

Full Length Research Paper

Isolation of a novel xyloglucan endotransglucosylase (*OsXET9*) gene from rice and analysis of the response of this gene to abiotic stresses

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To understand the mechanism(s) underlying stress responses and discover new stress-tolerance genes in rice (*Oryza sativa* L.), expression profiles were obtained for leaf and panicle tissues at seedling, booting and heading stages of *indica* cultivar Pei'ai 64S plants under cold, drought or heat stresses using the GeneChip Rice Genome Array (Affymetrix) representing 51, 279 transcripts from *japonica* and *indica* rice. A large number of genes highly up regulated or down regulated were identified under the stresses. *OsXET9* (*O. sativa* L. xyloglucan endotransglucosylase 9, GenBank accession: NM_001060322) was highly induced in leaf and panicle at all the developmental stages, in response to all stresses, especially in seedling and booting stage under cold stress. Real-time quantitative PCR analysis showed that the result was consensus with GeneChip Rice Genome Array, suggesting that *OsXET9* is a multiple stress responsive gene in rice. In order to study its function in stress tolerance, we cloned the cDNA of the gene through amplification by PCR. Sequence analysis showed that the cDNA encodes a protein of 284 amino acid residues with M.W. \approx 31kD and pI \approx 5.2. The gene encodes a protein with several conserved domains. Comparison of protein sequences indicates that *OsXET9* is closely related to the xyloglucan endotransglucosylase. Analysis of the putative promoter region for candidate cis-regulatory elements using Plant CARE software (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) identified some cis-elements related to stress responses. Based on these analyses and results obtained, we propose that *OsXET9* is a novel candidate gene involved in stress tolerance in rice in addition to the function in metabolism of cell wall.

Keywords: Rice, abiotic stress, microarray, xyloglucan endotransglucosylase.

INTRODUCTION

Various abiotic stresses such as low and high temperature, and drought, have negative impact on plant growth and productivity of crops (Nakashima et al., 2009). Plants have adapted to respond to these stresses at the molecular, cellular, biochemical and physiological level, enabling them to survive (Nakashima et al., 2009). Various adverse environmental stresses induce the

expression of a variety of genes in many plant species (Xiong et al., 2002; Shinozaki et al., 2003; Bartels and Sunkar, 2005). Numerous stress-induced genes have been identified using microarray experiments (Kreps et al., 2002; Seki et al., 2002). The products of these genes are thought to promote stress tolerance and regulate gene expression through signal transduction pathways (Xiong et al., 2002; Shinozaki et al., 2003). Researches have indicated that part of the plants genome participate in the self-response of drought, cold and heat stress (Shinozaki and Yamaguchi-Shinozaki, 2000; Zhu, 2001; Xiong et al., 2002; Shinozaki et al., 2003). Change in the expression of

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a single gene can enhance the ability of drought and salt stress (Xu et al., 1996; Kasuga et al., 1999; Garg et al., 2002; Haake et al., 2002; Shi et al., 2002; Zhang et al., 2004).

The xyloglucan endotransglucosylase/hydrolases (XTHs) are a family of enzymes that specifically use xyloglucan as a substrate and catalyze xyloglucan endotransglucosylase (XET) and/or xyloglucan endohydrolase activities (Yokoyama et al., 2004). The primary enzyme activity of XTH proteins is xyloglucan endotransglucosylase (XET) (Vissenberg et al., 2003). XTHs typically, are encoded by large multigene families in dicotyledons (Rose et al., 2002). In *Arabidopsis*, 33 open reading frames (ORFs) potentially encoding XTH proteins have been identified from the genome sequence database (Yokoyama and Nishitani, 2001). In monocotyledons such as rice, analysis of the draft sequences of its genome has revealed a large rice XTH (*OsXTH*) gene family with 29 members, a number that is similar to that of the *AtXTH* gene family (Yokoyama et al., 2004). Yokoyama et al. (2004) have also examined the expression patterns of all 29 *OsXTH* genes using a quantitative DNA microarray procedure with gene-specific oligonucleotide probes and the analysis showed that most members of the rice XTH family exhibited organ- and growth stage-specific expression. This was confirmed by quantitative real-time reverse transcriptase-polymerase chain reaction analysis of representative *OsXTH* members and the result revealed in more detail the temporally and spatially controlled expression profiles of individual *OsXTH* genes at particular sites in rice (Yokoyama et al., 2004).

The *Arabidopsis* XET-related genes are regulated by abiotic factors, such as touch, darkness, cold-shock, heat shock (Xu et al., 1995, 1996), wind (Antosiewicz et al., 1997), flooding (Saab and Sachs, 1995), drought and salt tolerance (Schopfer and Liszskay, 2006), light quality (Sasidharan et al., 2010). The XET-related genes are also regulated by several hormones, example abscisic acid (Wu et al., 1994), ethylene (Saab and Sachs, 1996), brassinosteroids (Zurek and Clouse, 1994), gibberellins (Genovesi et al., 2008) and auxins (Potter and Fry, 1994). Expression analysis of these genes in *Arabidopsis* has revealed that most of the family members exhibit distinct expression patterns in terms of tissue specificity and that they respond differently to hormonal signals (Xu et al., 1996; Akamatsu et al., 1999; Yokoyama and Nishitani, 2001; Nakamura et al., 2003). 32 genes that potentially encode XTH family proteins in the genome of *Physcomitrella patens* were indentified and their expression profiles are tissue-dependent (Yokoyama et al., 2010).

In this study, GeneChip Rice Genome Array and Real-time quantitative PCR were used to screen a stress tolerance candidate gene *OsXET9* from cultivar Pei'ai64s rice *Liang-You-Pei-Nine* (*LYP9*). Among the genes identified as responsive to stresses, the *OsXET9* gene which is the maternal parent of the super hybridization encoding xyloglucan endotransglucosylase was highly

induced by cold stress in the leaves of seeding and booting stage and was chosen for further study.

MATERIALS AND METHODS

Indica cultivar Pei'ai 64S, which is the maternal parent of the super hybridization rice *Liang-You-Pei-Jiu* (*LYP9*) was used.

Stress treatments, sample preparation, isolation of total RNA, GeneChip Rice Genome Array (Affymetrix) and quantitative real-time RT-PCR

The process was according to protocols previously described by Xu et al., 2008; Jiang et al., 2011. Microarray analysis was performed according to the GeneChip[®] Expression Analysis Technical Manual (2005 version) provided by Affymetrix (Affymetrix, Inc., Santa Clara, CA, USA). The main operation procedures were as follows: (1) Extraction and purification of total RNA, (2) synthesis and purification of cDNA, (3) synthesis and segmentation of cRNA, (4) array hybridization and washing, (5) array scanning and (6) data analysis.

Real-time PCR primers were designed by Primer Expression 3.0 software, the target gene (*OsXET9*) primers were *OsXET9*t-F: 5'-ACC GAC TTC CAC ACC TAC TCC AT -3'; *OsXET9*t-R: 5'-CCT TGT CCG CGT GGT TCT T -3', with amplified fragment length 99 bp. The specific primers for internal control gene 18S rDNA used were 18S-F: 5'-CGT CCC TGC CCT TTG TAC AC-3', 18S-R: 5'-CGA ACA CTT CAC CGG ATC ATT-3'.

Cloning of cDNA

Full-length of *OsXET9* cDNA was amplified using high fidelity HiFi taq DNA polymerase (Transgen). Special primers were designed using the software primer-premier 5.0 after searching homology cDNA sequence that were *OsXET9*-F: 5'-AAG CTT AAC ACA TGG GGT TCG GCT -3' with a unique HindIII restriction site upstream from the translational start codon, *OsXET9*-R: 5'-GGA TCC CTC TAC GGC ATG GAG CAC TC -3' with a unique BamHI restriction site downstream from the termination codon. The PCR cyclers was programmed as follows: an initial denaturation for 5 min at 94°C, 30 amplification cycles, [30 s at 94°C (denaturation), 30 s at 55°C (annealing), and 1 min at 72°C (polymerization)], followed by a final elongation for 10 min at 72°C. All the PCR products were purified using Gel Extraction Mini Kit (Biomed, China), the amplified product was ligated into vector pMD18-T (TaKaRa, Dalian, China), then cloned into *Escherichia coli* strain Top 10. The positive transformants were screened by using ampicillin selection. And restriction enzymes HindIII and BamHI were used for double cuts for confirmation. Restricted fragments were analyzed on 1.0% agarose gel. Positively screened clone was sequenced by invitrogen.

Sequence analysis

The analysis and comparison of the deduced amino acid sequence with published sequences were performed with BLASTp (Standard Protein-Protein BLAST) on the NCBI server (<http://www.ncbi.nlm.nih.gov/>). Promoter analysis of 2000 bp, upstream of *OsXET9* gene, was performed with PlantCARE on the web (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>).

Conserved domains in *OsXET9* were identified with online protein predict software InterProScan (<http://www.ebi.ac.uk/InterProScan/>). *OsXET9* gene was aligned with other XTH proteins from different species using DNAMAN, and then the phylogenetic tree was generated by the Maximum Parsimony method using Mega4.1.

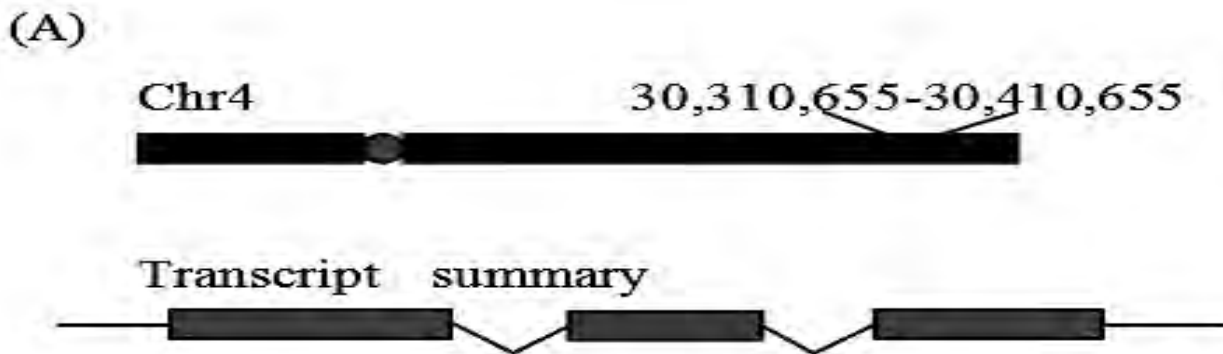


Figure 1. Location of *OsXET9* in rice genome and candidate cis-elements of *OsXET9* in the putative promoter region (the putative start codon ATG is denoted with +1). (A) Location and transcript summary of *OsXET9*. (B) Sequence of *OsXET9*, candidate cis-elements in the putative promoter region. The putative translation initiation site (ATG) are underlined with continuous lines. ABRE, HSE, MBS, C-repeat/DRE, TCA-element, G-box, P-box, CGTCA-motif, WUN-motif, LTR, TGACG-motif and ARE are cis-elements involved in abscisic acid, heat stress, drought condition, cold and dehydration, salicylic acid, light, gibberellin, MeJA, wound, low temperature, MeJA and anaerobic induction responsiveness, respectively. “...” Represents bases without print.

Bootstrap (1000 replications).

RESULTS

Cloning and sequence analysis of *OsXET9*

Using GeneChip Rice Genome Array (Affymetrix) representing 51, 279 transcripts from *japonica* and *indica* rice, we identified *OsXET9*, a gene highly induced by these stresses. To further analyze *OsXET9*, we designed and synthesized two specific primers based on the conserved region after searching GenBank, and then cloned the cDNA sequence of *OsXET9* containing the complete ORF by RT-PCR from rice Pei'ai 64S. *OsXET9*, which is located in chromosome 4, was found to have two introns (Figure 1A). Sequence analysis showed that the cloned cDNA is 874 bp in length containing a 854 bp ORF which encodes a protein of 284 amino acids and shared 99.64% identity to the corresponding sequence of Nipponbare (Genbank accession: NM_001060322). Comparison result showed that there were 3 bp difference and a 12-bp surplus, and in ORF there were 4 amino acids surplus. With comparison of *OsXET9* with xyloglucan endotransglycosylase (AF443603) of Nipponbare which is published in GenBank, there were 8 bp different, 1 amino acid different and 4 amino acids surplus, the similarity was 99.06% with comparison of *OsXET9* with corresponding sequence of Guang Lu Ai 4 (CT834497) which is published in GenBank, there were 3 bp different, but only 1 amino acid different in ORF exhibited 99.65% similarity. The small differences between them were most probably due to the different rice subspecies.

Several putative cis-elements related to stress responses were identified in the putative promoter region

of *OsXET9* about 2.0 kb upstream of the ATG site using PlantCARE (Figure 1B), including 27 TATAbox, 28 CAATbox (common cis-acting element in promoter and enhancer regions), 2 ABRE (ABA-responsive element), 1 ARE (cis-acting regulatory element essential for the anaerobic induction), 1 C-repeat/DRE (regulatory element involved in cold- and dehydration-responsiveness), 2 CGTCA-motif (MeJA-responsive element), 2 TGACG-motif (MeJA-responsive element), 4 G-box (light responsive element), 3 HSE (cis-acting element involved in heat stress responsiveness), 1 LTR (cis-acting element involved in low-temperature responsiveness), 3 MBS (MYB binding site involved in drought-inducibility), 1 P-box (gibberellin-responsive element), 2 TCA-element (cis-acting element involved in salicylic acid responsiveness), 1 WUN-motif (wound-responsive element) and so on. The existence of these stress related cis-elements, showed that with the promoter region of *OsXET9* responses to various kinds of stress signals, the expression of *OsXET9* is regulated by several stress factors. In order to get protein structure information of *OsXET9*, protein predict software online (<http://www.ebi.ac.uk/InterProScan/>) was used to deduce its secondary structure (Figure 2), and there were 1 glycoside hydrolase family 16 domain, 1 glycoside hydrolase family 16 active site domain, 3 β -glucanase, 1 concanavalin A-like lectin/glucanase, 1 xyloglucan endo-transglycosylase C-terminal, 1 concanavalin A-like lectin/glucanase subgroup, 1 xyloglucan endo-transglycosylase, 1 signal-peptide and 1 transmembrane-regions.

Phylogenetic analysis of *OsXET9*

The database search and analysis with BLASTp through

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(B)
-1929
+CGGTTAGC CCAAGTACTC CAGTCCAGGC AAGTGACGGT GGCAGGATGT AAGTGGCCCA .....
-GCCAATCG GGTC AATGAG GTCAGGTCCG TTCACTGCCA CCGTCT ACAATCACCC GGT .....
                                         ABRE

-1859
                                         HSE
+CGCTGAAT GGCCTTATCC TCCTCTACCA AAAAGA AAAAAACTTC TGTA .....
-GCGACTTA CCGGAATAGG AGGAGATGGT TTTCTTTTT TTGAAGACAT G .....

-1359
+ACATGATCGT ATCGCCCA AATCCAATAGA AAAATACACAA CGTAGTACT CCGTATTACC .....
-TGTACTAGCA TAGCGGGTGT TAGGTATCT TTAATGTGT GCGATCATGA GG ATAAT GG .....
                                         TATA-box

-1209
+CCAGTTGGCATCCGGCCACCGTCTTCAATCCGGTGGTGGGGCCGGCGGTCGGCCAGG .....
-G GTCAAAC CGTAGCCCGTGGCAGAAGTTAGGCCACCAACCCGGCCGGC CAGCCGGT CC .....
MBS                                     C-repeat/DRE

-989
TATA-box                                TCA-element
+GACC TATA TAAITGTAAA GAGTATCTA TTAATGGGT AGATTGA GAGAAGACT TAAA .....
-CTGGGATATA ATTAACATTT CTC AATAGAT AATATAACCA TCTAACCTCTTCTGATATT .....

-799
                                         G-box
+CGCTTGCTGC GCGCAGCGAGAAAAAAGC CACGAC CAG AATTCCA .....
-GGAAACGACG CCGCTCGCGC TCTTTTTTCG TGTGTGGTC TAAAGGTGT .....

-729
P-box                                     ABRE
+CTCCCCA CTTTT TCGTGT CTCCCCTCTTCACTTGT TCTCGCCAAA TCC TACCT A GTC .....
-GAGGGGTGGA AAACAGCACAGAGGGGTAGA AGTGAACAAA AGAGCGGTTT AGGATGCAT .....

-519
                                         CGTCA-motif
+ACACACTCT CCAACCACT CCTCA CCCT CAC .....
-TGTGTAGAG GGTGGTGA GGCAGTGGGA GTG .....

-449
+TATGGCTGT TTAGTGGG GAAAAGGAAA TTTTGGGTG TCACATCAGA CGTTGACCG GA .....
-ATACCGACAC AAATCAACCC CTTTT CTTT AAAAA CCCAC AGTGTAGTCT GCAA ACTGGC CTA .....
                                         WUN-motif   HSE                                     MBS

-379
+GGGGTTTTCG GACACGAATG AAAAAACTAA TTTCAGAAC .....
-CCCA AAAGC TGTGCTTAC TTTTGTGATT AAAGTCTGA GCGG .....
LTR

-309
CGTCA-motif
+GCCTACTTAA GC CGTCA TTA GCACATGTGG GTTACTGTAG CACT .....
-CGGATGAATT CG CGCAT AAT CGTGTACACC CAATGACATC GTGAATA .....
TGACG-motif

-239
TATA-box                                ARE
+TCAAAAAGATT CGTCTCGCGA TT TATA GCTA AACTGTGCAA T TGGT TTC TT .....
-AGTTTTCTAA GCAGAGCGCT AAATATCGAT TTGACACGTT AACCAAAAAG AA .....

-169
+TCCATGCA TA TGCCAAAAG ATCGATGTGA TAGGTGAAGG GGAAAATTT TGAGAACTA .....
-AGTACGTAT ACAGGTTTCT AAGCTACACT ATCCACTTCC C TTTTAAAAA CTCTTGATT .....
                                         HSE

-99
TATA-box
+ TATAA CCCT GCGAGGCACG CACCGATCGA TCACTACACC TTC .....
-ATATTGGGA CGCTCCGTGC GTGGTAGCT AGTGATGTGG AAG .....

-29
                                         +1
+GATCGATCAC AAAGAGGTAA ACAGTAAACAC ATGGGGTT .....
-CTAGTAGTG TTTCTCCAAT TGTCATTGTG TACCCCAA .....

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Figure 1. Contd.

the NCBI website showed that the deduced amino acid sequence of *OsXET9* has higher homology (98%) with xyloglucan endotransglycosylase (AAL35903) from *japonica* and has 99% homology with OSIGBa0118P15.8 (CAH66718) from *indica*. BLASTp showed that *OsXET9* has the highest identity with xyloglucan endotransglycosylase from other plant species. The percentages of identity were from 81 to 51%, such as 81% (SORBIDRAFT_06g027670), 78% (*ZmXTH15*), 57% (*DcXTH*), 56% (*SaXET*), 56% (*MdXET*), respectively (Figure 3). Comparison of *OsXET9* with several similar proteins from other species revealed that GH16_XET (xyloglucan endotransglycosylases) motifs were highly conserved in these proteins, again suggesting that they have the same functions, probably associated with cleave and religate xyloglucan polymers in plant cell walls via a transglycosylation mechanism. Thus, XET is a key

enzyme in all plant processes that require cell wall remodeling and also have the functions, probably associated with survival in a harsh stress environment.

A list of putative *OsXTHs* was compiled by searching databases for ORFs with characteristic sequences that are highly conserved among XTHs. To elucidate phylogenetic relationships between the deduced protein sequences of *OsXET9*, several known plant XET genes and the XTH family genes in rice, a phylogenetic analysis was performed using full-length protein sequences (Figure 4). The result shows that *OsXET9* was more closely related to *OsXTH9*.

Expression analysis of *OsXET9*

In order to find and separate genes in relation to stress

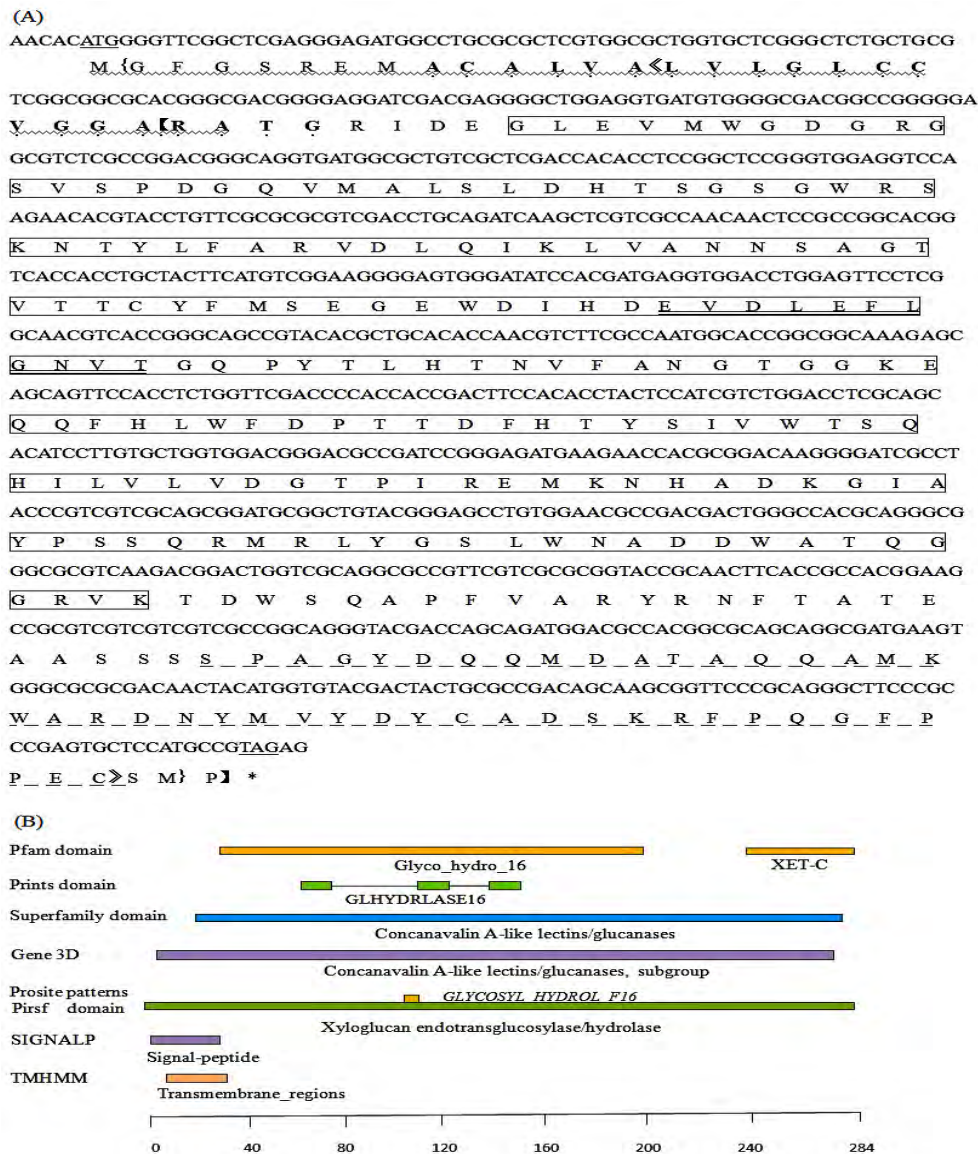


Figure 2. The deduced ORF and the deduced secondary structure of the OsXET9 protein. (A) The deduced ORF and the conserved secondary structure of the OsXET9 protein. The predicted amino acid sequence : sequence between “ **【** ” is the concanavalin A-like lectin/glucanase super family; the glycoside hydrolase, family 16 domain is boxed; sequence between “ **《** ” is the concanavalin A-like lectin/glucanase, subgroup; sequence between “ **{ }** ” is the xyloglucan endotransglucosylase/hydrolase; sequence which is double underlined is glycoside hydrolase, family 16, active site; sequence which is underlined with discontinuous line is xyloglucan endo-transglycosylase, C-terminal; sequence which is underlined with wavy line is signal-peptide; the transmembrane_region is underlined with emphasis symbol and is shown in bold letters. (B) The visual map of the structure information of the OsXET9 protein.

tolerance, we used GeneChip Rice Genome Array (Affymetrix) representing 51, 279 transcripts from *japonica* and *indica* rice to analyze expression levels of the whole genome of super hybrid rice maternal plant - Pei'ai 64s in leaves and panicles of seedling, booting and flowering stage, and obtained the up-regulated gene

OsXET9 (Figure 5). Expression levels of OsXET9 were up-regulated obviously in low and high temperature, and drought conditions of seedling stage, booting stage and flowering stage. According to the result of microarray analysis, the expression levels of OsXET9 were elevated in different tissues of rice at different stages under the

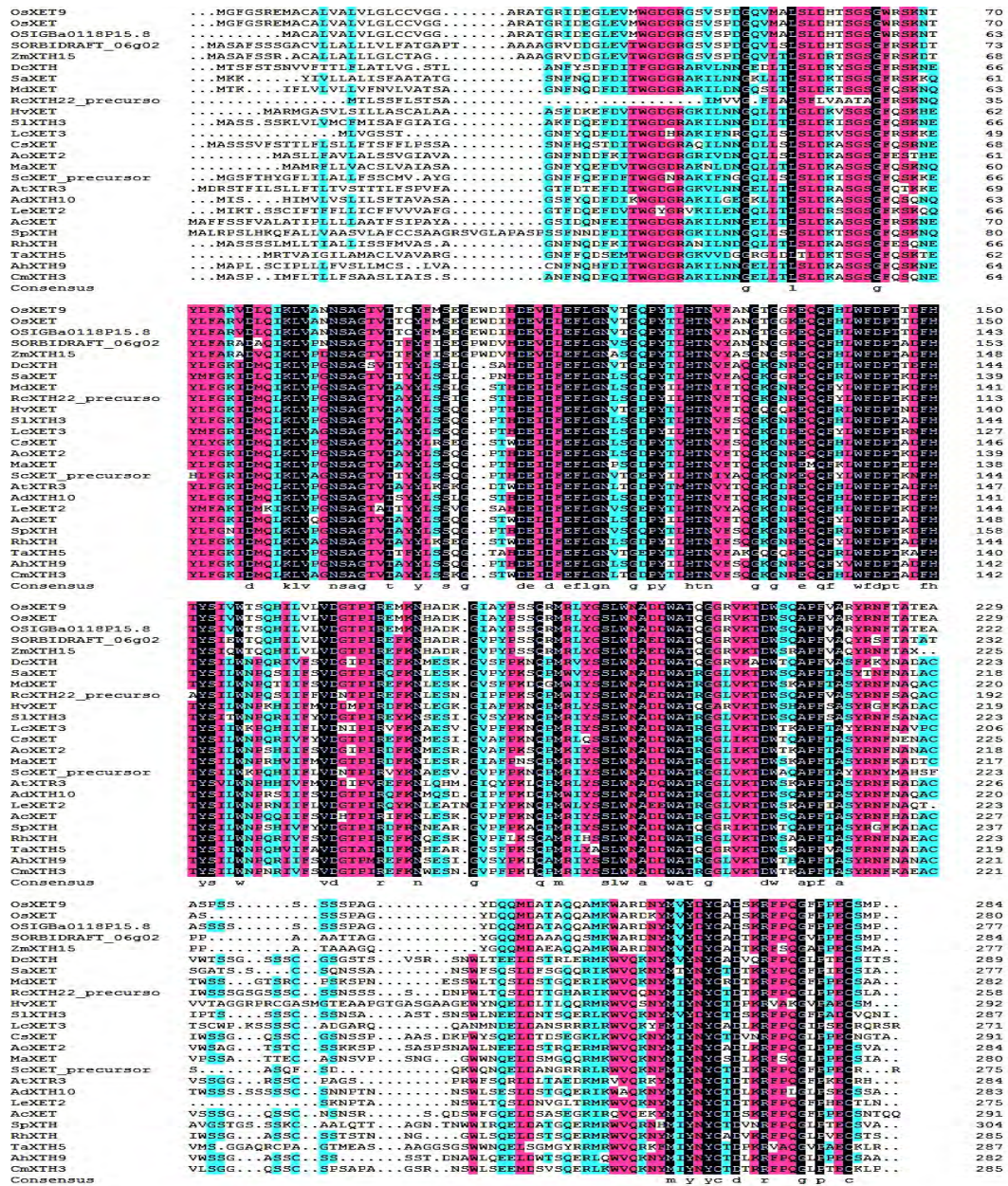


Figure 3. Multiple amino acid sequence alignment of the xyloglucan endotransglycosylases (prepared using software DNAMAN, Version 6). The amino acid sequence of OsXET9 from the rice Pei'ai64s (*indica*) is aligned with that of xyloglucan endotransglycosylase from Nipponbare (*japonica*) (OsXET, GenBank: AAL35903), Guanglu ai 4 (*indica*) (OSIGBa0118P15.8, GenBank: CAH66718), *Sorghum bicolor* (SORBIDRAFT_06q027670, GenBank: XP_002448474), *Zea mays* (ZmXTH15, GenBank: NP_001149692), *Dianthus caryophyllus* (DcXTH, GenBank: BAJ10395), *Striga asiatica* (SaXET, GenBank: ABD98046.1), *Malus x domestica* (MdXET, GenBank: AAN07898.1), *Ricinus communis* (RcXTH22 precursor, GenBank: XP_002532673.1), *Hordeum vulgare* subsp. *vulgare* (HvXET, GenBank: CAA63662.1), *Solanum lycopersicum* (SIXTH3, GenBank: AAS46241.1), *Litchi chinensis* (LcXET3, GenBank: ABK30789.1), *Cucumis sativus* (CsXET, GenBank: CAD87536.1), *Asparagus officinalis* (AoXET2, GenBank: AAF80591.1), *Musa acuminata* (MaXET, GenBank: ABL10090.1), *Solanum chacoense* (ScXET precursor, GenBank: ABE11608.1), *Arabidopsis thaliana* (AtXTR3, GenBank: NP_568859), *Actinidia deliciosa* (AdXTH10, GenBank: ACD03220.1), *Solanum lycopersicum* (LeXET2 GenBank: AAG00902.1), *Annona cherimola* (AcXET, GenBank: ACK36945.1), *Sagittaria pygmaea* (SpXTH, GenBank: BAE06063.1), *Rosa hybrid cultivar* (RhXTH, GenBank: BAH36875.1), *Triticum aestivum* (TaXTH5, GenBank: AAT94297.1), *Actinidia hemsleyana* (AhXTH9, GenBank: ACD03219.1) and *Cucumis melo* (CmXTH3, GenBank: ABI94063.1), using the multiple sequence alignment program. Letters denote residues identical between all of the protein sequences.

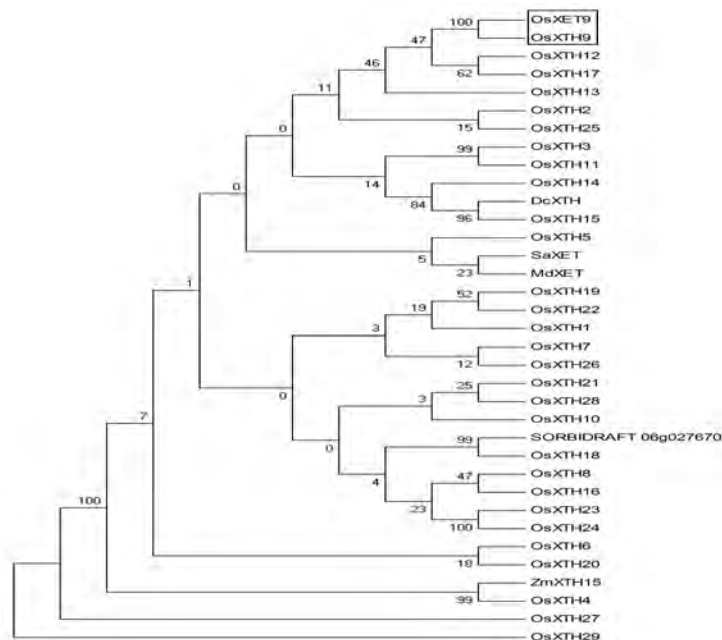


Figure 4. Phylogenetic-alignment of the *OsXET9* deduced amino acid sequence with other plant XTHs and *OsXTHs*. The phylogenetic-tree was generated by the maximum parsimony method using the Mega4.1. bootstrap (1000 replications). Bootstrap values are shown in the nodes of the tree. Details and GenBank accession numbers are: SORBIDRAFT_06g027670, XP_002448474; *ZmXTH15*, NP_001149692; *DcXTH*, BAJ10395; *SaXET*, ABD98046.1; *OsXTH1*, AP003899; *OsXTH2*, AC136481; *OsXTH3*, AL606650; *OsXTH4*, AP003907; *OsXTH5*, AP004657; *OsXTH6*, AL606638; *OsXTH7*, AL662996; *OsXTH8*, AP004705; *OsXTH9*, AL606638; *OsXTH10*, AP005445; *OsXTH11*, AP005445; *OsXTH12*, AP005445; *OsXTH13*, AP005398; *OsXTH14*, AP005398; *OsXTH15*, AP004784; *OsXTH16*, AL662996; *OsXTH17*, AP004705; *OsXTH18*, AP005445; *OsXTH19*, AC113930; *OsXTH20*, AC025783; *OsXTH21*, AP003839; *OsXTH22*, AP004026; *OsXTH23*, AP005859; *OsXTH24*, AC120506; *OsXTH25*, AC018929; *OsXTH26*, AP004886; *OsXTH27*, AP079037; *OsXTH28*, AC118981; *OsXTH29*, AP005430.

stresses, with an average increase of 5.20-fold, ranging from 0.016- to 26.86-fold increases. Under cold treatment, the expression level of *OsXET9* increased 26.86- and 9.96-fold in the leaf of seedling and booting stages, respectively, so tissue in these two stages are hypersensitive to low temperature. In the panicle of heading and flowering stage, expression level of *OsXET9* up-regulate 8.02-fold under drought conditions, so panicles in this stage may be hypersensitive to water-deficient. Expression level of *OsXET9* changed slightly in high temperature, and only up-regulate 1.85-fold in the panicle of heading and flowering stage, so *OsXET9* is insensitive to high temperature.

To verify the expression profile of *OsXET9* obtained by the microarray analysis, quantitative real-time RT-PCR analysis of the gene was conducted (Figure 5). The result shows that expression levels of *OsXET9* were increased at low and high temperature, and drought conditions; moreover, the tendency to increase was in concurrence

with genechip microarray, suggesting that *OsXET9* is a multiple stress-responsive gene in rice. However, some variations in the amplitude of expression levels were observed between the two sets of data. This most likely resulted from differences in the technologies used and also from the use of the plant materials sampled at different times.

DISCUSSION

Genes encoding members of the xyloglucan endotransglycosylase/hydrolase enzyme family have been characterized in a number of plants recently, including rice (Yokoyama et al., 2004), club moss (Van Sandt et al., 2006), tomato (Saladie et al., 2006), maize (Genovesi et al., 2008), kiwifruit and grape (Atkinson et al., 2009), *Fragaria chiloensis* (Opazo et al., 2010), nasturtium (Stratilova et al., 2010), *Arabidopsis thaliana*

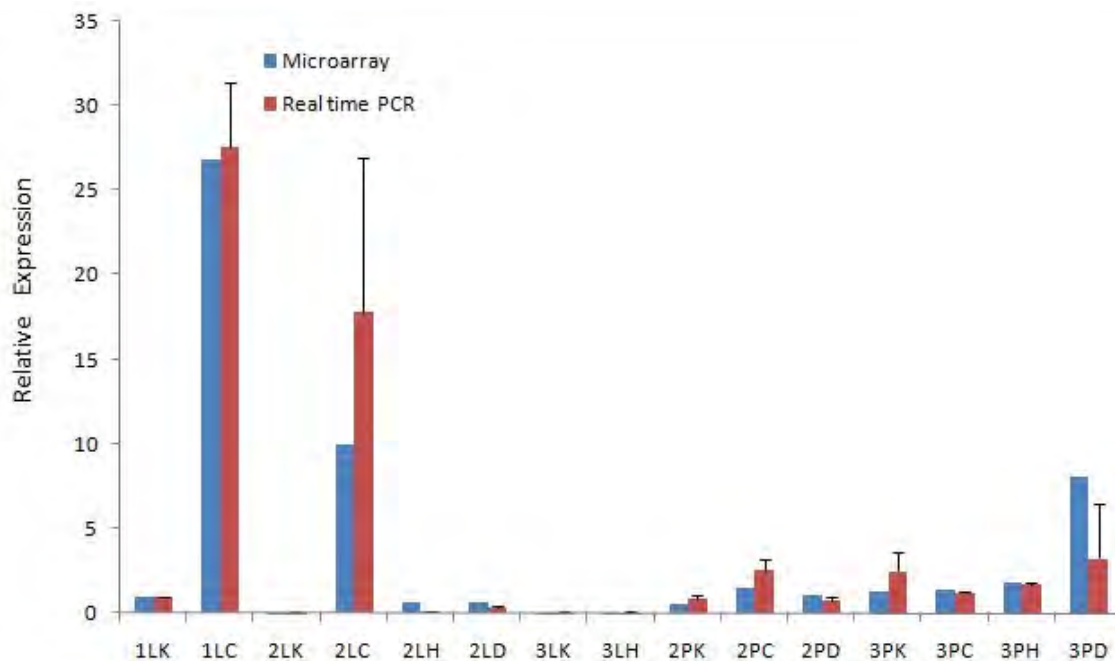


Figure 5. The relative expression of *OsXET9* in different tissues of Pei'ai64s at different development stages under stresses. 1: Seedling stage; 2: booting stage; 3: heading and flowering stage; L: leaf; P: panicle; K: control; C: cold; H: heat; D: drought.

(Maris et al., 2011). In our study, the cDNA sequence of *OsXET9* gene was cloned and its upstream region include imaginable promoter that was analyzed (Figure 1). The result shows that it was cloned successfully, and several cis-elements that responded to abiotic stresses were found in the promoter region.

The database search and analysis with BLASTp of *OsXET9* gene showed that it belongs to the family of xyloglucan endotransglycosylases (Figures 3 and 4). Xyloglucan endotransglycosylases participate in modification of cell walls via cleave and relegate xyloglucan polymers (Genovesi et al., 2008). Some researches showed that diverse factors regulate the expression of XETs and XET related (XTR) proteins, including auxin (Xu et al., 1995, 1996), abscisic acid (Wu et al., 1994), gibberellins (Genovesi et al., 2008), ethylene (Saab and Sachs, 1996) and brassinosteroids (Zurek and Clouse, 1994). Upregulation of XETs is also triggered by various environmental stimuli, including touch, darkness, temperature shock (Xu et al., 1995, 1996), wind (Antosiewicz et al., 1997) and flood (Saab and Sachs, 1995). This can also provide evidence for expression of *OsXET9* under stress treatment.

In this study, we obtained the expression profile of *OsXET9* in cold and high temperature, and drought conditions, and saw that it was unregulated obviously in the leaves of seeding and booting stage after short time cold treatment, also, upregulation was observed in the

panicles of booting stage under cold treatment and heading and flowering stage under drought treatment (Figure 5). The average upregulation in the leaf under cold treatment with GeneChip Rice Genome Array and real-time quantitative PCR was 18.26- and 22.69-fold. The expression of XTH genes has been found particularly in elongating tissues. Multiple isoforms of XTHs are expressed in different organs of different plant species in response to many hormonal, environmental and developmental stimuli (Xu et al., 1995; Akamatsu et al., 1999; Yokoyama and Nishitani, 2001). RT-PCR analysis showed that three CaXTH mRNAs were concomitantly induced by a broad spectrum of abiotic stresses, including drought, high salinity and cold temperature, and in response to stress hormone ethylene, suggesting their role in the early events in the abiotic-related defense response (Cho et al., 2006). Thus, the characterization of individual XTH genes and their corresponding proteins within a single species is essential if we are to understand their specific role (Jimenez et al., 2006). Due to the restriction of conditions, we cannot treat materials in each tissue of all the developmental stages to analyze their expression level. But choose leaf and panicle in one of the development stage under 1 to 3 stress treatments to analyze expression patterns of *OsXET9*, which did not affect global recognition of its response to stress treatments. However, real-time quantitative PCR can be used to implement further analyses of stress. Now, the

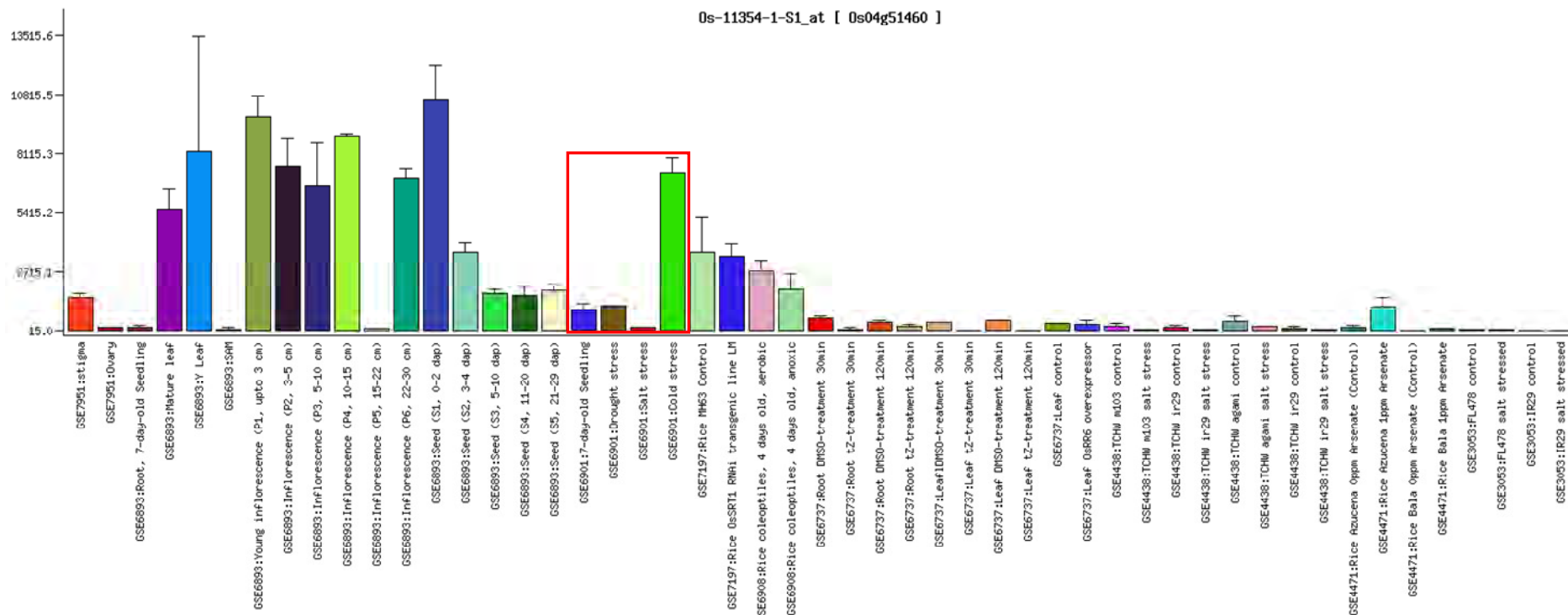


Figure 6. RiceGE: Gene Expression Atlas of *OsXET9* (from <http://signal.salk.edu/>). The columns in the red rectangle are expression data for stress treatment in rice seedlings of *Oryza sativa*. 1. GSE7951: Genome-wide gene expression profiling of rice stigma [*Oryza sativa*], 2. GSE6893: Expression data for reproductive development in rice [*Oryza sativa*], 3. GSE6901: Expression data for stress treatment in rice seedlings [*Oryza sativa*], 4. GSE7197: Down-regulation of OsSRT1 induces DNA fragmentation and cell death in rice [*Oryza sativa*], 5. GSE6908: Transcript profiling of the aerobic and anoxic rice coleoptile [*Oryza sativa*], 6. GSE6737: Over-expression of a type-A response regulator alters rice morphology and cytokinin metabolism [*Oryza sativa*], 7. GSE4438: Expression data from rice under salinity stress [*Oryza sativa* (*indica* cultivar-group), *Oryza sativa* (*japonica* cultivar-group)], 8. GSE4471: Expression data from rice varieties Azucena and Bala grown in 0 and 1 ppm arsenate [*Oryza sativa*], 9. GSE3053: Rice salt expression [*Oryza sativa* (*indica* cultivar-group)], 10. GSE661: ABA or GA calli treatment [*Oryza sativa*].

function of *OsXET9* for stress treatment is researched.

We also searched the internet (<http://signal.salk.edu/>) and found RiceGE: Gene Expression Atlas of *OsXET9* (Os.11354.1.S1_at). The columns in the red rectangle were expression data for stress treatment in rice seedlings of *Oryza sativa*. The results show that the expression level of *OsXET9* was unregulated obviously under cold

stress treatment, which was in concordance with the results of GeneChip Rice Genome Array (Affymetrix) and Real-time Quantitative PCR (Figure 6).

Conclusion

This study describes the identification and

molecular characterization of a new low-temperature regulated gene *OsXET9* from Pei'ai 64s, whose expression in leaves of seeding and booting stage is upregulated obviously after cold treatment. The complete cDNA which is 874 bp in length and G+C contents of 67.4% was cloned from Pei'ai64s. The longest open reading frame within encodes a putative protein of 284 amino acids, with a calculated molecular mass of

31 kDa and an isoelectric point of 5.2. The homology gene was found in rice, *Sorghum bicolor*, *Zea mays*, *Dianthus caryophyllus*, *Arabidopsis thaliana* and so on. They all belong to xyloglucan endotransglycosylases family, and involved in the alteration of architectonic of cell walls with the function of cleaving and relegating xyloglucan polymers of the cell wall. There were 12 kinds of cis-elements that are related to stress responses which were found in the predicted promoter region, and it was further certified that *OsXET9* is related to stress treatments.

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