Identification and characterization of a novel abiotic stress responsive OsTHIC gene from rice

Changqiong Hu, Changqian Quan, Jingmin Zhou, Qiang Yu, Zhigang Bai, Zhengjun Xu, Xiaoling Gao, Lihua Li, Jianqing Zhu and Rongjun Chen

Key Laboratory of Crop Genetic Resources and Improvement, Rice Research Institute of Sichuan Agricultural University, Chengdu, Sichuan, PR China

ABSTRACT
To better understand the mechanisms of plant abiotic stress responses and identify novel stress-related genes in rice, we performed global expression analysis in indica rice Pei’ai 64S under multiple stresses. Among numerous genes, a gene named OsTHIC was selected, which was highly induced in leaves and panicles in response to all stresses at different developmental stages, especially at the booting stage under cold stress. OsTHIC contains an open reading frame (ORF) of 1920 bp and encodes a predicted protein with 639 amino acid residues. The molecular weight and isoelectric point were predicted to be about 71.31 kD and 6.41, respectively. The results of quantitative real-time polymerase chain reaction (qRT-PCR) analysis were almost identical to those from the GeneChip Rice Genome Array. The sequence alignment showed about 99% similarity to rice phosphate methylpyrimidine synthase. Promoter sequence analysis showed that various stress response-related cis-elements were contained in the promoter region. All the results suggested that OsTHIC may be involved in rice stress responses.

KEYWORDS
Oryza sativa L.; thiamine; OsTHIC; stress tolerance

Introduction
Abiotic stresses, such as heat, cold and drought, have severe impacts on plant growth, development and productivity [1–3]. As a major source of carbohydrates for over one-third of the world’s population, rice is regarded as a food crop with high importance and is widely cultivated around the world [1,4]. Exposure to extreme environmental conditions will lead to a dramatic decline in yield or even plant death [5]. To survive under these environmental stresses, plants have evolved the ability to respond to various abiotic stresses [6]. In recent years, the molecular and cellular mechanisms underlying the adaptation of higher plants to environmental stresses have attracted more attention [7–9]. To improve rice abiotic stress resistance, we need to understand the mechanisms by which plants respond to these abiotic stresses.

Thiamine is an essential compound for all living organisms, as it plays significant roles in plant metabolisms, including the Calvin–Benson cycle (C3), the pentose phosphate pathway (PPP), the tricarboxylic acid cycle (TCA) [10,11], NADPH and ATP synthesis and the formation of nucleic acids [12]. Unlike microorganisms and land plants, humans and other animals cannot de novo synthesize thiamine and only get it through food [13]. Previous studies [14–18] have demonstrated that thiamine biosynthesis is activated in the course of adaptation to persistent abiotic stresses, such as salt, osmotic, cold, heat, drought and oxidative stress. Additionally, thiamine enhances plant disease resistance initiation, allowing plants to fight back quickly against pathogen invasion and disease progression [19].

Chemically, thiamine consists of a thiazole HET-P (4-methyl-5-β-hydroxyethylthiazole) and a pyrimidine HMP-PP (4-amino-5-hydroxymethylpyrimidine) moiety. HET-P and HMP-PP are independently synthesized in plants, and then thiamine was produced by dephosphorylation and pyrophosphorylation [20,21]. In Escherichia coli, the thiazole moiety is formed from DXP (1-deoxy-D-xylulose-5-phosphate), tyrosine and cysteine [22–26], and five gene products (ThiF, ThiS, ThiG, ThiH and ThiI) are involved [13,27,28]. The pyrimidine unit is synthesized from AIR (5-aminoimidazole ribotide), and ThiC is required for pyrimidine biosynthesis [29,30]. ThiE is required for the linking of the thiazole and the pyrimidine to form TP. Four gene products (ThiD, ThiM, ThiL and PdxK) are kinases to TPP formation [13]. However, the mechanism of thiamine biosynthesis is still unclear in plants.
In this paper, we screened a stress tolerance candidate gene OsTHIC from cultivar Pei’ai 64S (Oryza sativa L.) by GeneChip Rice Genome Array and quantitative real-time polymerase chain reaction (qRT-PCR). OsTHIC was highly induced in leaf and panicle under abiotic stresses during all the developmental stages and may play a significant role in the response to diverse abiotic stresses in rice.

Materials and methods

Plant materials and growth conditions

According to the method described in previous studies [31,32], we obtained the experimental materials as follows. Seeds of cultivated Indica rice Pei’ai 64S were sterilized in 0.1% HgCl₂ for 10 min and washed three times under double-distilled water, immersed for 3 days under 25 °C with water changed every day; then they were selected and germinated in liquid culture medium at 37 °C for 2–3 days. The seeds were sowed in batches in the net basin of the Rice Research Institute, Sichuan Agricultural University. The plants were divided into one control and three treatment groups. The control group was maintained under non-stress conditions and the stress treatment groups were exposed to heat, cold and drought stresses. At the five-leaf stage, part of the plants, as the test material from the seedling stage, were taken; whereas other parts were transplanted to the other pots as test material for the booting and flowering stage, with five seedlings for each plastic pot. They were put in a greenhouse under non-stress conditions of regular water, fertilizer management, disease and pest control. For the heat stress tests, the seedlings were exposed to 45 °C for 2 h. For the cold stress tests, the plants at the five-leaf stage were exposed to 4 °C for 12 h. The plants from the booting, heading and flowering stages were treated for 16 h at 12 °C, and the materials were harvested. All rice plants of the tested and control groups were grown at 28 °C day/25 °C night under natural light conditions. For the drought stress tests, the water was poured out from the pots, which were put in a dry shed. The materials were collected after 16 h, when their leaves started curling. The control and treatment groups were under the same conditions, but the control group was watered. We used leaf and panicle tissue samples throughout the genome expression profiling.

Extraction of total RNA and cDNA cloning

The procedure was done according to protocols described previously [4,33]. Leaves were collected from the experimental and control groups. The materials were cut into pieces, and then put into dry and clean 1.5-mL centrifuge tubes. The samples were stored at −70 °C until required. In this study, total RNA was extracted from the frozen samples using TRIzol (Invitrogen). The samples stored at −70 °C were taken out; then ground to powder at low temperature, and immediately divided into pre-installed 1.0 mL TRIzol extraction reagent. The samples were vortexed to homogeneity, then chloroform (200 µL) was added, followed by vigorous shaking for 15 s and centrifugation at 12 000 × g for 15 min at 4 °C. The supernatant was carefully removed from each tube and transferred to another 1.5 mL centrifuge tube and isopropanol (500 µL) was added. The samples were precipitated at −40 °C for at least 1 h and then centrifuged to isolate the RNA. The RNA pellets were washed twice with 75% ethanol, air-dried and dissolved in a suitable volume of RNase-free water. The purity of the RNA was determined by the A₂₆₀/A₂₈₀ absorbance ratio (1.9 to 2.0). Isolated RNAs were stored at −70 °C, after checking the integrity and purity of 5S, 18S and 28S rRNA bands in 1.5% agarose gel. Full-length of OsTHIC cDNA was amplified using high fidelity HiFi taq DNA polymerase (TransGen). Special primers were designed using the software Primer Premier 5.0 after searching homologous cDNA sequences. The primers were OsTHIC-F: 5’-AAGCTTGGAGGAAATGGCTGCCCTGC-3’ with a unique Hind III restriction site upstream from the translational start codon, OsTHIC-R: 5’-GAATTCACATAGGATGGTGAG-GAGTGC-3’ with a unique EcoR I restriction site downstream from the termination codon. The PCR cycler was programmed as follows: an initial denaturation step for 5 min at 95 °C, 30 amplification cycles [30 s at 95 °C (denaturation), 30 s at 58 °C (annealing) and 1.5 min at 72 °C (polymerization)], followed by a final elongation step for 7 min at 72 °C. All the PCR products were purified using a Gel Extraction Mini Kit (Biomed, China). The amplified product was ligated into vector pMD18-T (Takara, Dalian, China), then cloned in Escherichia coli strain DH5α. The positive transformants were screened by using ampicillin selection. Restriction enzymes Hind III and EcoR I were used for double cuts for confirmation. Restricted fragments were analyzed in 1.0% agarose gel. Five positively screened clones were sequenced by Invitrogen.

Microarray and microarray data analysis

According to the experimental manual of the Affymetrix expression microarray provided by GeneTech (Biotechnology Limited Company, Shanghai, China), the procedure was performed on the basis of protocols described previously [6,34]. In brief, the following steps are: (1) Total RNA extraction and purification; (2) cDNA synthesis
and purification; (3) cRNA synthesis and transcription purification in vivo; (4) cRNA fragmentation, preparation of hybridization solution; (5) chip hybridization; (6) elution chip; (7) scan chips; (8) data analysis.

Results and discussion

Expression analysis of OsTHIC under stresses

In order to identify genes that respond to heat, cold and drought stress, the GeneChip Rice Genome Array (Affymetrix) representing 51,279 transcripts from Japonica and Indica rice were used to analyze the expression levels of the whole genome of super hybrid rice maternal plant Pei’ai 64 s in leaves and panicles at the seedling, booting and flowering stages. The microarray analysis revealed genes that were significantly up-regulated or down-regulated. According to the microarray analysis, we screened a gene named OsTHIC, whose expression level in leaves and panicles was significantly different compared to that in the control group plants at the seedling, booting, heading and flowering stages under cold, heat and drought conditions. As shown in Figure 1, the expression level of OsTHIC in leaves at the seedling and booting stage were 33.26-fold and 13.69-fold higher, and 2.21-fold and 4.60-fold higher in panicles at the booting and flowering stages under cold conditions. However, the expression level of OsTHIC was down-regulated slightly in leaves and panicles at all the tested stages under drought conditions. The expression profile of OsTHIC obtained by the microarray analysis was verified by quantitative RT-PCR (Figure 1). The expression level of OsTHIC was increased under cold conditions and decreased under heat and drought conditions, which was generally consistent with the microarray analysis, suggesting that OsTHIC was a multiple stress-responsive gene in rice. The other gene expression data are available at a public microarray database, the National Center for Biotechnology Information (NCBI) gene expression omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) [35]: the comparisons of tissues (that is, seedlings, ovary, leaf, inflorescence and seed) versus untreated control (NCBI GEO accession no.GSE6893), abiotic stresses (that is, drought, salt and cold) versus untreated control (GSE6901), trans-zeatin versus mock dimethyl sulfoxide in root and leaves at 30 and 120 min after treatment (GSE6737; Figure 2) [36].

Cold is one of the major environmental stress factors, and it adversely affects the growth and development of plants and significantly constrains crop distribution and agricultural productivity [37]. Recent studies have reported some genes associated with cold discovered using molecular and generic approaches as microarray [38,39].

Cloning and sequence analysis of OsTHIC

To further analyze OsTHIC, we designed and synthesized two specific primers (OsTHIC-F and OsTHIC-R) for the amplification of OsTHIC, and then cloned the cDNA sequence containing the complete ORF. OsTHIC was located in chromosome 3, consisted of five exons but no introns (Figure 3). Sequence analysis showed that the cloned cDNA was 2074 bp in length with a 1920-bp ORF and encoded a protein with 639 amino acid residues. Using an online bioinformatics tool (http://us.expasy.

![Figure 1. Relative expression of OsTHIC in leaves and panicles of Pei’ai 64S at different developmental stages under various stresses.](image)

Note, 1: Seedling stage; 2: booting stage; 3: heading and flowering stage; L: leaf; P: panicle; K: control; C: cold; H: heat; D: drought.
we predicted that the molecular weight of the protein is about 71.31 kD and the isoelectric point is about 6.41.

To understand the organization of the regulatory region of OsTHIC, the 1500-bp promoter sequence of OsTHIC was analyzed by plantCARE. The possible promoter region (Nipponbare 3: 26954 104 – 26955 603) contains about 30 different cis-acting elements and there may be 11 cis-acting elements related to abiotic stress, such as 1 ABRE (cis-acting element involved in the abscisic acid responsiveness), 1 ARE (cis-acting regulatory element essential for the anaerobic induction), 1 CGTCA-motif (cis-acting regulatory element involved in the MeJA-responsiveness), 1 GARE-motif (gibberellin-responsive element), 2 MBS (MYB binding site involved in drought-inducibility), 1 TCA-element (cis-acting element involved in salicylic acid responsiveness), 1 O2-site (cis-acting regulatory element involved in Zain metabolism regulation) and so on (Figure 4). The presence of these stress-related cis-elements showed that OsTHIC was involved in multiple abiotic stress responses.

Phylogenetic tree analysis of OsTHIC

In view of the predicted protein sequence of OsTHIC and analysis with BLASTp through the NCBI website (http://www.ncbi.nlm.nih.gov/), the full-length protein sequence of OsTHIC was identified to be consistent with a predicted protein (XM_015777 485.1) encoded by LOC4333719 from Nipponbare. BLASTp analysis showed that OsTHIC also shares high similarity with genes from other plant species (Figure 5). To construct a phylogenetic tree, the predicted full-length amino acid sequence of OsTHIC, several putative rice a methylpyrimidine phosphate synthase proteins and the corresponding homologues in other species were used. The obtained dendrogram indicated that OsTHIC has the highest homology with sequences from Oryza sativa Indica,
Glycine max and Arabidopsis thaliana. Moreover, OsTHIC was also revealed to have high homology with sequences from Medicago truncatula, Macleaya cordata, Oryza sativa Japonica Group, Pseudomonas aeruginosa PAO1 and Escherichia coli str. K-12 substr. MG1655 (Figure 6). By analyzing the promoter region of OsTHIC, we found some cis-elements related to plant stress responses. Among these cis-elements, the GARE motif is a cis-acting regulatory element relevant to gibberellin response, MBS is involved in drought-inducibility, the O2-site is a cis-acting regulatory element involved in zein metabolism regulation. It has been reported that ABRE showed response to cold stress [40] and ARE was involved in the anaerobic induction [41,42]. In addition, the CGTCA-motif and TCA-element have also been associated with stress response [43–45]. The existence of these stress-related cis-elements indicated that OsTHIC may be involved in the adaptation process of abiotic stress tolerance in rice.

The database search and analysis with BLASTp through the NCBI website showed that the deduced amino acid sequence of OsTHIC had higher homology (99%) with a predicted protein (XM...015777 485.1) encoded by LOC4333719 from Nipponbare. The protein is predicted to be a methylpyrimidine phosphate synthase associated with thiamine biosynthesis and to be located in the chloroplast. According to previous studies [46,47], the synthesis of thiamine occurs in plastids and

Figure 4. Cis-elements of OsTHIC in the putative promoter region. Note: The putative start codon ATG is denoted with +1.

Figure 5. Multiple amino acid sequence alignment of the OsTHIC protein and corresponding protein sequences. Note: The boxed sequences are the conserved THIC domain. Gm, Glycine max; Mt, Medicago truncatula; At, Arabidopsis thaliana; Mc, Macleaya cordata; Os, Oryza sativa Japonica Group; Pa, Pseudomonas aeruginosa PAO1; E. coli, Escherichia coli str. K-12 substr. MG1655.
chloroplasts in plants, and the AtTHIC protein is also targeted in plastids and chloroplasts [48]. Thus, the OsTHIC protein is likely to be located in the chloroplast.

Conclusions

The OsTHIC gene was screened in Pei’ai 64S by Gene-Chip. It was responsive to heat, cold and drought at different growth stages. The expression level at the seedling and the booting stage was significantly higher than that of other stages. The full-length ORF of OsTHIC was 1920 bp with 46% GC content. OsTHIC encoded a putative protein with 639 amino acid residues and the relative molecular weight and isoelectric point were 71.31 kD and 6.41. Multiple sequence alignment revealed that many genes from other species showed high similarity to OsTHIC, such as genes from rice, maize, wheat and so on. The promoter sequence of OsTHIC was analyzed by plantCARE and 11 stress-related cis-elements were found in the promoter region. These results laid the foundation for further analysis of OsTHIC function. Furthermore, it is also highly important to increase the content of thiamine through genetic modification of vegetables and grains to improve human thiamine deficiency.

Disclosure statement

The authors declare that they have no conflict of interest.

Funding

This research was supported by Fund (An international cooperation Project Supported by the Science and Technology Department of Sichuan Province) [2015HH0032] to RJ Chen.

References

[1] Jain M, Nijhawan A, Arora R, et al. F-box proteins in rice. Genome-wide analysis, classification, temporal and spatial gene expression during panicle and seed development, and regulation by light and abiotic stress. Plant Physiol. 2007;143:1467–1483.
[2] Nakashima K, Ito Y, Yamaguchi-Shinozaki K. Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. Plant Physiol. 2009;149:88–95.
[3] Sweetlove LJ, Heazlewood JL, Gerald V, et al. The impact of oxidative stress on Arabidopsis mitochondria. Plant J. 2002;32:891–904.
[4] Liao Y, Liu S, Jiang Y, et al. Genome-wide analysis and environmental response profiling of dirigent family genes in rice (Oryza sativa). Genes Genomics. 2016;39:47–62.
[5] Jones RA, Qualset CO. Breeding crops for environmental stress tolerance. 1984:305–340.
[6] Liao Y, Liu S, Jiang Y, et al. Genome-wide analysis and environmental response profiling of dirigent family genes in rice (Oryza sativa). Genes Genomics. 2016;39:47–62.
[7] Cushman JC, Bohnert HJ. Genomic approaches to plant stress tolerance. Curr Opin Plant Biol. 2000;3:117–124.
[8] Mittler R. Abiotic stress, the field environment and stress combination. Trends Plant Sci. 2006;11:15–19.
[9] Zhu JK. Salt and drought stress signal transduction in plants. Annu Rev Plant Biol. 2002;53:247–273.
[10] Begley TP. The biosynthesis and degradation of thiamin (vitamin B1). Nat Prod Rep. 1996;13:177–185.
[11] Wang L, Ye X, Liu H, et al. Both overexpression and suppression of an Oryza sativa NB-LRR-like gene OsLSR result in autoactivation of immune response and thiamine accumulation. Sci Rep. 2016 [cited 2018 Mar 20];6:24079. DOI: 10.1038/srep24079.
[12] Rapala-Kozik M, Wolak N, Kujda M, et al. The upregulation of thiamine (vitamin B1) biosynthesis in Arabidopsis thaliana seedlings under salt and osmotic stress conditions is mediated by abscisic acid at the early stages of this stress
response. BMC Plant Biol. 2012 [cited 2018 Mar 20];12:2. DOI: 10.1186/1471-2229-12-2.

[13] Begley TP, Downs DM, Ealick SE, et al. Thiamin biosynthesis in prokaryotes. Arch Microbiol. 1999;171:293–300.

[14] Ribeiro DT, Farias LP, de Almeida JD, et al. Functional characterization of the thi1 promoter region from Arabidopsis thaliana. J Exp Bot. 2005;56:1797–1804.

[15] Ferreira S, Hjerno K, Larsen M, et al. Proteome profiling of Populus euphratica Oliv. upon heat stress. Ann Bot. 2006;98:361–377.

[16] Wong CE, Li Y, Labbe A, et al. Transcriptional profiling implicates novel interactions between abiotic stress and hormonal responses in Thellungiella, a close relative of Arabidopsis. Plant Physiol. 2006;140:1437–1450.

[17] Rapala-Kozik M, Kowalska E, Ostrowska K. Modulation of thiamine metabolism in Zea mays seedlings under conditions of abiotic stress. J Exp Bot. 2008;59:4133–4143.

[18] Tunc-Ozdemir M, Miller G, Song L, et al. Thiamin confers enhanced tolerance to oxidative stress in Arabidopsis. Plant Physiol. 2009;151:412–432.

[19] Ahn IP, Kim S, Lee YH. Vitamin B1 functions as an activator of plant disease resistance. Plant Physiol. 2005;138:1505–1515.

[20] Goyer A. Thiamine in plants: aspects of its metabolism and functions. Phytochemistry. 2010;71:1615–1624.

[21] Spenser ID, White RL. Biosynthesis of vitamin B1(thiamin): an instance of biochemical diversity. Angewandte Chemie Int Ed English. 1997;36:1032–1046.

[22] Bernard E, Michel T. Tyrosine as a factor of biosynthesis of the thiazole moiety of thiamine in Escherichia coli. Biochim Biophys Acta. 1972;273:275–282.

[23] Bellion E, Kirkley DH, Faust JR. The biosynthesis of the thiazole moiety of thiamine in Salmonella typhimurium. Biochim Biophys Acta. 1976;437:229–237.

[24] White RH, Rudolph FB. The origin of the nitrogen atom in the thiazole ring of thiamine in Escherichia coli. Biochim Biophys Acta. 1978;542:340–347.

[25] DeMoll E, Shive W. Determination of the metabolic origin of the sulfur atom in thiamin of Arabidopsis coli by mass spectrometry. Biochim Biophys Res Commun. 1985;132:217–222.

[26] Tazuya K, Yamada K, Nakamura K, et al. The origin of the sulfur atom of thiamin. Biochim Biophys Acta. 1987;924:210–215.

[27] Webb E, Downs D. Characterization of thii, Encoding thiamine-monophosphate Kinase, in Salmonella typhimurium. J Biol Chem. 1997;272:15702–15707.

[28]Taylor SV, Kelleher NL, Kinsland C, et al. Thiamin biosynthesis in Escherichia coli. J Biol Chem. 1998;273:16555–16560.

[29] Zhang Y, Begley TP. Cloning, sequencing and regulation of thiA, a thiamin biosynthesis gene from Bacillus subtilis. Gene. 1997;198:73–82.

[30] Zhang Y, Taylor SV, Chiu HJ, et al. Characterization of the Bacillus subtilis thiC operon involved in thiamin biosynthesis. J Bacteriol. 1997;179:3030–3035.

[31] Cao XF, Liao YR, Rong SH, et al. Identification and characterization of a novel abiotic stress responsive sulphotransferase gene (OssSOT9) from rice. Biotech Biotech Equip. 2016;30:227–235.

[32] Jiang Y, Chen R, Dong J, et al. Analysis of GDSL lipase (GLIP) family genes in rice (Oryza sativa). Plant Omics. 2012;5:351–358.

[33] Chen R, Jiang Y, Dong J, et al. Genome-wide analysis and environmental response profiling of SOT family genes in rice (Oryza sativa). Genes Genomics. 2012;34:549–560.

[34] Yang C, Li D, Mao D, et al. Overexpression of microRNA319 impacts leaf morphogenesis and leads to enhanced cold tolerance in rice (Oryza sativa L.). Plant Cell Environ. 2013;36:2207–2218.

[35] Kreps JA, Wu Y, Chang HS, et al. Transcriptome changes for Arabidopsis in response to salt, osmotic, and cold stress. Plant Physiol. 2002;130:2129–2141.

[36] Seki M, Narusaka M, Ishida J, et al. Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J. 2002;31:279–292.

[37] Liao Y, Zou HF, Wei W, et al. Soybean GmbZIP44, GmbZIP62 and GmbZIP78 genes function as negative regulator of ABA signaling and confer salt and freezing tolerance in transgenic Arabidopsis. Planta. 2008;228:225–240.

[38] Doferus R, Jacobs M, Peacock WJ, et al. Differential interactions of promoter elements in stress responses of the Arabidopsis Adh gene. Plant Physiol. 1994;105:1075–1087.

[39] Olive MR, Peacock WJ, Dennis ES. The anaerobic responsive element contains two GC-rich sequences essential for binding a nuclear protein and hypoxic activation of the maize Adh1 promoter. Nucleic Acids Res. 1991;19:7053–7060.

[40] Fujita Y, Fujita M, Shinozaki K, et al. ABA-mediated transcriptional regulation in response to osmotic stress in plants. J Plant Res. 2011;124:509–525.

[41] Merlot S, Gosti F, Guerrier D, et al. The ABI1 and ABI2 protein phosphatases 2C act in a negative feedback regulatory loop of the abscisic acid signalling pathway. Plant J. 2001;25:295–303.

[42] Rowland O, Ludwig AA, Merrick CJ, et al. Functional analysis of Avr9/CF-9 rapidly elicited genes identifies a protein kinase, ACIK1, that is essential for full CF-9-dependent disease resistance in tomato. Plant Cell. 2005;17:295–310.

[43] Juillard JH, Douce R. Biosynthesis of the thiazole moiety of thiamin (vitamin B1) in higher plant chloroplasts. Proc Natl Acad Sci U S A. 1991;88:2042–2045.

[44] Belanger FC, Leustek T, Chu B, et al. Evidence for the thiamine biosynthetic pathway in higher-plant plastids and its developmental regulation. Plant Mol Biol. 1995;29:809–821.

[45] Kong D, Zhu Y, Wu H, et al. AtTHIC, a gene involved in thiamine biosynthesis in Arabidopsis thaliana. Cell Res. 2008;18:566–576.