAGCAATGCAAT-3′, in which the restriction sites of *Bam*HI and *Eco*RI were introduced in the forward primer and reverse primer, respectively. The PCR amplification condition was as follows: at 94°C for 3 min, then 35 cycles at 94°C for 1 min, 56°C for 1 min and 72°C for 2 min, with another extension at 72°C for 7 min. The PCR products were then cloned into vector PUCm-T (Sangon, Shanghai, China) and transformed to *E. coli* DH5α. The plasmid of a positive clone was isolated and double digested by *Bam*HI and *Eco*RI, the fragment of *Sphy1* was then inserted into the pET28a (+) which was also double digested by above restriction enzymes. The ligated products were then used to transform the host BL21 (DE3). The induction and polyaclimide gel electropheresis analysis of *Sphy1* were followed with the method described by Sambrook et al. (1989). For the activity assay of the induced protein, the hosts after growing and protein induction were sonicated broken and centrifuged for 10 min at 10000 g at 4°C. The supernatants were used to assay the phytase activities according to the method of Xiao et al. (2005).

### 2.5 Assay of phytase activity in germinated cotyledons and various tissues of the seedlings

At each time point of 10, 20 and 30 days after seed germination, various tissues of the soybean seedlings including cotyledons, leaves, stems and roots of plants were separately harvested. Another time point of five days after germination was also selected for harvest of the cotyledon samples. The phytase activities of each sample were assayed also following the descriptions of Xiao et al. (2005).

## 3 Results

### 3.1 Cloning of a novel soybean phytase gene

Based on screening of a cDNA library which was constructed from germinated soybean codyledons, in which a cDNA

fragment of *M. truncatula* phytase gene (GenBank accession No. AY878355) labeled with $\alpha^{32}$-dATP was used as the probe. Three positive clones with the same sequence insertions were identified based on sequencing analysis. The one holding the whole ORF was used for further analysis. According to the sequence, a specific primer pair for amplifying the whole ORF was designed and PCR amplification was carried out. The size of the PCR fragment was in accordance with the ORF length of this gene (Fig. 1).

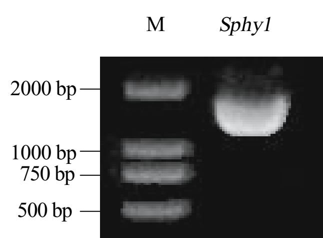


**Fig. 1** Agarose gel electropheresis of the PCR products of *Sphy1* ORF

### 3.2 Characterization of the novel soybean phytase gene

The ORF sequence of this phytase gene and its encoded protein are shown in Fig. 2. To our knowledge, it has not been reported in the previous studies. Therefore, we name it *Sphy1*. The whole ORF of *Sphy1* was 1644 bp predicated to encode 547 amino acids. The molecular weight of *Sphy1* was 62.13 kD with an isoelectric point 5.21. Subcellular analysis indicated that there was a 27-aa signal peptide at the N-terminal in *Sphy1*, indicating that it could be targeted at the cytoplasmic membrane or secreted into the rhizosphere after post-translation modifications.

Phylogenetic analysis indicated that *Sphy1* had high similarities with the phytases from *M. truncatula* (GenBank accession No. AAK1117) and rice (GenBank accession No. AAO37938). Among them the phytase gene of *M. truncatula* was used as the probe to screen the *Sphy1*. It was also found that *Sphy1* had relative high similarities with some purple acid phosphatase genes from *M. truncatula* (GenBank accession No. ABE89961, and ABP96799) and Arabidopsis (GenBank accession No. CAN65638, and AAM16284*)*. A Ser/Thr protein phosphatase family protein gene from rice (GenBank accession No. ABF99890) was also homologous to *Sphy1* (Fig. 3). The ClustalW analysis results of *Sphy1* and four other phyatse genes and purple acid phosphatase genes from *M. truncatula,* rice and *Arabidopsis* are listed in Fig. 4, indicating that *Sphy1* is possibly an important member of the phytase gene family in soybean.

### 3.3 Expression of *Sphy1* in *E. coli* and activity assay of the induced phytase

The ORF without 27-aa signal peptide sequence at the N terminal of *Sphy1* was inserted into the bacterial expression

```
   1 ATGGCGTCAATTACTTTTTCTCTTCTTCAATTTCATCGTGCTCCTATTCTTCTGCTAATTCCGCTCGCGGGTTTCGGTCACTGCCATATTCCG
      M  A  S  I  T  F  S  L  L  Q  F  H  R  A  P  I  L  L  L  I  P  L  A  G  F  G  H  C  H  I  P
  94 TCAACCCTCGAAGGTCCCTTTGATCCCGTCACCGTTCCGTTCGACCCCGCCTTGCGCGGCGTCGCCGTCGACTTGCCGGAAACCGATCCTCGA
      S  T  L  E  G  P  F  D  P  V  T  V  P  F  D  P  A  L  R  G  V  A  V  D  L  P  E  T  D  P  R
 187 GTTCGCCGCCGCGTCCGGGGTTTCGAGCCCGAACAGATTTCGGTTTCTCTCTCTACCTCCCATGACTCCGTTTGGATATCTTGGGTTACAGGG
      V  R  R  R  V  R  G  F  E  P  E  Q  I  S  V  S  L  S  T  S  H  D  S  V  W  I  S  W  V  T  G
 280 GAGTTCCAAATAGGTCTCGACATCAAGCCTTTAGACCCTAAAACTGTATCAAGTGTTGTTCAATATGGAACTTCAAGATTTGAATTAGTGCAT
      E  F  Q  I  G  L  D  I  K  P  L  D  P  K  T  V  S  S  V  V  Q  Y  G  T  S  R  F  E  L  V  H
 373 GAAGCTAGAGGCCAGTCTCTCATCTACAACCAGCTCTATCCTTTCGAAGGCCTTCAGAATTACACATCTGGAATCATCCATCACGTTCAACTC
      E  A  R  G  Q  S  L  I  Y  N  Q  L  Y  P  F  E  G  L  Q  N  Y  T  S  G  I  I  H  H  V  Q  L
 466 AAAGGATTGGAACCAAGCACACTATACTATTATCAATGTGGAGATCCTTCATTGCAAGCCATGAGTGATATATACTATTTCAGGACCATGCCA
      K  G  L  E  P  S  T  L  Y  Y  Y  Q  C  G  D  P  S  L  Q  A  M  S  D  I  Y  Y  F  R  T  M  P
 559 ATTTCTGGTTCAAAGAGCTACCCAGGCAAAGTAGCTGTAGTAGGAGATCTTGGTCTTACTTATAATACAACTACTACCATCGGTCACCTGACT
      I  S  G  S  K  S  Y  P  G  K  V  A  V  V  G  D  L  G  L  T  Y  N  T  T  T  T  I  G  H  L  T
 652 AGTAATGAACCTGATCTTCTTCTATTGATTGGTGATGTAACCTACGCGAATCTGTACCTCACAAATGGAACTGGCTCTGATTGTTATAGTTGC
      S  N  E  P  D  L  L  L  L  I  G  D  V  T  Y  A  N  L  Y  L  T  N  G  T  G  S  D  C  Y  S  C
 745 TCGTCTCCACTAACTCCTATACATGAAACCAACCAGCCTCGATGGGATTATTGGGGAAGGTTTATGCAGAATCTAGTTTCTAACGTTCCAATA
      S  S  P  L  T  P  I  H  E  T  N  Q  P  R  W  D  Y  W  G  R  F  M  Q  N  L  V  S  N  V  P  I
 838 ATGGTGGTAGAAGGAAATCATGAAATAGAAAAACAGGCTGAAAACAGGACATTTGTGGCCTACAGTTCTAGGTTTGCATTCCCCTCTCAAGAA
      M  V  V  E  G  N  H  E  I  E  K  Q  A  E  N  R  T  F  V  A  Y  S  S  R  F  A  F  P  S  Q  E
 931 AGTGGATCTTCATCTACATTCTACTATTCTTTCAATGCTGGAGGCATTCATTTTATTATGCTTGGGGCTTATATTAACTATGATAAAACGGCT
      S  G  S  S  S  T  F  Y  Y  S  F  N  A  G  G  I  H  F  I  M  L  G  A  Y  I  N  Y  D  K  T  A
1024 GAACAATACAAGTGGTTGGAGAGAGATCTGGAAAATGTTGATAGATCAATAACTCCCTGGCTTGTAGTTACTTGGCATCCACCATGGTATAGT
      E  Q  Y  K  W  L  E  R  D  L  E  N  V  D  R  S  I  T  P  W  L  V  V  T  W  H  P  P  W  Y  S
1117 TCTTATGAAGCCCATTACAGAGAAGCAGAGTGCATGAGGGTGGAGATGGAGGACCTATTATACGCATATGGTGTGGATATAATATTTAATGGA
      S  Y  E  A  H  Y  R  E  A  E  C  M  R  V  E  M  E  D  L  L  Y  A  Y  G  V  D  I  I  F  N  G
1210 CATGTTCATGCCTATGAGAGGTCAAACCGAGTTTACAATTACAATTTAGATCCATGTGGTCCTGTATATATTACAGTTGGGGATGGGGGCAAC
      H  V  H  A  Y  E  R  S  N  R  V  Y  N  Y  N  L  D  P  C  G  P  V  Y  I  T  V  G  D  G  G  N
1303 AGAGAGAAGATGGCAATCAAATTCGCAGACGAGCCTGGTCATTGTCCCGATCCATTAAGTACTCCTGATCCTTATATGGGTGGCTTTTGTGCA
      R  E  K  M  A  I  K  F  A  D  E  P  G  H  C  P  D  P  L  S  T  P  D  P  Y  M  G  G  F  C  A
1396 ACAAATTTTACGTTTGGTACAAAAGTGAGTAAGTTTTGTTGGGATCGCCAGCCAGATTACAGTGCTTTCAGAGAAAGTAGCTTTGGCTATGGG
      T  N  F  T  F  G  T  K  V  S  K  F  C  W  D  R  Q  P  D  Y  S  A  F  R  E  S  S  F  G  Y  G
1489 ATTCTAGAGGTGAAAAATGAAACTTGGGCTTTGTGGAGTTGGTATCGTAATCAGGACTCTTACAAGGAAGTTGGGGATCAAATTTACATAGTG
      I  L  E  V  K  N  E  T  W  A  L  W  S  W  Y  R  N  Q  D  S  Y  K  E  V  G  D  Q  I  Y  I  V
1582 AGACAACCTGATATATGCCCCATCCATCAAAGGGTGAACATAGATTGCATTGCTTCGATATAA
      R  Q  P  D  I  C  P  I  H  Q  R  V  N  I  D  C  I  A  S  I
```

**Fig. 2** Open reading frame (ORF) sequence of *Sphy1* and its translated amino acids. Note: A 27-aa signal peptide at the N-terminal was underlined. The translation start condon ATG and termination codon TAA were also underlined.

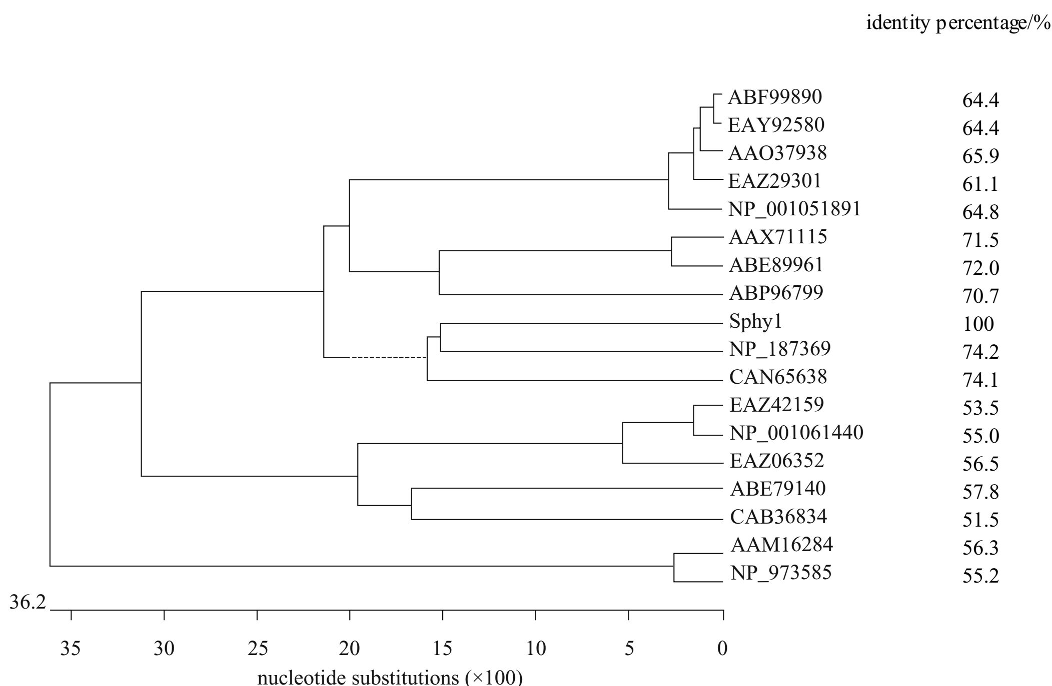


**Fig. 3** Phylogenetic analysis of *Sphy1* and other proteins from plant species that have high similarities with *Sphy1*. Note: The identities in the phylogenetic tree were calculated based on the comparison with *Sphy1*. The proteins used to construct the phylogenetic tree were represented by the GenBank accession numbers. The gene functions corresponding to the GenBank accession numbers were separately as follows: ABF99890, *O. sativa* Ser/Thr protein hosphatase family protein; EAY92580, *O. sativa* hypothetical protein OsI_013813; AAO37938, *O. sativa* putative phytase; EAZ29301, hypothetical protein *OsJ*_012784; NP_001051891, *Os*03g0848200; AAX71115, *M. truncatula* phytase; ABE89961, *M. truncatula* Purple acid phosphatase; ABP96799, *N. tabacum* purple acid phosphatase; NP_187369, *A. thaliana* PAP15; CAN65638, *V. vinifera* hypothetical protein; EAZ42159, *O. sativa* hypothetical protein *OsJ*_025642; NP_001061440, *O. sativa Os*08g0280100; EAZ06352, *O. sativa* hypothetical protein *OsI*_027584; ABE79140, *M. truncatula* Purple acid phosphatase; CAB36834, *A. thaliana* putative protein; NP_973585, *A. thaliana* PAP13; AAM16284, *A. thaliana* truncated putative purple acid phosphatase.

vector pET-28a(+) and transformed to the host BL21 (DE3). The *Sphy1* was induced by 0.4 mmol/L isopropylthio-β-galactoside (IPTG) for 16 h at 20°C. The sodium dodecyl sulfate (SDS) polyacrylamide gel electropheresis analysis showed the target protein of *Sphy1* was induced (Fig. 5). After the hosts were sonicated broken and centrifuged, the supernatants were used to assay the phytase activity. It is found that the phytase activity in the transformants was 4.73-fold higher than that in the non-transformed control hosts. Therefore, the *Sphy1* belongs to a category of plant phytase and has the capability to hydrolyze the phytic acid and its derivatives in plant.

### 3.4 Expression patterns of *Sphy1* in various tissues of seedlings

Semi-quantitative RT-PCR was used to elucidate the expression levels of *Sphy1* in cotyledons, leaves, stems and roots of the seedlings. The transcripts of *Sphy1* could be detected in cotyledons five days after germination, although the level was low. At the time point of ten days after germination, the expression level of *Sphy1* in cotyledons was dramatically enhanced and kept an increasing trend from 10 to 30 days after seed germination. In the meantime, high expression levels of *Sphy1* could also be detected in leaves, stems and roots from 10 to 30 days after germination (Fig. 6). The detected transcripts of *Sphy1* in cotyledons at the time point of five days after germination indicated that this gene could be induced rapidly after seed germination. Meanwhile, higher expression levels in cotyledons from 10 to 30 days after seed germination implied that *Sphy1* has possibly played an important role in hydrolyzing the phytic acids and its derivatives, the phosphorus reserve in soybean seeds, during the early growth stage of the seedlings.

### 3.5 Phytase activities in various tissues of seedlings

The phytase activities of cotyledons five days after seed germination, and the phytase activities of cotyledons, leaves, stems and roots at 10, 20 and 30 days after germination were assayed. It was found that the phytase activities in the above tissues were tightly in accordance with their corresponding transcripts at the same time point. For cotyledons, the phytase activity was low at the time point of five days after germination, and dramatically enhanced at ten days after germination with an increasing tendency till 30 days after germination.

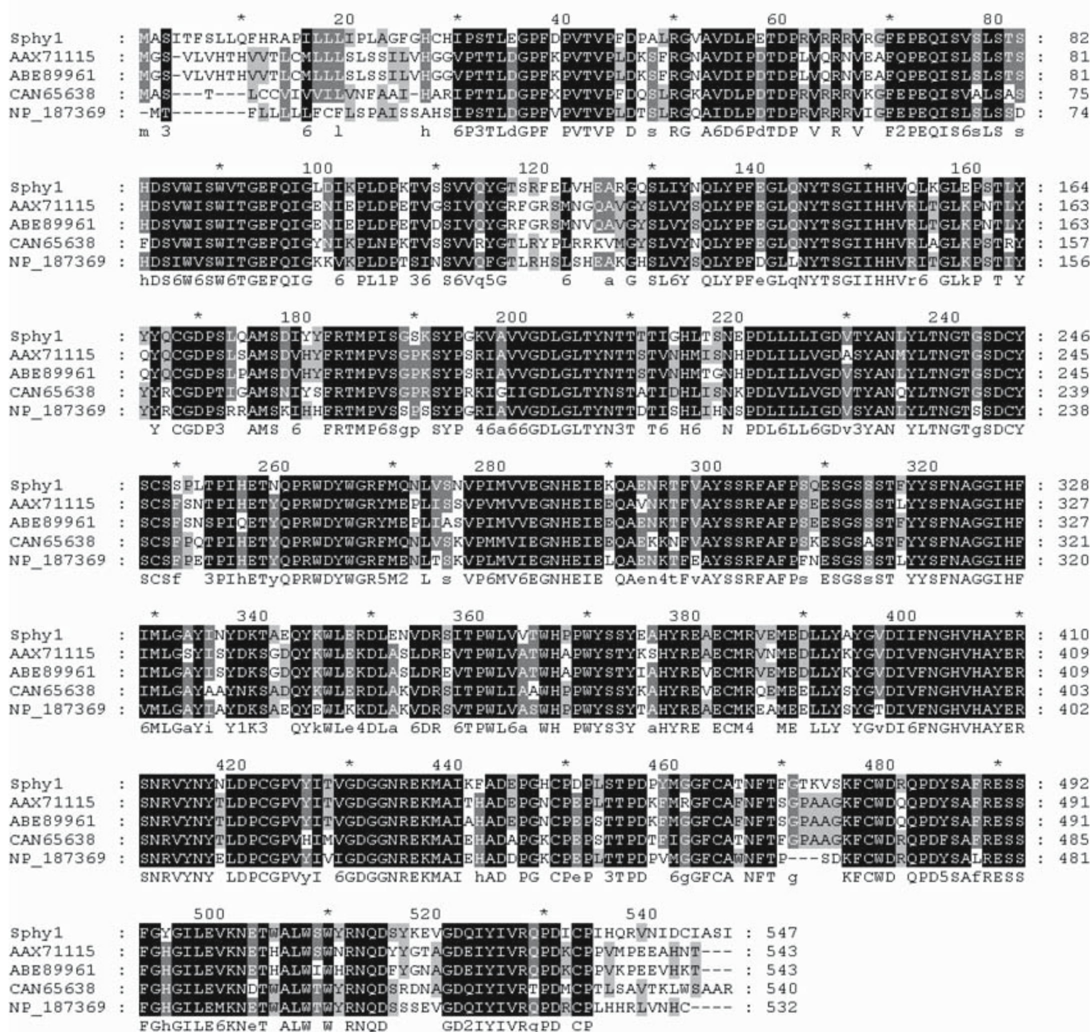

**Fig. 4** Clustal W analysis of *Sphy1* and other four proteins from plant species with high similarities with *Sphy1*. Note: The protein accession number and its corresponding putative function in the Clustal W analysis were separately as follows: AAX71115, *M. truncatula* phytase; ABE89961, *M. truncatula* Purple acid phosphatase; CAN65638, *V. vinifera* hypothetical protein; NP_187369, *A. thaliana* PAP15.

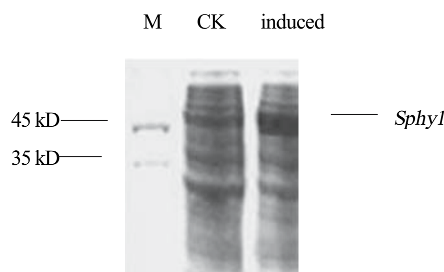


**Fig. 5** Sodium dodecyl sulfate (SDS) polyacrylamide gel electropheresis of the induced *Sphy1*

The phytase activities in leaves, stems and roots were also high for all the assayed time points (Fig. 7). Therefore, *Sphy1* could be one of the important components for the phytase activities in germinated cotyledons and in various tissues of the soybean seedlings.

## 4 Discussion

Phytases have been identified in the roots of plants in several species (Hubel and Beck, 1996; Li et al., 1997; Hayes et al., 1999; Richardson et al., 2000). However, the cloning and characterization of phytase genes in plant species are less until now. Only two phytase genes from maize (Maugenest et al., 1997; 1999) and one from *M. truncatula* (Xiao et al., 2005) have been isolated, mainly resulting from the low abundances at transcription level and translation level of the phytase genes in the plant species.

In this study, the novel soybean phytase gene *Sphy1* has been cloned based on screening a cDNA library which was constructed from the germinated cotyledon of soybean (cv. Kefeng 6), by using a fragment of *M. truncatula* phytase gene as the probe. Blast analysis by using *Sphy1* and querying the international bioinformatics website (National Center for

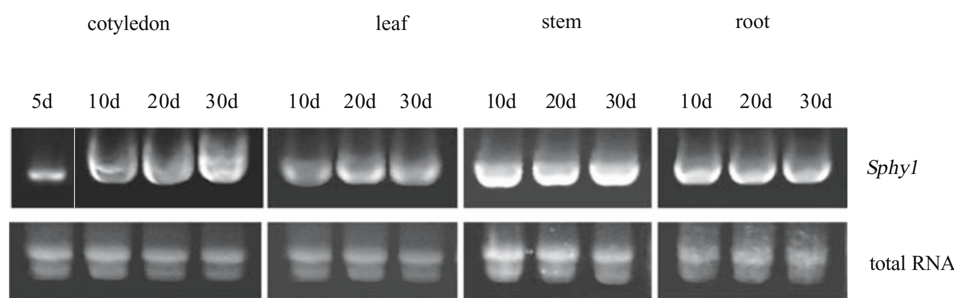


**Fig. 6** Expression patterns of *Sphy1* in cotyledons, leaves, stems and roots at different time points. Note: 5, 10, 20 and 30 days represent the days after seed germination, respectively.

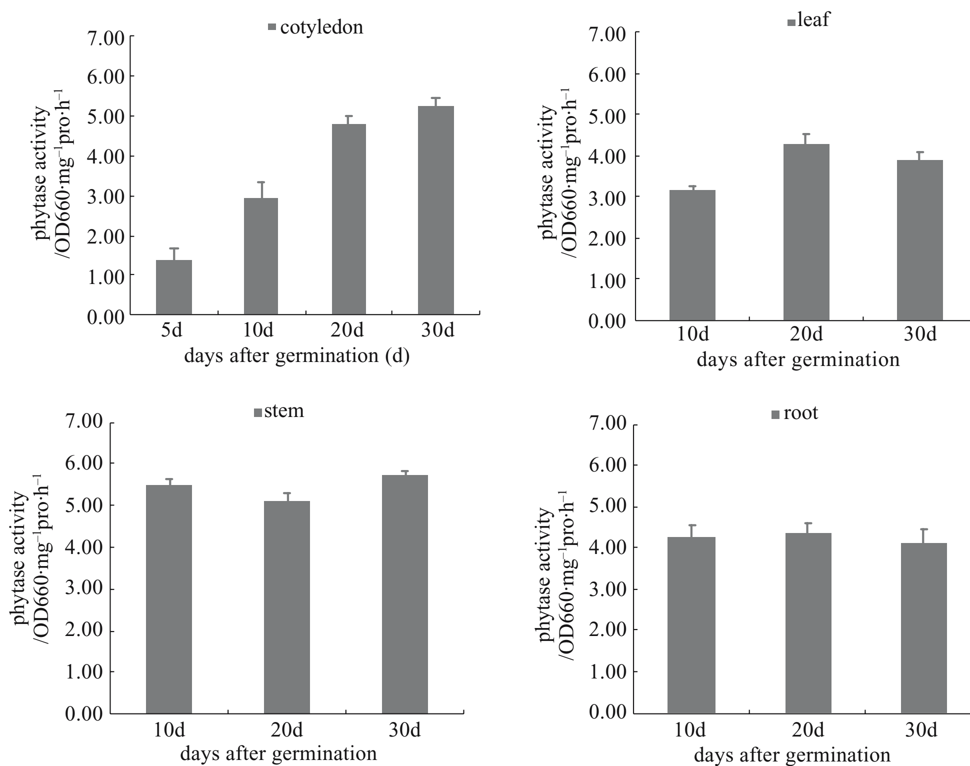


**Fig. 7** Phytase activities in various tissues of seedlings at different time points after germination

Biotechnology Information (NCBI)) identified high similarities of some proteins with *Sphy1*. Among them there were two phytases from *M. truncatula* and rice, respectively. We also expressed the *Sphy1* in *E. coli* and carried out the phytase activity analysis. The results showed that the induced *Sphy1* had a strong phytase activity. Therefore, the novel soybean phytase gene *Sphy1* could be one important member of phytase gene family in soybean.

The gene expression of *Sphy1* in cotyledons was induced at the early germination stage of five days after seed germination. At the time of ten days after germination, the expression level of *Sphy1* in cotyledons was dramatically enhanced with an increasing trend till 30 days after germination. Thus, *Sphy1* could be functional for hydrolysis of the phytic acids and its derivatives stored in seeds during the seed germination and the early growth stage of seedlings. The concurrent changes of phytase activities in cotyledons at the same assayed time point, combining the results of the induced phytase activities by *Sphy1* in *E. coli*, suggested that *Sphy1* has played an important role in releasing $P_i$ from the organic phosphorus compounds of the seed so as to supply $P_i$ for the growth of the seedlings. It is interestingly found out that the leaves, stems and roots of the seedlings also have high expression levels form 10 to 30 days after germination. The functions of the expressed *Sphy1* in the above tissues need to be further explored in the future.

The organic phosphorus compounds generally makes up 30% to 80% of the total phosphorus in the surface layer of the soil, which, after mineralization or degradation, can contribute considerably inorganic phosphorus ($P_i$) nutrition

to the plants (Dadal, 1977; Iyamuremye and Dick, 1996). The predominant form of organic P in the soil is phytate (inositol hexa- and penta-phosphates), which constitutes up to 60% of soil organic P and is poorly utilized by plants (Mudge et al., 2003). Recently, the potential for producing phytase in plant roots and improving $P_i$ uptake has been recognized. It is found that application of a fungal phytase to sterile cultures of subterranean clover (*Trifolium subterraneum*) may enable the seedlings to use phytate as the only source of phosphorus (Hayes et al., 1999). Ectopic expression of a fungal phytase gene (Richardson et al., 2001; Mudge et al., 2003; Han et al., 2007) or a synthetic phytase gene (Zimmermann et al., 2003) may result in acquisition of the increased $P_i$ and biomass production in transgenic plants. In the present study, the induced novel soybean phytase *Sphy1* has higher activities of phyase based on *E. coli* expression analysis. Therefore, it can be promisingly applied as an important function gene in establishing the transgenic crop variety with high phosphorus efficiency by improving the organic phosphorus compounds of soil in future.

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