

Full Length Research Paper

# Isolation of a novel PP2C gene from rice and its response to abiotic stresses

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**Biotic and abiotic stresses adversely affect plant growth and improvement. In order to discover new stress-tolerance genes in rice (*Oryza sativa* L.), we analyzed a global genome expression profiling of the *indica* cultivar Pei'ai 64S, using Affymetrix rice expression chip, subjected to cold, drought or heat stress. A large number of genes highly up regulated or down regulated were identified under the stresses. One of these genes, *O. sativa* L. protein phosphatase2C-I (*OsPP2C1*), was highly induced in leaf and panicle at the heading and flowering stages, in response to all stresses. The expression profile of *OsPP2C1* obtained by the microarray analysis was confirmed by quantitative real-time PCR (qRT-PCR). The two sets of data matched very well, suggesting that *OsPP2C1* is a multiple stresses responsive gene in rice. In order to study its function in stress tolerance, the cDNA of the gene was cloned by reverse transcription (RT-PCR). Sequence analysis showed that there is 3bp discrepancy between the cDNA of *japonica* and *indica* group. Deduced protein from *OsPP2C1* cDNA has molecular weight about 37.6 kD and isoelectric points about 6.16. Analysis of the putative promoter regions showed that there were about 10 kinds of stress induced by cis-acting elements. Based on the mentioned analyses and results obtained, we propose that *OsPP2C1* is a novel candidate gene involved in stress tolerance in rice.**

**Key words:** Rice, stress, microarray, real-time PCR, analysis function.

## INTRODUCTION

Rice (*Oryza sativa*) is an essential crop, providing a carbohydrate source for more than one-third of the world's population and it is widely cultivated in arable land worldwide (Jain et al., 2007). Low temperature, drought and high salinity are common stress conditions that adversely affect plant growth and crop production (Duan et al., 2009; Xiong et al., 2002). Some researches have showed that plants subjected to stress conditions, is not a passive defense, but to take proactive measures to deal with stress, hence, it is necessarily developed high-quality, high yield varieties (Beck et al., 2007; Duan et al., 2009; Rao et al., 2006; Serraj et al., 2009; Yokoi et al., 2002).

The reversible phosphorylation of proteins is a fundamental mechanism by which living organisms modulate

cellular processes including cell cycle events, growth factors responses, hormone and environmental stimuli responses, metabolic control, and developmental processes. Protein phosphatases (PPs), by reversing the action of protein kinases, provide modulations of protein phosphoregulation. Protein phosphatases can be divided into two major classes: protein tyrosine phosphatases (PTPs) and protein serine/threonine phosphatases. Based on enzymological criteria using specific inhibitors or according to distinct amino acid sequences and crystal structures, most serine/threonine protein phosphatases have been placed into two subcategories: the protein phosphatase 1 (PP1) and protein phosphatase 2 (PP2). PP2 proteins were further distinguished by metal ion requirements: while protein phosphatases 2A (PP2As) have no ion requirement, PP2Bs require  $\text{Ca}^{2+}$  and PP2Cs require  $\text{Mg}^{2+}$  or  $\text{Mn}^{2+}$  (Cohen, 1997; Schweighofer et al., 2004; Xue et al., 2008; Yang et al., 2009).

The PP2C gene family is one of the largest families in the plant kingdom, 76 genes from *Arabidopsis* were iden-

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tified as PP2C candidates and 78 genes were in rice (Kerk et al., 2002; Schweighofer et al., 2004; Singh et al., 2010; Xue et al., 2008), but only six PP2Cs were found in the yeast (*Saccharomyces cerevisiae*) genome and no more than fifteen are present in the human genome (Cheng et al., 2000; Stark, 1996; Xue et al., 2008). These 78 rice genes were grouped into 11 subfamilies and PP2Cs belong to subfamily A (Singh et al., 2010; Xue et al., 2008). Group A contains most of the identified genes that are associated with abscisic acid (ABA) signal transduction, such as ABI1 and ABI2 (Baudouin et al., 1999; Cheng et al., 2000; Kerk et al., 2002; Merlot et al., 2001; Schweighofer et al., 2004; Song et al., 2006; Stark, 1996; Xue et al., 2008). Recent studies show that a pathway based on pyrabactin resistance 1/PYR1-like (PYR/PYL) ABA receptors, PP2Cs and SNF1-related protein kinase 2s (SnRK2s) forms the primary basis of an early ABA signaling module. In the presence of ABA, the PYR/PYL receptor proteins can disrupt the interaction between the SnRK2s and PP2Cs, the PP2C-mediated dephosphorylation of the SnRK2s and resulting in the activation of the SnRK2 kinases (Fujii et al., 2009a, b; Hirayama and Umezawa, 2010; Hubbard et al., 2010; Tougane et al., 2010; Umezawa et al., 2009; Yang et al., 2010).

A common feature of all PP2C-type phosphatases is the presence of 11 characteristic subfamilies in the catalytic part of the protein (Bork et al., 1996; Schweighofer et al., 2004). Eukaryotic PP2Cs carry the catalytic domain at either the N- or at the C-terminus. The catalytic domain of most (44 out of 76) *Arabidopsis* PP2Cs is located at the C-terminus, with different N-terminal extensions; 24 PP2Cs have N-terminal and C-terminal extensions, however in a few cases, PP2C genes start with the catalytic part (Rodriguez, 1998; Schweighofer et al., 2004).

Here, we present the discovery of gene *OsPP2C1* response to cold, heat and drought stress by array. Expression of *OsPP2C1* was analyzed by GeneChip rice genome array and qRT-PCR in leaf and panicle tissues at seedling, booting and heading stages from plants under no stress or cold, drought or heat stresses. cDNA of the gene was cloned by RT-PCR.

## MATERIALS AND METHODS

### Plant materials growth and management

The germinal seeds of cultivated rice *Pei'ai* 64S (*O. sativa* L.) were suspended in a sterile solution of 0.1% HgCl<sub>2</sub> for 10 min, washed 3 times using running water, immersed for 3 days under 25°C and changed water once a day, then were germinated and grown in distilled water at 37°C for 2 to 3 days. They were partly sown in pots that put in net room at Institute of Subtropical Agriculture of Chinese Academy of Science. Plants were divided into one control and two treatment groups. The control group was maintained under normal growth conditions and the treatment groups were exposed to drought, heat and cold stresses. The leaf samples were from one treatment group at the five-leaf stage, another group plants were put in other pots which contained five ones, as the test materials of the booting and flowering stages.

### Rice cold, heat and water-deficit treatment

For the drought tests the water was poured away from the basin, the treatment groups were put in scaffold to dry out meanwhile the control group was kept the water level. The leaves were harvested when they started curling after 16 h. For the heat tests, the treatment group was exposed to 45°C for 2 h then plants were harvested for the cold tests, the treatment group was harvested after exposed to 4°C for 12 h at seedling stage and to 12°C for treatment for 16 h at booting and flowering stages. All the rice was seedlings in U.S. Percival produced PGC15.5 artificial climate chamber, while the control group was in another chamber at 28°C. Test groups and control groups were in the dark.

### Sample preparation

Four or five countdown second leaves were collected from treatment and control group and four or five leaves were harvested which were not out of the young panicle or the middle of spiked out flower. The materials were cut into pieces, then ground into a dry powder with liquid nitrogen and immediately divided into pre-installed 1.0 ml TRIzol extraction (Invitrogen) in 1.5 ml centrifugal tubes, about 100 mg each tube. We used low temperature marker pen to mark labels, tightly closed lid, then vigorously vortex to sure that the samples were mixed with TRIzol extraction, seal the tubes with Parafilm wrap. The samples were stored at -70°C until required.

### Total RNA isolation

Total RNA was isolated from the frozen samples using TRIzol. Extract method using TRIzol reagent (Invitrogen). The samples saved in -70°C were taken out; vortexed to homogeneity, chloroform (200 µl) was added, vigorously shaken for 15 s and then centrifuged at 12 000 × g for 15 min at 4°C. The upper layer was carefully removed from each tube, was transferred to another centrifuge tube, isopropanol (500 µl) was added, precipitated for at least 1 h at -40°C, then centrifuged to separate the RNA. The RNA pellets were twice washed by 75% ethanol, air dried and dissolved in the appropriate volume of RNase-free water. The purity of RNA was determined by the A260/280 absorbance ratio (1.9 to 2.0). Isolated RNAs were stored at -70°C, after checking the purity and integrity of 18 S, 5 S and 28 S rRNA bands on 1.5% agarose gel.

### Real-time quantitative PCR

The reagent of total RNA extraction was purchased from Fermentas, which could remove genomic DNA with DNase (operational method was provided by factory). In order to detect the quantitative real-time fluorescent PCR products, QIAGEN's SYBR green qRT-PCR one step kit (Cat. No.204243) and RoterGene3000 PCR instrument was used. Real-time PCR primers were designed by primer expression 3.0 software, the target gene (*OsPP2C1*) primers were *OsPP2C1*-F: 5'-GCC GCA GCT CCG ACA A-3'; *OsPP2C1*-R: 5'-CTA CAG CAT CAG CTG GGT GAC A-3', amplified fragment length 874 bp; actin gene was *18S*, the primers *18S*-F: 5'-CGT CCC TGC CCT TTG TAC AC-3'; *18S*-R: 5'-CGA ACA CTT CAC CGG ATC ATT-3'. To detect possible residual DNA, PCR system was without RT mix (containing reverse transcriptase) as a reference. Thermal cycle programmed as follows: incubation for 30 min at 48°C and an initial denaturation for 10 min at 95°C, 40 amplification cycles (15 s at 95°C, 40 s at 58°C and 20 s at 72°C).

The computational method of real-time quantitative PCR was: Rel. Exp (the expression of relative target genes) = 2<sup>-ΔΔCt</sup>, where ΔΔCt = (unknown sample ΔCt) - (Calibrator ΔCt), unknown sample

$\Delta Ct$  = (internal reference gene Ct) - (target gene Ct), Calibrator  $\Delta Ct$  = (reference sample internal reference gene Ct) - (reference sample of target gene Ct).

### Cloning of cDNA

Sequences used in this study for PCR from Gremene and NCBI and the gene PCR primers were designed by Primer Premier5 that were *OsPP2C1-F*: 5' -AAG CTT AGG TGT TTG ATG TCG ATG GC -3', *OsPP2C1-R*: 5' -GGA TCC CAT CAG CTG GGT GAC ACT CT -3'. The PCR cycle programmed as follows: an initial denaturation for 5 min at 95°C, 30 amplification cycles [30 s at 95°C (denaturation), 30 s at 58°C (annealing) and 110 s at 72°C (polymerization)], followed by a final elongation for 10 min at 72°C. The PCR products were electrophoresed through 1.0% agarose and visualized with ethidium bromide. The amplified product was eluted from the gel by gel extraction kit. Purified PCR product was ligated in cloning vector PMD19-T. The competent cells (Li et al., 2010) of *Escherichia coli* top10 were transformed with the ligation mixture containing the recombinant DNA. The positive transformants were screened by using ampicillin selection. Colony PCR was done for confirmation and restriction enzymes BamHI and HindIII were used for double cuts. Restricted fragments were analyzed on 1.0% agarose gel. Positively screened clone was sent to YINGJUN, for nucleotide sequencing. *E. coli* top10 was prepared in our laboratory and stored at -70°C.

### Identification of PP2C genes in rice and other species

To collect all members of the PP2C gene family in *indica cultivar*, all predicted *OsPP2C* protein sequences were initially used as query sequences to search against the NCBI using BLASTP. Multiple BLAST searches using protein sequences of significant hits and removal of redundant sequences from our data set.

### Alignment and phylogenetic analysis of PP2C sequences

Multiple alignments of amino acid sequences were generated using ClustalW and were manually corrected. Phylogenetic trees were constructed by neighbor-joining logarithms of Mega (5.02). Bootstrapping was performed 1000 times to obtain support values for each branch.

## RESULTS

### *OsPP2C1* gene cloning

The cDNA extracted from locally isolated leaves of Nipponbare and 93-11 was PCR amplified using the same *OsPP2C1* gene specific primers. PCR product was about 1300 bp amplified from Nipponbare genomic as well as the 93-11 by analyzing on 1.0% agarose gel. The results of nucleotide sequencing showed that the PCR product sequences of Nipponbare (LOC\_Os09g15670, 9:9565870-9568504:1) and 93-11 (BGIOSIBCE029483, 9:8372263-8374530:1) were similar 99.0%.

### *OsPP2C1* protein structure

*OsPP2C1* is 1435 bp in length and it has an open reading

frame (ORF) of 1077 bp nucleotides of Nipponbare encoded deduced protein is 358 amino acid residues and about in 9311 the corresponding genomic DNA sequence as a template for a complete ORF; a length of 1074 bp, encoding a protein of deduced amino acid residues 357 and with intron less in these genes (Figure 1). The calculated molecular mass of the matured proteins are approximately 37.7 and 37.6 kDa each with a predicted isoelectric point of 6.16. There is a S more at 227 site base on 358 amino acid, which 74-352 region is for PP2Cc (Serine/threonine phosphatases, family 2C, catalytic domain); 73-345 PP2C region for the (protein phosphatase2C). The analysis showed that this one amino acid difference does not affect the function of protein, because the active sites are R84, E88, D89, D117, G118, H119, D298 and D343. But the length of protein sequence is 356 aa in indica group, short at two beginning positions according to NCBI and GRAMENE. The study of protein function domains indicates that the purpose protein contains seven domains (Figure 2). These results indicate clearly that this DNA encodes protein phosphatase 2C.

### Promoter

The 1500 base-pair (bp) upstream promoter sequences from the ATG site of these *OsPP2C* genes used the Plant-CARE database and online Plant-CARE software analysis (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>). The possible promoter region (Nipponbare: 9:9564780:9566852:1 and 93-11:9:8371060:8373159:1) contains about 30 sorts of cis-acting elements; there may be 10 kinds related to stress-inducible. Stress inducible related to cis-acting elements: ABRE (cis-acting element involved in the abscisic acid responsiveness), ACE (cis-acting element involved in light responsiveness), AT-rich (sequence element for maximal elicitor-mediated activation (2 copies)), CAT-box (cis-acting regulatory element related to meristem expression), CCAAT-box (MYBHv1 binding site), CE3 (cis-acting element involved in ABA and VP1 responsiveness), CGTCA-motif (cis-acting regulatory element involved in the MeJA-responsiveness), GARE-motif (gibberellin-responsive element), HSE (cis-acting element involved in heat stress responsiveness), O2-site (cis-acting regulatory element involved in zein metabolism regulation) (Figure 3).

### Evolution and divergence of PP2C genes in rice and other species

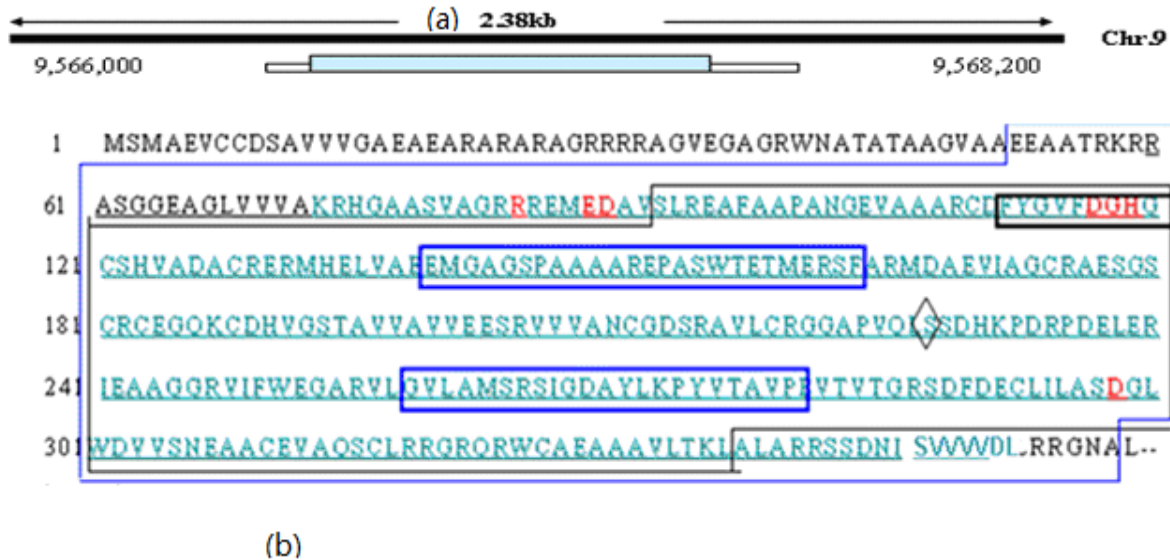
Analysis of the evolution and divergence of genes is important to know the function of proteins and the sole. All of the *OsPP2C* genes of *Japonica* cultivar had been studied (Xue et al., 2008); in this article, we analyzed 78 *OsPP2C* genes of *indica* cultivar using GRAMENE and orthologous genes of *OsPP2C1* between 27 different

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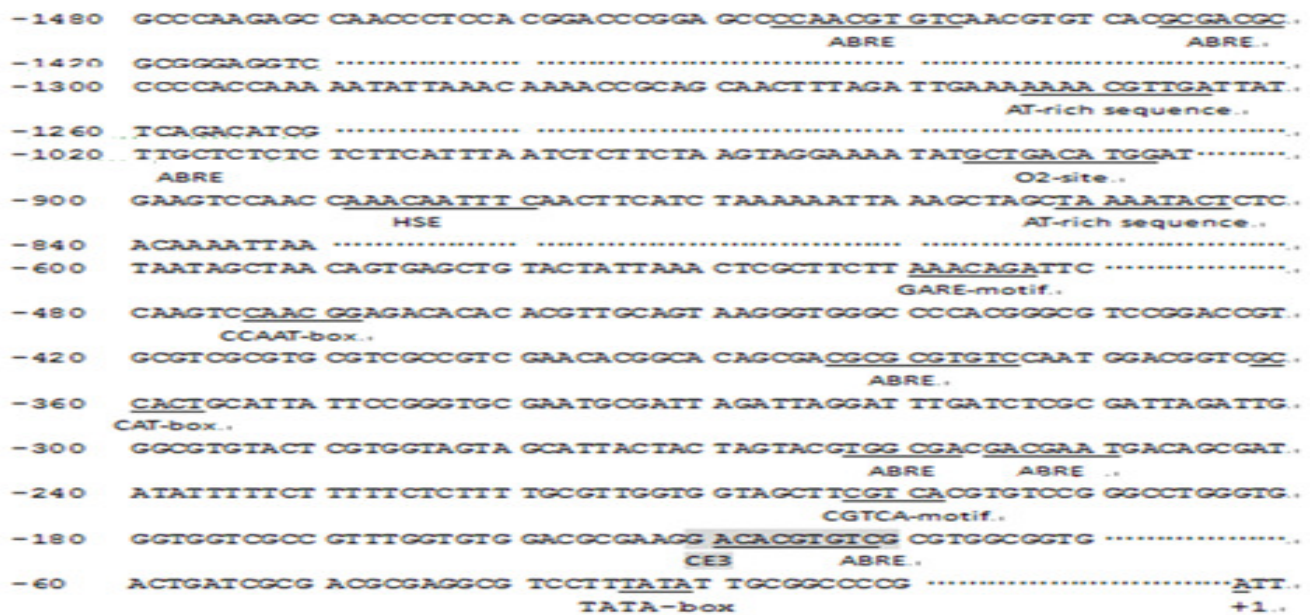
1      ATTCTCTCTCTCGTTGCTCCTGAGATCGTCTCGTCTCGTGGTGGTGGTGGTGGCCATTGC
61     CGGAGCTTCCAAGGTGTTTGATGTCGATGCGCGGAGGTGTGCTGTGATTCTGCGGTGGTGG
1      M S N A E V C C D S A V V
121    TCGGGCCGGAGGCCGGAGGCCGAGGGCCGAGGGCCGCGCGCGCGGGAGGCCGGCGGGCGGGAG
14     V G A E A E A R A R A R A G R R R R A G
181    TTGAGGGGGCAGGGAGGTGGAACGCCACGGCCGACGGCTGCGGGTGTGGCCGGCCGGAGGAGG
34     V E G A G R U N A T A T A A G V A A E E
241    CCGCGACGAGGAAGCGGAGGGCCGAGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGG
54     A A T R K R R A S G G E A G L V V V A K
301    GGCACGGGGCCGGCTCGGTGCCCGGGAGGAGGAGGAGATGGAGGACGCCGTGTCGCTGC
74     R H G A A S V A G R R R E M E D A V S L
361    GCGAGGCGTTCCGCCGCCCGGCCGAACGGGGAGGTTGCGGGCCGGCGAGGTGTGACTTCTACG
94     R E A F A A P A N G E V A A A R C D F Y
421    GGGTGTTCGACGGGCACGGCTGCTCGCACGTGGCCGACCGCTGCAGGGAGCCGGATGCACG
114    G V F D G H G C S H V A D A C R E R M H
481    AGCTCGTCCGGAGGAGATGGGCCGCCGGGTACCCGGCGGGCGGGCGCGCGAGCCGGCGT
134    E L V A E E H G A G S P A A A A R E P A
541    CTTGGACGGAGACGATGGAGCCGAGCTTCGCGAGGATGGACGCCGAGGTGATCGCCGGCT
154    S U T E T M E R S F A R M D A E V I A G
601    GCCGAGCCGAGAGCGGCAGCTGCCGGTGGGAGGGGCCAGAAGTGGGACCCAGTGGGGTCCA
174    C R A E S G S C R C E G Q K C D H V G S
661    CCGCGGTGGTGGCGTCTGTTGGAGGAGAGCCCGCTCGTGTGCGCCAACTGGCGGCGACTCCC
194    T A V V A V V E E S R V V V A N C G D S
721    GCGCCGTGCTCTGCCCGGGGGCGCGCCCGTGCAGCTATCCCGGACCACAAGCCCGACC
214    R A V L C R G G A P V Q L S S D H K P D
781    GCCCCGACGAGCTGGAGCCGATCGAGGCCGCCGGCGGGCGCGTCACTCTCTGGAGGGCG
234    R P D E L E R I E A A G G R V I F U E G
841    CCGTGTCTCGGGCTCCTCGCCATGTCCGGCTCCATCGGGCAGCCCTACCTGAAGCCCT
254    A R V L G V L A M S R S I G D A Y L K P
901    ACGTCAACGCCGTGCCGGAGGTGACGGTACCGGCAGGTCCGACTTCGACGAGTGCCTCA
274    Y V T A V P E V T V T G R S D F D E C L
961    TCCTCGCCAGCGACGGCTCTGGGACGTCTGTCAGCAACGAGGCCCGCGTGGGAGGTGGCCG
294    I L A S D G L W D V V S N E A A C E V A
1021   AGTCGTGCCTCCGCCCGGGCAGGCAGCGTGGTGGCGCCGAGGCCGGCGGGTGTGTGACCA
314    Q S C L R R G R Q R U C A E A A A V L T
1081   AGCTGGCCCTCGCTCGCCGACGCTCCGACAACATCTCTGTCTGTCTGTCTGTCTGTCTGTCTGTCT
334    K L A L A R R S S D N I S V V V V D L R
1141   GAGGCAACGCCTTGTAGAGAGAGTGTCAACCAGCTGATGCTGTAGCATCAGTACTAGTAT
354    R G N A L *
1201   TGCAGATATTAGCTAAGCTAATGGTTCCTACTAATCTTTTTTTTTTCAITTCCTTGTGTAA
1261   AAATCATTTTTTTCTCTCTAATTTTCTCCCTGAATTAACCAAGTTCAGTGGAGCACATA
1321   GATCAGGTGATGGGTTGTGCAACTGCTAGTTGGTGTACCAGAATTCATCTGGATACCA
1381   ACTGAAATGAAATGAATATTAAAGCAAGATTATTTGAGA

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Figure 1. Structure of the *OsPP2C1* DNA (Nipponbare).



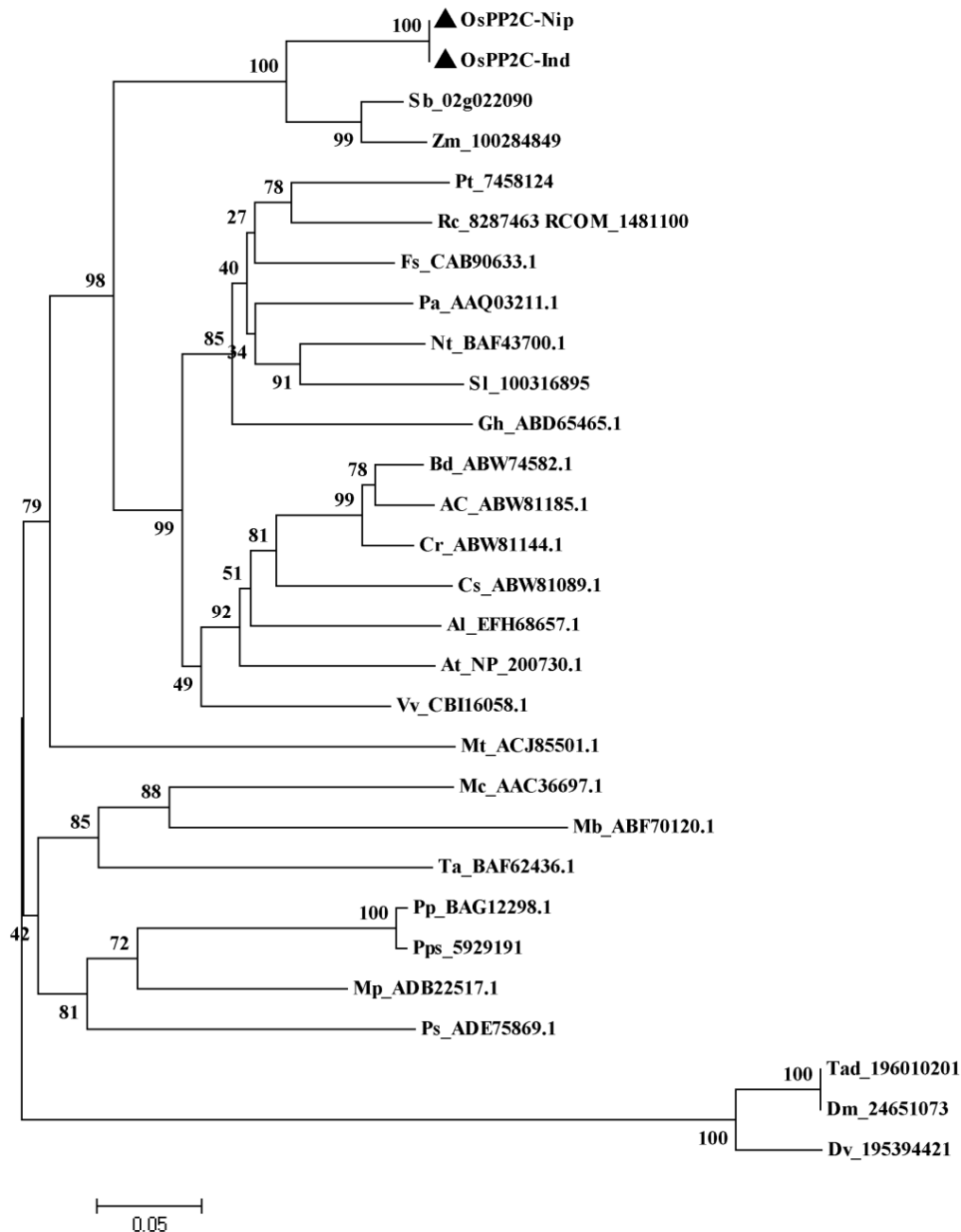
**Figure 2.** Location of *OsPP2C1* and prediction of the function of the putative *OsPP2C1* protein. (a) Location of *OsPP2C1* on rice chromosome 9; (b) PP2C: protein phosphatase2C; PP2C superfamily: serine/threonine phosphatases superfamily2C catalytic domain; PS01032 (prosite\_patterns): PS01032, InterPro, little thick black frame: PP2C\_Mn2\_Asp60\_BS, 112-120aa; large thin black frame: PF00481, InterPro, PP2\_N, 73-345aa; little thick blue frames: PR01608, InterPro, BacInvasinC, 139-163aa, 258-281aa; underline: SM00332, InterPro, PP2C\_related, 60-350aa; green words: SM00331, InterPro, PP2C\_related, 92-352aa; large thin blue frame: 81606(superfamily), InterPro, PP2C\_related, 53-357aa; red characters indicate active sites; rhombus indicates difference between species.



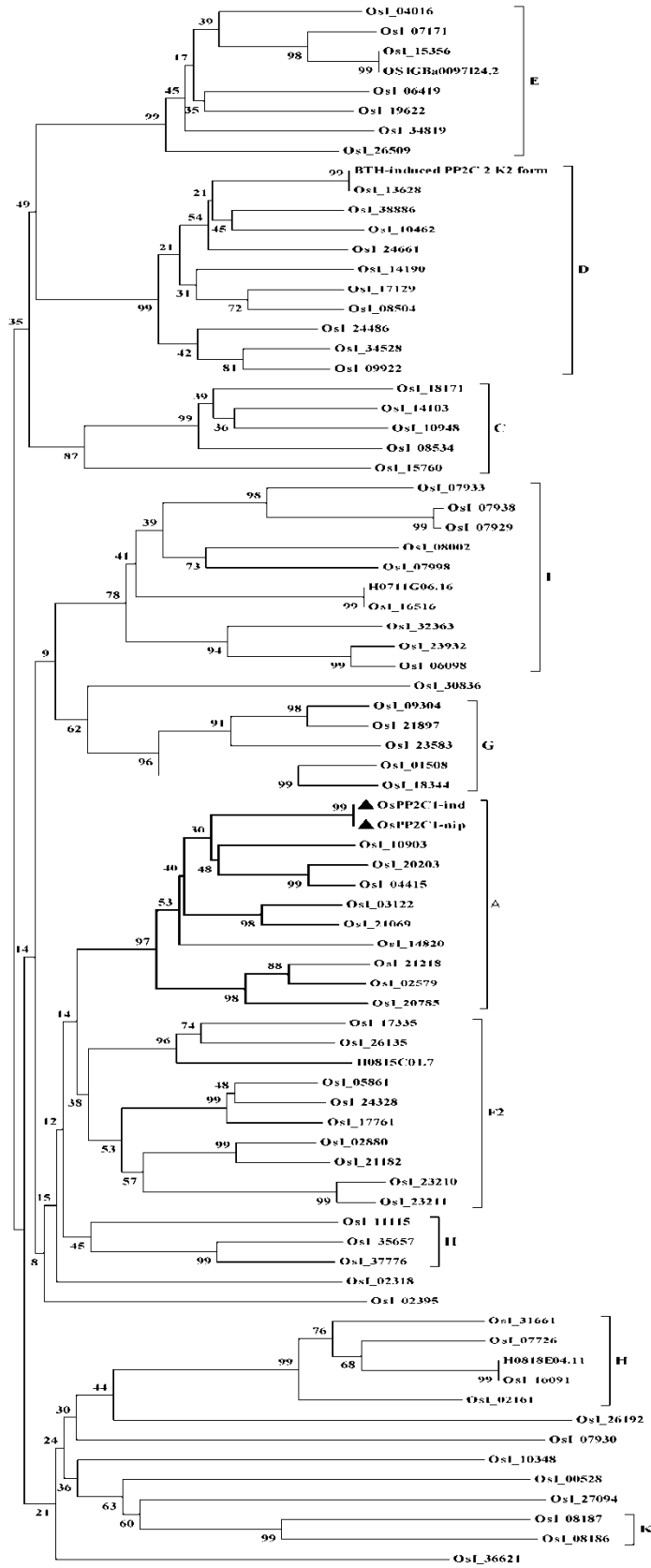
**Figure 3.** Sequences of *OsPP2C1* candidate *cis*-elements in the putative promoter region. The identified matches to stress related *cis*-elements and TATA box in the putative promoter region are highlighted and underlined. “...” represents bases without print.

species though NCBI. Subsequently, multiple sequence alignment and phylogenetic analysis was performed to construct a paralogous tree (Figure 4a) and an orthologous tree (Figure 4b), from which it can be seen that the

PP2C proteins fall into ten major groups (group A to group K). To identify the motifs (Table 1) shared among related proteins within the PP2C family, the MEME motif search tool was employed. Also, the specific proteins of



**Figure 4.** (a). Analytical view on the mostly PP2C gene family among different species. (b). analytical view on the mostly PP2C gene family in *Oryza sativa indica* cultivar group. Protein distance tree: the unrooted tree was constructed using aligned full-length amino acid sequences and summarizes the phylogenetic relationship among the 78 members of PP2C family in rice and 27 kinds of plants. The numbers beside the branches represent bootstrap values ( $\geq 50\%$ ). The phylogenetic test used was the Bootstrap test by Neighbor-Joining evolution method, as implemented by MEGA version 5.02 (Kumar et al., 2008). Dendrogram branches are labeled with percentage of 1,000 iterations supporting each branch. The scale bar at bottom reflects the frequency of amino acid substitutions between sequences as determined by the Poisson corrective distance model. Pp: *Physcomitrella patens*; Pps: *Physcomitrella patens* subsp. *Patens*; Mt: *Medicago truncatula*; Ps: *Picea sitchensis*; Mc: *Mesembryanthemum crystallinum*; Mb: *Musa balbisiana*; Dm: *Drosophila melanogaster*; Dv: *Drosophila virilis*; Tad: *Trichoplax adhaerens*; Ta: *Triticum aestivum*; Cr: *Capsella rubella*; Bd: *Boechera divaricarpa*; Vv: *Vitis vinifera*; Ac: *Arabidopsis cebernensis*; Al: *Arabidopsis lyrata* subsp. *Lyrata*; Cs: *Cleome spinosa*; Nt: *Nicotiana tabacum*; Rc: *Ricinus communis*; Pt: *Populus trichocarpa*; Fs: *Fagus sylvatica*; Sl: *Solanum lycopersicum*; Gh: *Gossypium hirsutum*; Pa: *Prunus avium*; Mp: *Marchantia polymorpha*; Zm: *Zea mays*; Sb: *Sorghum bicolor*.



b. 0.05  
**Figure 4. Contd.**

**Table 1.** Sequences and lengths of conserved motifs in the amino acid sequences of *O. sativa indica* cultivar-group PP2C genes.

Motif	Multilevel consensus sequence
1	LFLVLLASDGLWQVYKNDVAVY
2	GLAVSRAIGDLK
3	LVANSGDSRAVLR
4	LEEGYFDGHGG
5	ALRYKAALFAABKREMYPLKSLKSG
6	AVLSLPHKPEE
7	LELSLDRITLVVVYF
8	GLSTAVYA
9	DGLLWVLELAAAGEFSMAAXXQANLEDAQVESPAA
10	VAAYSSSLLEAVKPEE
11	LALHPDDESLVYLKHSXWRYK

motifs in the proteins were shown (Figure 5). Tongtong (2008) reported that there were 11 motifs in PP2C Nipponbare and our research showed that there were 11 motifs similar to them. Some motifs are widespread among selected proteins (for example, motifs 1, 2, 3, 4 and 8). According to the classification based on domain composition and the phylogenetic classification, not all the proteins that belong to one predict subfamily completely match the reported classification.

### The expression levels of *OsPP2C1* under stresses

In order to find effective stress tolerance genes, the rice genome was studied at different growth stages and different tissues that respond to different stresses, using Affymetrix gene chip system and containing 51,279 rice transcripts expression chips (GeneChip rice genome array). Analysis of the expression of the super rice 'Liangyoupei nine' *Pei'ai 64S* female parent seedling, booting, heading and flowering leaf and panicle gene in low-temperature, high temperature and drought stress, selected a significant part of genes up-regulated expression or down-regulated expression. *OsPP2C1* is one of the genes, in which the expression levels of leaves and spikes are compared with that of the control in the seedling, booting, heading and flowering stage under low-temperature, high temperature and drought conditions according to microarray analysis, at low temperature and drought conditions of up-regulated expression, while in high temperature of down-regulated expression (Figure 6). Low temperature conditions of the seedling stage reduced the expression with 0.86-fold, while the average of the up-regulated expression of the booting and heading up expression of flowering was 12.30 times, and the highest increases by 15.84 times; although, the minimum was 7.26 times. Under drought conditions, the average of the up-regulated expression was 10.74 times,

while the maximum was 13.5 times, and the minimum was 9.36 times; but under high temperature conditions, the average was 1.62 times lower, the maximum was 1.80 lower, and the minimum was 1.29 times lower.

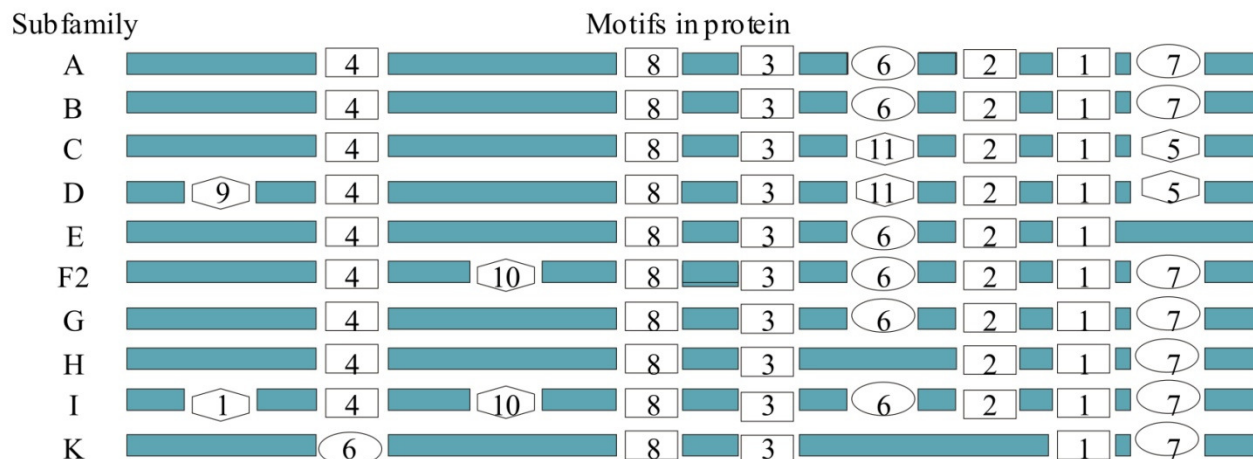
To verify this result, using real time quantitative PCR method analysis, the material of microarray (the same stress conditions, developmental stages, tissues and organs) and *OsPP2C1* expression levels were further analyzed and were basically consistent with the results of DNA microarray (Figure 1), which showed that *OsPP268* gene activity was induced by the changes of two sets of data variation by low temperature, drought and heat stress factor. The two sets of data changed the size of the differences, perhaps because the technology itself or the method of taking the collection of plant material caused the difference.

Other gene expression data are available in a public microarray database, the National Center for Biotechnology Information (NCBI) gene expression omnibus (GEO; <http://www.ncbi.nlm.nih.gov/geo/>; Edgar et al., 2002): the comparisons of tissues (that is, stinging, 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), transzeatin versus mock dimethyl sulfoxide in root and leaf at 30 and 120 min after treatment (GSE6737 Figure 7; Xue et al., 2008).

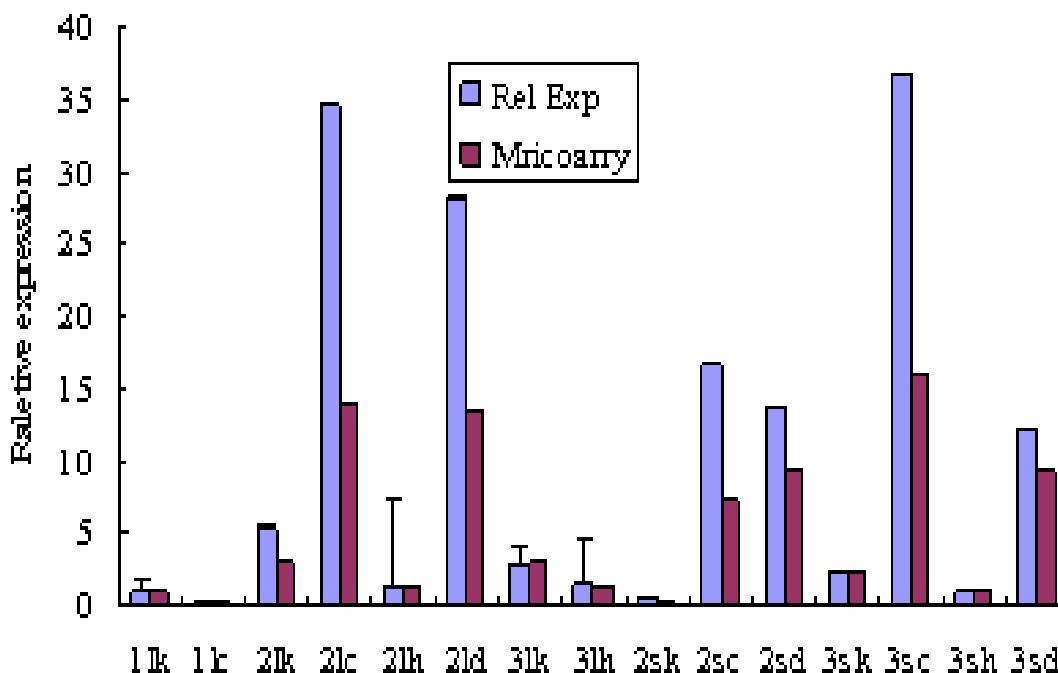
### DISCUSSION

Plant growth and crop production are adversely affected by low temperature, drought and high temperature stress conditions. The product of many genes involved in molecular processes through hormone (ABA, ethylene and salicylic acid, etc.) to resist stresses (Hu et al., 2010; Xiong et al., 2002). The protein structure determines the function, from the NCBI, GRAMENE and trans-





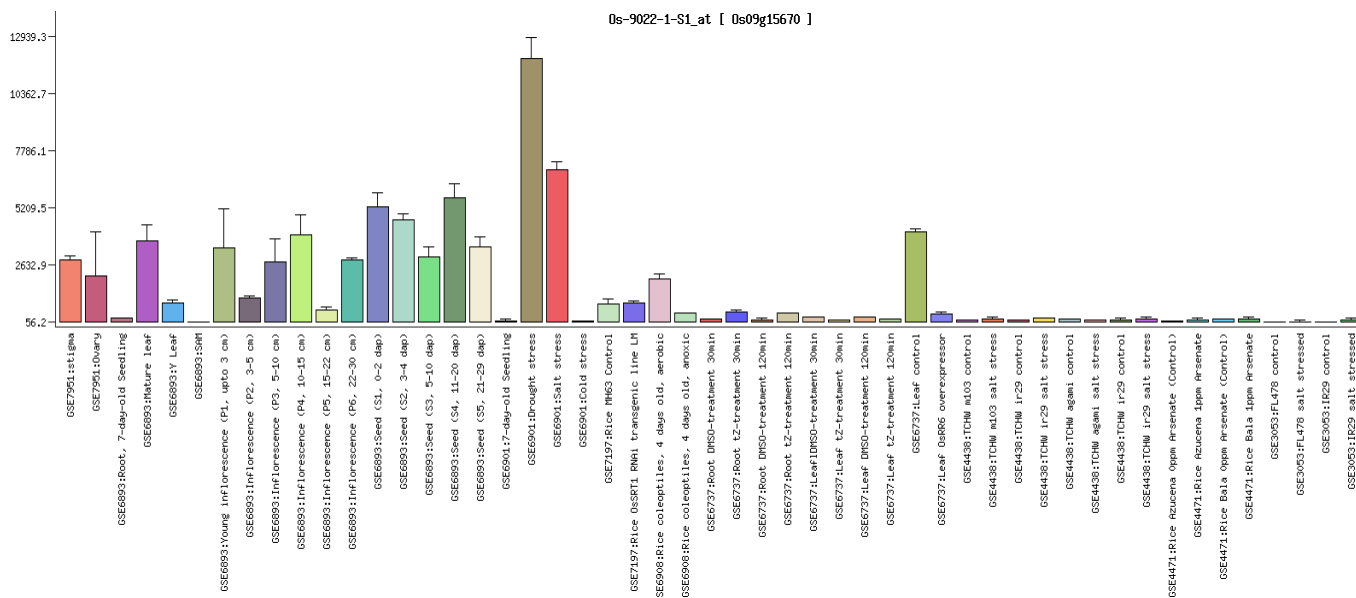
**Figure 5.** The specific motifs in the proteins. Each box with a number represents the occurrence of a specific motif in the protein.



**Figure 6.** Relative expression of *OsPP2C1* in leaves and panicles of *indica* rice cultivar Pei'ai 64S under the various stresses and in normal growth conditions, at different developmental stages. 1: seedling stage; 2: booting stage; 3: heading and flowering stage; L: leaf; S: spike; K: control; C: cold; H: heat; D: drought.

membrane helix prediction (TMHMM); the *OsPP2C1* protein contains one PP2C N-terminal domain, one Mn<sup>2+</sup> binding region and one of the three PP2C relative domains is PP2C-superfamily, which indicated that *OsPP2C1* gene codes a kind of PP2C protein and it belongs to PP2C subfamily A, which is relative to ABA (Xue et al., 2008). The *OsPP2C1* homologous sequence alignment, the ORF sequence of the *O. sativa Japonica* group rice and the *O. sativa indica* group rice had

differences of 3 bp nucleotides, using the same primers that predicted the protein sequence of amino acids difference between species. Although, these differences may not influence the protein function, the active points did not vary. As one kind of important signaling molecule, PP2Cs are numerous in plants, and have conserved structural features and physicochemical properties. A large number of PP2C genes have been cloned, and so, this study focused on their role in different signaling



**Figure 7.** RiceGE: Gene expression atlas for *OsPP2C1*. X axis: different series and relative tissues; Y axis: total RNA expression level. GSE7951 genome-wide gene expression profiling of rice stigma [*O. sativa*]; GSE3053 rice salt expression [*O. sativa* *O. sativa* (*indica* cultivar-group)]; GSE4438 Expression data from rice under salinity stress [*O. sativa* *O. sativa* (*indica* cultivar-group) *O. sativa* (*japonica* cultivar-group)]; GSE4471 Expression data from rice varieties Azucena and Bala grown in 0 and 1 ppm arsenate [*O. sativa*]; GSE6737 Over-expression of a Type-A response regulator alters rice morphology and cytokinin metabolism [*O. sativa*]; GSE6893 expression data for reproductive development in rice [*O. sativa*]; GSE6901 expression data for stress treatment in rice seedlings [*O. sativa*]; GSE6908 transcript profiling of the aerobic and anoxic rice coleoptiles [*O. sativa*]; GSE7197 down-regulation of OsSRT1 induces DNA fragmentation and cell death in rice [*O. sativa*]. GEO: Gene expression omnibus; SAM: shoot apical meristem; tZ: trans-zeatin; DMSO: dimethyl sulphoxide; web link: platform gpl2025.

pathways. However, a small number of genes, such as ABI1 and ABI2 are used to understand the biological function more clearly (Leung et al., 1997; Merlot et al., 2001).

Gene expression patterns can provide important clues for gene function. So a comparative analysis of the expression patterns of the whole PP2C gene family has been performed. The expression of AtPP2C and *OsPP2C* genes was examined in inflorescence, leaf, root and silique tissues using microarray data from Geneves tigator that have massively parallel signature sequencing (MPSS) data and EST abundance data from NCBI, but *OsPP2C1* is only expressed in leaves and roots (Xue et al., 2008). Moreover, most protein phosphatases are broadly expressed based on the expression data acquired with three methods (microarray, MPSS and EST) from four different tissues (root, leaf, inflorescence and seedling) and Os09g15670 wide expression (Yang et al., 2010). Also, from NCBI and Gene Expression Atlas Data Sources (RiceGE), the result show slight difference, with a highly extended expression in Figure 6 (Hirose et al., 2007; Huang et al., 2007; Jain et al., 2007; Li et al., 2007; Norton et al., 2008; Rasika et al., 2007; Sharma et al., 2009; Walia et al., 2007). The RiceGE indicated that the gene expression exist specifically in tissues and developmental stages.

The upstream promoter sequence analysis found about

10 kinds of stress induced by cis-acting elements, there are a number of ABRE (ABA cis-element) (Leung et al., 1997; Merlot et al., 2001), MRE (MYB binding site involved in light responsiveness) (Du et al., 2009; Jia et al., 2009), CGTCA-motif (cis-acting regulatory element involved in the MeJA-responsiveness), TCA-element (Chen et al., 2005; Lim et al., 2006) (cis-acting element involved in salicylic acid responsiveness) and HSE (cis-acting element involved in heat stress responsiveness) that shows that the gene may responses to multiple stresses and tolerance.

Through the NCBI gene prediction on *OsPP2C1* protein active sites, superfamily regional and other species of the conserved region of the forecast comparison showed that *OsPP2C1* gene protein is one of protein phosphatase2C. Predict sequence length and other species have high similarity. Analysis of the phylogenetic trees displays the evolution relationship among 78 *OsPP2C* genes of *O. sativa indica* cultivar-group and divergence in plants.

The public microarray database showed that *OsPP2C1* expression was evident to up-regulation in different series, tissues and under various development periods. It has been analyzed that the expression of 10 *OsPP2C* genes, including *OsPP2C1*, in rice plants treated with ABA, salt, osmotic (mannitol) and cold stress by reverse transcriptase polymerase chain reaction (RT-PCR) and the results of this study indicate that the 10 genes are

related to stress responses (Singh et al., 2010; Xue et al., 2008). In our study, the *OsPP2C1* microarray gene expression results in variations in different stress periods at low temperature, while the seedlings appeared down-regulated in the booting up expression of flowering. However, rice seedlings may be reduced as a result of their response to low temperature or as a result of different treatment temperatures. Expression under drought conditions and the high temperature reduction indicate a wide range of adversities by *OsPP2C1* gene response. Thus, the regulation of this gene can be predicted in different ways to have a variety of stress response. To further analyze the function of protein, we need to continue a research into the expression of the gene and the phenotype in transgenic plants.

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