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Characterization and expression analysis of the *SNF2* family genes in response to phytohormones and abiotic stresses in rice

X.-Y. LI[1], C. WANG, P.-P. NIE, X.-W. LU, M. WANG, W. LIU, J. YAO, Y.-G. LIU and Q.-Y. ZHANG*

State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, College of Life Sciences, South China Agricultural University, Wushan, Guangzhou 510642, P.R. China

Abstract

The function of SNF2 ATPases, the major catalytic subunits of chromatin remodeling complexes, in plants is not sufficiently understood. Here we identified 39 putative *SNF2* genes of rice (*Oryza sativa* L.) by homology analyses and analyzed the expression profiles of eight of them in response to phytohormones and abiotic stresses. Our results indicated that expression of the *SNF2* genes was affected by auxin, gibberellin, cytokinin, abscisic acid, ethylene, and some abiotic stresses such as heat, chilling, darkness, drought and salinity. It suggests that, like *Arabidopsis* SNF2s, rice SNF2 proteins may function in phytohormone signaling pathways and/or be associated with the resistance to abiotic stresses, but in distinct manners from their *Arabidopsis* orthologs. Some SNF2 proteins in rice may be involved in cross-talk of the signaling pathways between phytohormones and abiotic stresses.

Additional key words: abscisic acid, auxins, chilling, cytokinins, ethylene, gibberellins, *Oryza sativa,* osmotic stress, salinity.

Introduction

It has been demonstrated that the chromosome structure is highly dynamic, changing rapidly for transcription regulation according to the needs of the cell. In this process two main active players are involved: *1*) chromatin modifying complexes, which introduce covalent modifications on histone tails or the histone core and *2*) chromatin remodeling complexes, which alter the DNA-histone interaction noncovalently (Kwon and Wagner 2007). The ATP-dependent chromatin remodeler binds to both the protein core of the nucleosome and the DNA which winds around it. By using the energy of ATP hydrolysis, the complex changes the structure of a nucleosome temporarily, catalyzes nucleosome sliding, and makes the nucleosomal DNA accessible to regulatory proteins.

The catalytic subunits of chromatin remodeling complexes belong to the SNF2 family of DNA-dependent ATPases (Kwon and Wagner 2007). Their catalytic core consists of two characteristic domains, the SNF2_N domain located at the N-terminus and the Helic C domain located at the C-terminus. Now there are five classes of ATP-dependent chromatin remodelers operating in eukaryotes: SWI/SNF, ISWI, CHD1, SWR1 and RAD54 (Flaus *et al.* 2006). Since more recent discoveries concentrating on SWI/SNF complexes have advanced our understanding of the roles of SNF2 ATPases, SWI/SNF ATPases will be focused in this introduction.

The first SWI/SNF ATPase was found in *Saccharomyces cerevisiae* mutants which showed sucrose-nonfermenting and mating type switching defective phenotypes, thus the SNF2 family has got its name (sucrose-non-fermenting; Winston and Carlson 1992). The studies in single-cell organisms, invertebrates and mammals have indicated that SWI/SNF ATPases are essential for transcriptional reprogramming, morphogenesis, early embryo development, patterning and differentiation throughout development (Kwon and Wagner 2007). In plants, detailed research on two viable null mutants of SWI/SNF ATPases, SPLAYED (SYD) and BRAHMA (BRM), in *Arabidopsis thaliana* (Wagner and Meyerowitz 2002,

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Abbreviations: ABA - abscisic acid; 6-BA - 6-benzyladenine; 2,4-D - 2,4-dichlorophenoxyacetic acid; GA_3 - gibberellins; IBA - 3-indolebutyric acid; KT - kinetin; NAA - 1-naphthaleneacetic acid; Pac - paclobutrazol; PEG - polyethylene glycol; SA - salicylic acid.

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[1] Present address: Department of Biology, The Chinese University of Hong Kong, Shatin, Hong Kong, China

* Author for correspondence; fax: (+86) 20 85282180, e-mail: zqy@scau.edu.cn

Farrona *et al.* 2004, Kwon *et al.* 2005, 2006, Sarnowski *et al.* 2005, Hurtado *et al.* 2006, Su *et al.* 2006, Bezhani *et al.* 2007, Tang *et al*. 2008) improved our information on critical roles of plant SWI/SNF ATPases. Both SYD and BRM regulate stem cell maintenance, patterning (alteration of leaf polarity, flower morphogenesis and ovule development), develop-mental transitions (precocious onset of reproductive development) and growth (small stature, slow growth and reduced apical dominance) (Kwon and Wagner 2007), while BRM also controls root growth and male fertility (Bezhani *et al*. 2007). Recently SYD has been found to be also required for selective pathogen resistance and regulation of the specific pathways within biotic stress signaling networks (Walley *et al*. 2008). ATCHR12, another SWI/SNF ATPase in *A. thaliana*, has been demonstrated to play a vital role in mediating the temporary growth arrest of *Arabidopsis* upon perception of environmental stresses (Mlynárová *et al.* 2007). All these three SWI/SNF ATPases have an HSA domain at the N-terminus, lately shown to mediate critical interactions required for SWI/SNF-dependent trans-criptional activation (Trotter *et al.* 2008). BRM also have a C-terminal BROMO domain found to target remodeling complexes to hyperacetylated chromatin in yeast (Kasten *et al*. 2004, Jerzmanowski 2007).

Although these studies have provided important information of SNF2 ATPases in *A. thaliana*, what we know about their functions in the whole plant kingdom is still much limited. In this work, we identified 39 genes of the SNF2 family in rice (*Oryza sativa* L.) by homology analyses, and selected eight genes for further characterization which showed protein identity to their *Arabidopsis* orthologs and determined their tissue-specific expression patterns. In view of the involvement of some chromatin remodeling complexes in phyto-hormone and environmental stress signaling pathways (Ogas *et al.* 1997, Brzeski *et al.* 1999, Fukaki *et al*. 2006, Mlynárová *et al.* 2007, Walley *et al*. 2008), the expression profiles of these eight genes were investigated in rice seedlings treated with different phytohormones and under abiotic stresses.

Materials and methods

We used the amino acid sequence of SYD SNF2_N domain from *A. thaliana* for *BLAST* searches in the *NCBI* database (http://blast.ncbi.nlm.nih.gov/blast.cgi) to identify putative SNF2 family genes in rice. All predicted *SNF2* genes were used for similarity searches again to confirm these predicted genes and detect new candidates. All the domains of the SNF2 family were detected by the *Conserved domains* search program (Marchler-Bauer *et al*. 2009) in the *NCBI* database under a default E-value level (0.01).

Phylogenetic analysis was performed with the *MEGA 4.0* program (Tamura *et al.* 2007) by the Neighbor-Joining method. Bootstrap analysis was carried out with 1000 replicates based on the complete amino acid sequences. Subcellular localization of the rice SNF2 proteins was predicted *in silico* using the programs *WOLF PSORT* (Horton *et al.* 2007), *ProtComp8.0* (http://linux1. softberry.com/berry.phtml) and *NUCLEO* (Hawkins *et al.* 2007).

The seeds of a *japonica* rice (*Oryza sativa* L.) cultivar Zhonghua11 (ZH11) were germinated at 30 °C on wet filter paper in Petri dishes and then grown in liquid Murashige-Skoog (MS) medium at temperature of 28 °C and 14-h photoperiod with irradiance of 245 μmol m^{-2} s^{-1}. Two-week-old seedlings were used for all treatments. For phytohormone treatments, roots of the seedlings were submerged for 24 h to liquid MS media without and with 3.7 μM kinetin (KT), 13.3 μM 6-benzylaminopurine (6-BA), 1.5 μM paclobutrazol (Pac), 2.7 μM 1- naphtha-leneacetic acid (NAA), 10 μM 2,4-dichlorophenoxyacetic acid (2,4-D), 2.5 μM 3-indolebutyric acid (IBA), 100 μM abscisic acid (ABA), 1 mM salicylic acid (SA), 5 mM ethrel, and 50 μM gibberellic acid (GA_3). The concentrations were selected according to Nie *et al.* (2011) The abiotic stresses were: heat (42 °C for 2 h and recovery at 28 °C for 24 h), chilling (4 °C for 12 h), dark treatment for 24 h (at 28 °C) (Nie *et al.* 2011), drought (23 % PEG 6000 solution for 12 h at 28 °C) (Guo *et al.* 2006) and salinity (150 mM NaCl for 12 h at 28 °C) (He *et al.* 2002). Leaf samples were frozen in liquid nitrogen for RNA preparation.

Whole-genome microarray analyses were performed on *Affymetrix Genechips* (*Affymetrix,* Santa Clara, USA) containing features representing 48 564 *japonica* transcripts. All transcriptional units were represented by 25-mer probes (probeset). Total RNA extractions from six major tissues (roots, leaves, floral primordia, young panicles, mature panicles and anthers), cRNA syntheses, labeling, hybridizations and scanning were accomplished by *Capitalbio* (*Capitalbio Corporation*, Beijing, China).

For semiquantitative RT-PCR, total RNA was isolated using *TRIzol* reagent (*Invitrogen*, Carlsbad, USA). First-strand cDNAs were synthesized from DNaseI-treated total RNA using *Superscript II* reverse transcriptase (*Invitrogen*) and oligonucleotide *dT as* primers for reverse transcription, according to the instruction (Kim *et al*. 2010). The specificity of all used primers (Table 1) was confirmed by a *BLAST* search in the *NCBI* database. *OsAct1* (GenBank accession no. NM_001057621.1) was used as an internal control for each PCR reaction. The number of PCR cycles were 30 ~ 35 for each gene and 23 for *OsAct1*. The expression levels of the genes were

tested in three independent experiments.

The 5' and 3' rapid amplification of cDNA end (RACE) were performed to determine the full-length cDNAs of the eight SNF2 genes using *SMART RACE* cDNA amplification kit (*Clontech*, Mountain View, USA) with some modification (Wang *et al*. 2009). First-strand cDNAs of total RNA from leaves were synthesized following the *SMART* protocol. The gene-specific primers were designed according to the *EST* data of each gene at *NCBI* (Table 2). All the PCR products were cloned into the pMD18-T vector (*Takara*, Kyoto, Japan) and sequenced. Each CDS was confirmed by amplification using the gene-specific primers at the 5' and 3' termini (Table 2) and sequencing.

Table 1. Primers used for RT-PCR analysis.

Gene	Primer sequence (5' to 3')
Os02g0114000	gttgaccaagacagcattcggctagagaggaaaaggatcc
Os03g0722400	atgccaccggattggtcaaacgagcgacgtgagcatgccg
Os06g0183800	aagcggccgctgtacacgcggtgcaacgaccagatgag
Os02g0689800	gcaaatcttgttctatcatcccatgactcataagaaagtgg
Os04g0629300	gttcagacagtgaagagtacccaaggccattgactcc
Os01g0636700	cgtcatatgctgacctagcagcatgaaggacaagctcttc
Os01g0779400	gtccatgcccctcggcgacttgatgagttacccagaatag
Os05g0144300	ttagaatgccgagcgccctttgaggttccagactgggtg
OsAct1	ccagactcgtcgtactcagcccagatcatgtttgagacc

Table 2. Primers used in 5' and 3' RACE, and complete CDS amplification.

Gene	Primer sequence (5' to 3')		
	5'RACE	3'RACE	Complete CDS
Os02g0114000	cgcccgatgggtaccccttgctgctg	tccacacccttctcagcagtctatg	atgcagcccggcgaggcacccccctc
	gatgcatcattgcctgattc	ttctgtgcagcaatctgatc	tcacatatggcttggccgtcttttg
Os03g0722400	gccgcaagttccacagtcagggatg	agacggacatcaccgacgaggatc	atggtggtcgctgtggcaaatgggac
	ttacattcagaaatcttctc	cgaccttacaggccctcctg	ctagctggtgagcgacgtgagcatg
Os06g0183800	tttccaaatattcactttctgaaac	aggaagttatgctagatgcaagctc	atggaaaagatattagactgtgaag
	attcctttccattttataag	aaccgaggtgccttcgtcag	ttaactatcgatttccattttgtc
Os02g0689800	tccattttctctcagcatcaaactc	acattacccagtccctatctgac	atggcatcaaaaggtcctcgatc
	aagaagatggtcccagtgag	tcacctactttgataatcttc	tcatccgatgtctgtgaaatctg
Os04g0629300	agttatcgtcatccagcccgactag	aattggtcagacccgtcctgtaac	atggatattattgacttgtgttcag
	tgtgttgtgcaaaaccacctg	atcggattttagctctacag	tcatattccgaatagatattggag
Os01g0636700	agcagaatcccctgcctgggctgtg	tgaaaattgaagctaaaagggctg	atggtacagattaaggaacttg
	actatctttccatatttaatg	tgaacatatcaaggaagcag	ctaacccccttttccttttctttc
Os01g0779400	aacttgtgctgtccactggcatctc	acctgtcacagtgtcacggttaac	atggaggaagcggcggcggcgg
	tgctgctccttccagtcctc	atcgtattttggctctccag	ctaaaccataaacaaatagttc
Os05g0144300	acggcgggcggcagcgggaggttg	aaaaagtaagggctggggtttcttc	atggctgcctcggtggaggcgg
	ccgatgagcgtcctggcctg	tgccgaagatgaatccaccac	ttagaatgccgagcgccctttg

Results and discussion

By *BLAST* in the protein database at *NCBI*, using the amino acid sequence of SYD SNF2_N domain from *A. thaliana*, we detected 39 putative SNF2 genes in rice. They possess two characteristic domains belonging to the SNF2 family. One is the SNF2_N domain, namely SNF2 family N-terminal domain (Fig. 1), a variant of the typical DEXD/H domain with a conserved C-terminal extension of approximately 100 amino-acids, containing an ATP-binding pocket which mediates ATP hydrolysis (Jerzmanowski 2007). The other is the C-terminally located Helic C domain connected to SNF2_N by a flexible spacer of variable length (Jerzmanowski 2007) (Fig. 1). Many SNF2 proteins also have some important accessory domains involved in transcription regulation, DNA repair, DNA recombination, replication, and ubiquitination. The function of accessory domains is probably associated with SNF2 motors within the remodeling complexes. A sequence similarity tree using the predicted amino acid sequences of the rice SNF2s revealed relatively low sequence identity among the proteins except Os03g0722400 and Os09g0442700 (Fig. 1). This character of the rice SNF2 family facilitates our researches on distinct functions of single *SNF2* genes.

Prediction programs for subcellular localization based on the putative amino acids suggest that all the rice SNF2 proteins bear nuclear localization sequences (NLS). It is consistent with their functions in chromatin remodeling activities as DNA-dependent ATPases.

In some earlier papers, 41 SNF2 proteins encoded in the *A. thaliana* genome (Gendler *et al.* 2007) have been arranged in subfamilies and groups of subfamilies based on their domain architectures using advanced bio-informatics methods for distant homology detection (*Meta-BASIC*) and protein structure prediction (*3D-Jury*).

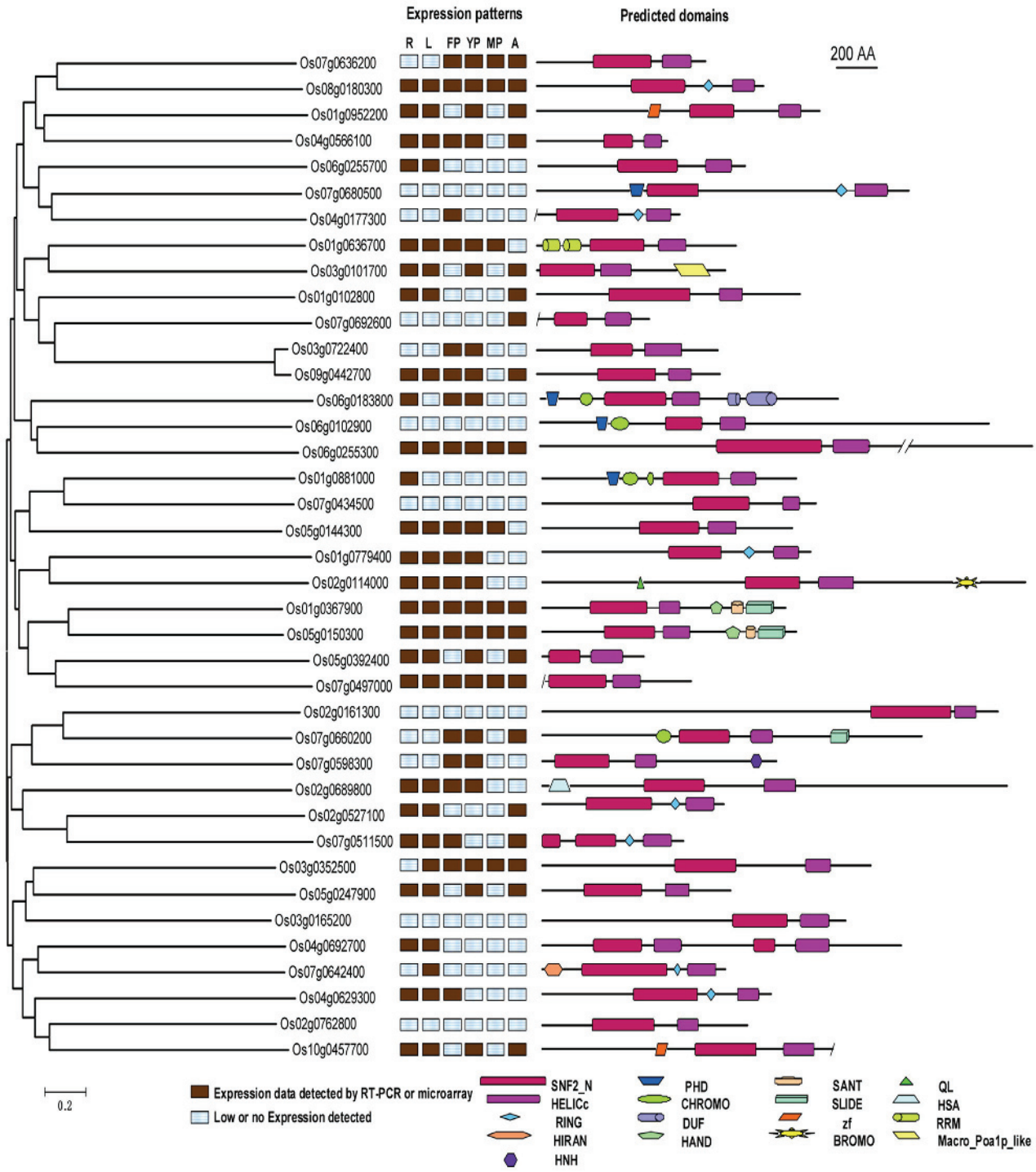

Fig. 1. Rice SNF2 protein domain architectures, sequence similarity tree and expression patterns for rice *SNF2* genes from microarray or RT-PCR. The letter R above the column of expression data refers to root, L refers to leaf, FP refers to floral primordium, YP refers to young panicle, MP refers to mature panicle and A refers to anther. Domains were recognized by CDD (Marchler-Bauer *et al*. 2009) at *NCBI* (http://www.ncbi.nlm.nih.gov/). Different domains are denoted by different shapes except SNF2_N and HELICc. The diagonal marks at the terminuses are the symbols of the truncated proteins. All the proteins were displayed in proportion. The protein sequence of Os06g0255300 was too long to show the full length in this figure, so part of it was replaced by the diagonal marks (//). The unrooted similarity tree was generated by aligning the predicted amino acid sequences of all rice SNF2 proteins using *MEGA 4.0* program (Kumar *et al.* 2007) by the Neighbor-Joining method. The bootstrap test of the inferred phylogeny was carried out with 1 000 replications and the bar indicates 0.2 substitution per site.

They were classified into 25 distinct subfamilies forming six larger groups (Flaus *et al.* 2006, Knizewski *et al.* 2008). We combined the amino acid sequences of all the SNF2 proteins from rice (*Oryza sativa* L.) and *A. thaliana* for phylogenetic analyses (Table 3). The result indicated that the 39 putative SNF2 proteins from rice also fell into these six larger groups and 18 sub-families showing different degrees of homology between rice and *A. thaliana*. Rice SNF2 proteins have most of the accessory motifs found in their orthologs from *A. thaliana* but some not, such as the RRM domain, on the basis of the *in silico* predictions from *CDD* (Marchler-Bauer *et al*. 2009) at *NCBI*.

Table 3. *SNF2* subfamily occurrences in *O. sativa* and *A. thaliana* genomes.

	O. sativa	*A. thaliana*		*O. sativa*	*A. thaliana*
Snf2	4	4	*Rad54*	1	1
Lsh	2	1	*ATRX*	1	1
Iswi	2	2	*DRD1*	6	6
Chd1	1	1	*Rad5/16*	4	5
Mi-2	4	3	*Ris1*	3	5
ALC1	1	1	*SHPRH*	1	2
Ino80	1	1	*Mot1*	1	1
Swr1	1	1	*ERCC6*	3	3
Etl1	1	1	*SMARCAL1*	2	2

So far the limited information on *Arabidopsis* SNF2s has revealed that some accessory motifs make great contribution to their functions (Hoege *et al*. 2002, Kasten *et al*. 2004, Ulrich 2005, Jerzmanowski 2007, Trotter *et al*. 2008). BRM, the only SNF2 protein bearing a BROMO domain in *A. thaliana* (Knizewski *et al.* 2008), controls growth, development (root, leaf and flower), male fertility, and repression of photoperiod-dependent flowering and seed maturation in leaves (Farrona *et al*. 2004, Hurtado *et al*. 2006, Bezhani *et al*. 2007, Kwon and Wagner 2007, Tang *et al*. 2008). PIE1 (photoperiod independent early flowering 1), the single ortholog in *A. thaliana* representative of SWR1 in yeast (Knizewski *et al.* 2008), is required for floral repression, petal development and immunity *via* establishing epigenetic memory in a manner of regulating the histone H2A.Z level in chromatin (Noh and Amasino 2003, Choi *et al*. 2007, Deal *et al*. 2007, March-Díaz *et al*. 2008). Interestingly, both BRM and PIE1 have an N-terminal HSA domain (Knizewski *et al.* 2008). PKL (Pickle), an SNF2 motor within a phytohormone-dependent chromatin remodeler, plays critical roles in gibberellin, auxin and ABA signaling pathways to regulate root development, transition from embryonic to vegetative development and embryogenesis osmotolerance programs during germination (Ogas *et al*. 1997, 1999, Fukaki *et al*. 2006, Perruc *et al*. 2007), harboring the PHD and CHROMO domains at its N terminus (Knizewski *et al.* 2008). The Ring domain within yeast Rad5 has been found to function in extending the ubiquitin chain on monoubiquitinated proliferating cell nuclear antigen (PCNA) (Hoege *et al.* 2002) for triggering error-free branch of post-replicative repair (PRR) (Ulrich 2005). The close Rad5 ortholog in *A. thaliana*, AtRad5a, also possessing a Ring domain embedded in the Snf2-helicase domain (Knizewski *et al.* 2008), is functionally conserved in PRR as yeast Rad5 (Chen *et al*. 2008). Consequently our research were focused on the rice SNF2s bearing some accessory motifs with biological information available. Here we selected eight *SNF2* genes from rice whose *Arabidopsis* orthologs belonged respectively to the six subfamilies (*Snf2, Lsh, Mi-2, Swr1, Ris1* and *ERCC6*) of four different groups (the *Snf2*-like, the *Swr1*-like, the *Rad5/16*-like and the *SSO1653*-like group), harboring some accessory motifs, such as BROMO, CHROMO, PHD, HSA, Ring and RRM, to explore biological roles of rice SNF2s (Table 4). The classification of SNF2 proteins in plants in recent studies is based only on the homology *BLAST*s and phylogenetic analyses utilizing various programs of prediction. Even so, these bioinformatics analyses can provide important information and useful clues for us to investigate biological functions of plant SNF2 proteins.

All the full-length cDNAs of eight *SNF2* genes were determined by 5' and 3' RACE. The PCR products were sequenced and compared with the EST data in the NCBI database. Our results indicated that most of the CDS for these eight genes at NCBI was complete, except for *Os02g0689800* (GenBank accession NM_001054321) and *Os01g0636700* (GenBank accession NM_001050202). The virtual full-length CDS of *Os02g0689800* and *Os01g0636700* were 6 066 bp and 2 688 bp respectively, and therefore the two characteristic domains within these two proteins recognized by CDD (Marchler-Bauer *et al*. 2009) were completed as well (Fig. 1).

To define the tissue-specific expression pattern of the eight *SNF2* genes in rice, we generated expression data using a combination of microarray analyses and semiquantitative RT-PCR. First we examined the transcript abundance for all the rice *SNF2* genes in six different tissues (roots, leaves, floral primordia, young panicles, mature panicles and anthers) in the microarray experiments. In general, transcript abundance for the *SNF2* genes was relatively low, especially for *Os06g0102900*. *Os01g0102800* was expressed at significantly higher level in vegetative tissues (roots and leaves) than in reproductive tissues (floral primordial, young panicles, mature panicles and anthers) while *Os03g0352500* was the opposite. It was likely that *Os04g0177300* was a floral-primordium-specific gene and *Os04g0629300* was a root-specific gene.

In order to confirm the expression data and obtain additional information about eight selected genes, we performed semiquantitative RT-PCR analyses in roots,

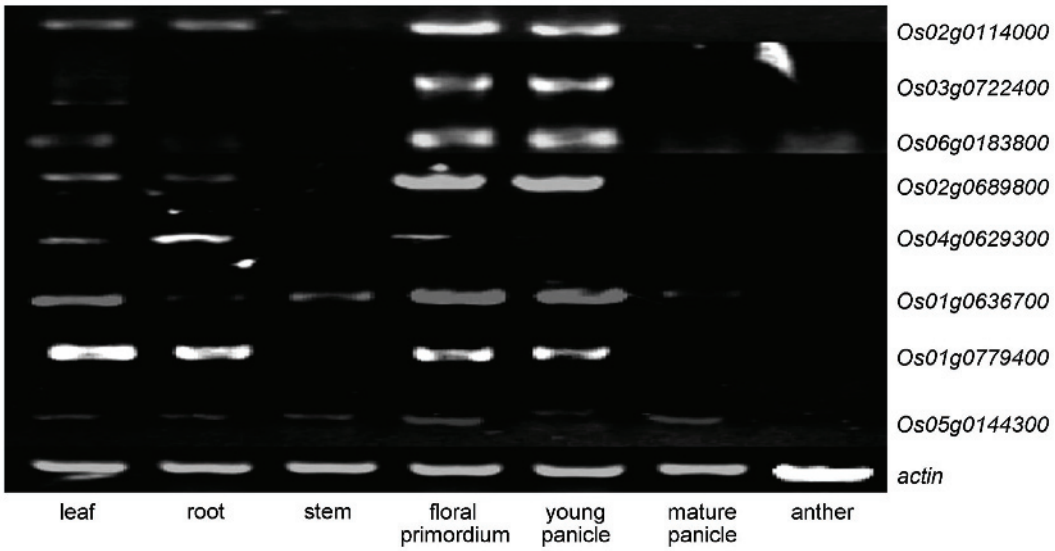

Fig. 2. Tissue-specific expression patterns of the eight *SNF2* genes detected by RT-PCR. The rice *Actin* gene (*OsAct1*) was used as an internal control.

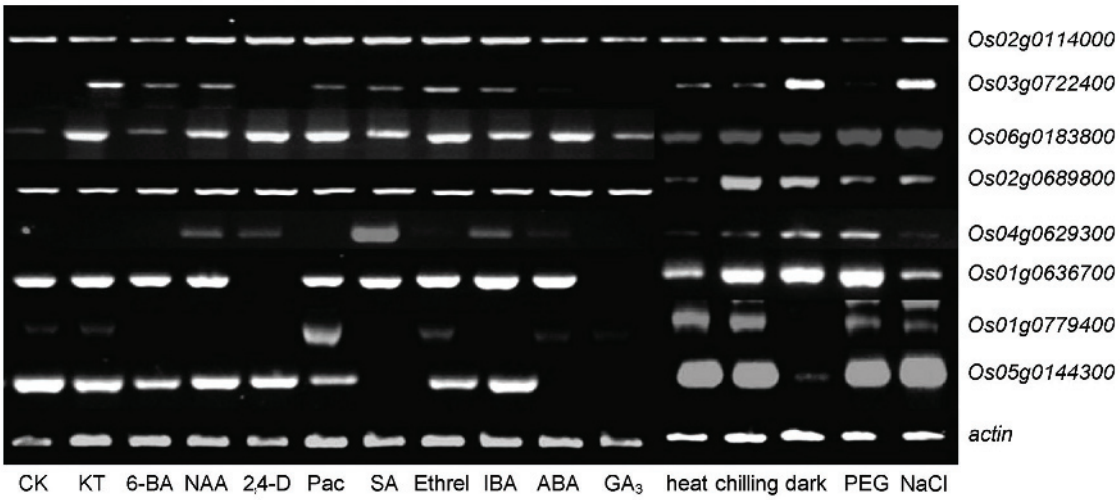

Fig. 3. Expression profiles of the eight *SNF2* genes detected by RT-PCR under phytohormone treatments and abiotic stresses. The rice *actin* gene (*OsAct1*) was used as an internal control. CK - mock-treatments, KT - kinetin, 6-BA - 6-benzyladenine, NAA - 1-naphthaleneacetic acid, 2,4-D - 2,4-dichlorophenoxyacetic acid, Pac - paclobutrazol, SA - salicylic acid, IBA - 3-indolebutyric acid, ABA - abscisic acid, GA_3 - gibberellic acid, PEG - polyethylene glycol.

stems, leaves, floral primordia, young panicles, mature panicles and anthers (Fig. 2). The results incorporated with the microarray analyses indicated that all these genes were expressed in multiple but not all of the tested tissues. Of the two selected SNF2 members, *Os02g0114000* was more abundant in floral primordial, young panicles, leaves and roots than in stems and mature mature panicles and anthers, while *Os05g0144300* showed no tissue preference except the low expression in anthers. Both the two selected Ris1 members, *Os04g0629300* and *Os01g0779400*, exhibited transcript abundance similar to *Os02g0114000*. All the other four selected genes, belonging to different subfamilies, showed tissue preference in young reproductive tissues. *Os02g0689800* and *Os01g0636700* also appeared abundant in leaves. Of eight SNF2 genes selected, *Os06g0183800* was the only gene whose expression in anthers was relatively high.

Our expression data revealed that many rice *SNF2* genes showed different spatial expression patterns from their *Arabidopsis* orthologs. For example, in anthers, *Os05g0144300* was expressed, albeit at a low level, while its *Arabidopsis* ortholog *ATCHR12* was not expressed (Mlynárová *et al*. 2007). Our phylogenetic analyses between rice and *A. thaliana* also indicated that some rice SNF2s bore accessory motifs different from their

Table 4. Eight *SNF2* genes selected in this study.

Gene	Subfamily	Group	Tissue-specific expression
Os02g0114000	Snf2	Snf2-like	roots, leaves, floral primordia and young panicles
Os03g0722400	Lsh	Snf2-like	floral primordia and young panicles
Os06g0183800	Mi-2	Snf2-like	foral primordia and young panicles
Os02g0689800	Swr1	Swr1-like	leaves, floral primordia and young panicles
Os04g0629300	Ris1	Rad5/16-like	roots, leaves, and floral primordia
Os01g0636700	ERCC6	SSO1653-like	leaves, floral primordia and young panicles
Os01g0779400	Ris1	Rad5/16-like	roots, leaves, floral primordia and young panicles
Os05g0144300	Snf2	Snf2-like	low expression in anthers

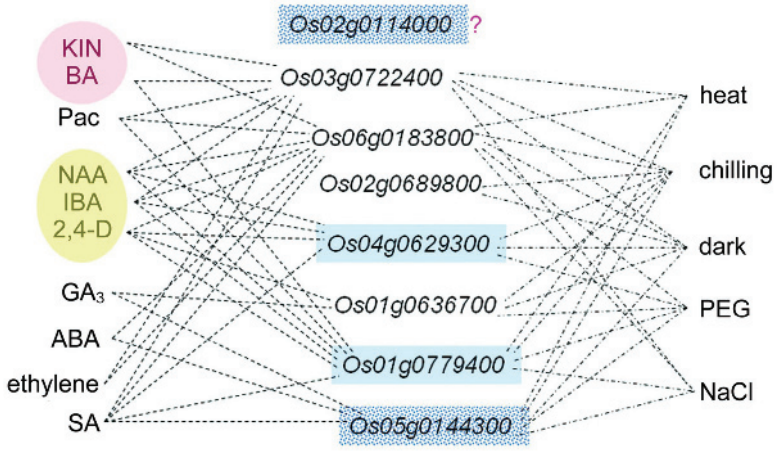

Fig. 4. The probable cross-talk draft in which the eight genes were involved. Their regulations in phytohormone and stress signaling pathways were designated with different types of broken lines. The two Snf2 members were displayed with speckle shadows while the two Ris1 subfamily genes were shown with plain shadows. The two cytokinin-category and three auxin-category phytohormones were oval-shaded, respectively.

Arabidopsis orthologs, such as *Os01g0636700* bearing the RRM domain not found within *Arabidopsis* SNF2s. Thus from the differences in domain architectures and tissue-specific expression patterns, it can be deduced that SNF2 proteins in rice may operate in distinct manner from their *Arabidopsis* orthologs, reflecting the differences between monocots and dicots.

Since some chromatin remodelers in *A. thaliana* play critical roles in phytohormone signaling pathways (Ogas *et al.* 1997, Brzeski *et al.* 1999, Fukaki *et al.* 2006), we investigated the effects of exogenous phytohormones on the expression of the eight *SNF2* genes in 2-week-old seedling leaves by semiquantitative RT-PCR (Fig. 3). Of the two genes in *SNF2* subfamily, *Os02g0114000* showed no response to any exogenous phytohormone treatment, while *Os05g0144300* revealed transcript reduction after SA, ABA and GA_3 treatments. Of the two members in Ris1 subfamily, *Os04g0629300* expression was up-regulated by auxins (NAA, 2,4-D, IBA) and SA treatments while *Os01g0779400* expression was induced by Pac but reduced after treatments with BA, auxins and SA. After most of the phytohormone treatments, both *Os03g0722400* and *Os06g0183800* displayed transcript accumulation while *Os01g0636700* exhibited no transcript abundance change except for 2,4-D and GA_3 treatments, which significantly decreased its expression level.

Responses to phytohormones and abiotic stresses are interrelated in plants. *SNF2* gene in *A. thaliana*, *AtCHR12*, has been considered perceiving environmental stresses (Mlynárová *et al.* 2007). In view of that, the expression profiles of the eight *SNF2* genes were analyzed in 2-week-old seedling leaves under heat, chilling, darkness, drought and salinity by semi-quantitative RT-PCR (Fig. 3). Of these eight genes, *Os02g0114000* was the only gene whose transcript abundance appeared not significantly changed under any stress, while *Os06g0183800*, a Mi-2 member, was the only one for which the transcript was accumulated under all the stresses. The other six genes exhibited transcript different accumulation under different stresses.

From our expression data in response to phytohormones and stresses, it can be deduced that most of the *SNF2s* in rice except *Os02g0114000* and

Os02g0689800, may be at the cross points of phytohormone signal transduction pathways affected by some abiotic stresses. The deduced cross-talk is outlined in a draft (Fig. 4) and needs more verification in the following research.

In conclusion, we have identified the members of the rice *SNF2* family. Eight members in this family were selected for further analyses. They show the features only similar but not identical to *Arabidopsis* SNF2s. Thus they may play different roles in rice. Of the eight SNF2 genes selected, *Os02g0114000* was the exception, which exhibited no responses to both phytohormones and abiotic stresses. We infer that this gene may be requisite for maintaining basic physiological activities. Although expression profiles in response to phytohormones and abiotic stresses can help us obtain insights into action modes of the rice SNF2 proteins, they are only restricted to mature leaves in this study. Role of SNF2s in the signaling pathways are our focus in the future.

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