Front. Agric. China 2008, 2(3): 253–261
DOI 10.1007/s11703-008-0052-0

RESEARCH ARTICLE

Yunfei CAO, Jiaojiao WANG, Li GUO, Kai XIAO

Identification, characterization and expression analysis of transcription factor (*CBF*) genes in rice (*Oryza sativa* L.)

Abstract The acclimation of plants to cold, salt and dehydration is involved in the action of the transcription factor (CBF) cold-response pathway. In this paper, nineteen rice *CBF* genes, including seven previously released and twelve unpublished novels, were identified and characterized. The multi-members of rice CBFs (*OsCBF1* to *OsCBF12*) were divergent at the nucleotide and amino acid level. Expression analysis shows that five novel rice *CBF* genes (*OsCBF1*, *OsCBF2*, *OsCBF3*, *OsCBF8*, and *OsCBF9*) responded to short-term (1 h or 3 h) stresses of low temperature, salt stress and dehydration. The transcripts of *OsCBF2*, *OsCBF8* and *OsCBF9* in the roots were rapidly elevated when the plants were exposed to low temperatures, suggesting that they were possibly involved in low temperature responses in rice plants. Meanwhile, the expression level of *OsCBF2* in leaves was enhanced when exposed to salt stress of 1–3 h, implying that *OsCBF2* functioned as a transduction component in the salt stress signal cascade. Various expression patterns in *OsCBF1*, *OsCBF2*, *OsCBF3*, *OsCBF8*, and *OsCBF9* under low temperature, salt and drought conditions, together with the different expression patterns between roots and leaves for each of these indicated that every rice *CBF* gene has unique and non-redundant functions in the response to the abiotic stresses.

Keywords rice (*Oryza sativa* L.), abiotic stress, *CBF* gene, identification, characterization, gene expression

1 Introduction

Abiotic stresses, such as low temperature, salt and drought, frequently occur during crop production (Nakashima et al., 2007; Redondo-Gomez et al., 2007; Drame et al., 2007; Xiong et al., 2002; Surjus and Durand, 1996). In the meantime, plants have evolved some corresponding acclimation mechanisms to the adverse environments (Guy, 1990; Ludlow and Muchow, 1990; Thomashow, 1999). In *Arabidopsis*, a lot of studies have shown that the cold acclimation is involved in the action of the CBF cold-response pathway (Gilmour et al., 1998; Thomashow, 2001). The transcripts of *CBF1*, *CBF2*, and *CBF3*, or *DREB1b*, *DREB1c*, and *DREB1a* (Liu et al., 1998), respectively, increased dramatically in plants when shortly exposed to the low temperatures (Gilmour et al., 1998; Jaglo-Ottosen et al., 1998; Medina et al., 1999). It is also suggested that CBFs have been involved in salt and drought acclimation processes (Haake et al., 2002).

CBF1, *CBF2* and *CBF3* encode the transcriptional activators that are members of the AP2/EREBP family of DNA-binding proteins (Riechmann and Meyerowitz, 1998). These transcription factors bind the cold- and dehydration-responsive DNA regulatory element which was designated by the CRT (C-repeat)/DRE (dehydration response element) (Baker et al., 1994; Yamaguchi-Shinozaki and Shinozaki, 1994; Stockinger et al., 1997). Generally, the CRT/DRE elements are present in the promoters of *COR* and many other cold or other abiotic-responsive genes stimulate their transcription (Stockinger et al., 1997; Haake et al., 2002) which leads to an increase in freezing tolerance (Gilmour et al., 1998; Jaglo-Ottosen et al., 1998; Liu et al., 1998) and dehydration resistance (Haake et al., 2002). Some studies have explored that multiple mechanisms possibly contribute to the enhancement of freezing-, salt- and drought- tolerances, including the synthesis of cryoprotective polypeptides, such as COR15a (Artus et al., 1996; Steponkus et al., 1998) and the accumulation of compatible solutes that have cryoprotective properties, including sucrose, raffinose and proline (Nanjo et al., 1999; Gilmour et al., 2000; Taji et al., 2002).

The *CBF* genes in plant species exist as multi-members at the genomic level. About ten *CBF* genes have been identified in *Arabidopsis* (Kaake et al., 2002; Liu et al.,

Received September 17, 2007; accepted March 17, 2008

Yunfei CAO, Jiaojiao WANG, Li GUO, Kai XIAO (✉)
College of Agronomy, Agricultural University of Hebei, Baoding 071001, China
E-mail: xiaokai@hebau.edu.cn

1998). In barley, about twenty *CBF* genes (*HvCBFs*) comprising three multigene phylogenetic groups (*HvCBF1*, *HvCBF3*, and *HvCBF4*) have been estimated. However, until now, only seven *CBF* genes have been released or characterized in rice (Skinner et al., 2005; Dubouzet et al., 2003). Therefore, other rice *CBF* genes need to be identified, by which to further elucidate the molecular mechanisms of rice plants to acclimate to the abiotic stresses, such as low temperature, salt and drought.

In our study totaled 19 rice *CBF* genes, including seven previously released and twelve unpublished novel genes, have been identified based on BLAST (Basic Local Alignment Search Tool) analysis and rice genome search in NCBI (National Center for Biotechnology Information). The characterizations of the rice CBFs were analyzed using a bioinformatics approach. The expression patterns of the rice *CBF* genes under low temperature, salt stress and drought conditions were studied. The systematic identification and preliminarily expression analysis of the rice *CBF* genes would be helpful for further exploring how rice plants acclimate to abiotic stresses, such as low temperature, salt stress and dehydration in the future.

2 Materials and methods

2.1 Plant materials and treatments

The seeds of rice (subsp. *japonica* cv. Nipponbare) were surface sterilized and germinated under darkness at 25°C, and then transferred onto steel mesh grids which was held in a tray. The tray was filled with Murashige and Skoog (MS) medium to supply nutrients for the young seedlings. The young seedlings were grown at 25°C/20°C (day/night) under a photoperiod of 12 h daytime (cool-white fluorescent light, photon flux of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$). During the plants' culture, the MS medium was replaced once every three days and the three-week-old plants were used for all experimental treatments. For low-temperature treatment, the plants were grown at 4°C for 1–3 h in a growth chamber. For salt stress treatment, the plants were grown in the MS medium supplemented with 100 $mmol \cdot L^{-1}$ NaCl for 1–3 h. For drought stress treatment, the plants were grown in the MS medium supplemented with 10% polyethylene glycol (PEG) 6000 for 3 h. In each treatment, the roots and leaves were harvested at the indicated times, immediately frozen in liquid nitrogen and stored at −80°C until use.

2.2 Characterization of the rice CBFs

BLAST analysis combined with rice genome search in the NCBI website (http://www.ncbi.nlm.nih.gov) was performed for the identification of the rice *CBF* genes. In total, 19 non-redundant *CBF* genes were identified, including seven that have been released previously (Skinner et al., 2005; Dubouzet et al., 2003) and twelve unpublished novel ones. In this study, the 19 rice *CBF* genes were named as *OsCBF1* to *OsCBF19*. The deduced translated amino acids of the *CBF* genes that were previously unpublished were analyzed by DNAStar software. The nuclear location signal (NLS) and the conserved AP2 domain for the 19 CBFs were predicted based on online analysis, in which the NLS prediction was performed by PSORT (http://psort.ims.u-tokyo.ac.jp/) and the AP2 conserved domain was identified based on the protein conserved domain analysis program in the NCBI website (http://www.ncbi.nlm.nih.gov/Structure/cdd/). The DNAStar tool was also used in the phylogenetic tree construction and the ClustalW analysis of the 19 rice CBFs.

2.3 Expression analysis of the rice *CBF* genes

The total RNA in roots and leaves was extracted with the TRIzol reagent (Invertrogen) by following the recommendations provided by the manufacturer. For the identification of the transcripts of the rice *CBF* genes in the roots and leaves under different abiotic stresses, semi-quantitative reverse transcriptase-polymerase chain reactions (RT-PCRs) were performed as follows: Firstly, the amount of normalized total RNA was reverse transcribed into cDNA (TaKaRa). After that, a polymerase chain reaction (PCR) was conducted with the gene specific primer pair and the transcribed cDNA. The specific primer pairs used in the PCR reactions for the twelve novel rice *CBF* genes were designed by DNAStar software and listed in Table 1. The PCR conditions consisted of a cycle at 95°C for 3 min, followed by 30 cycles at 95°C for 45 s, 55°C for 45 s and 72°C for 2 min. After an extra extension for 7 min, the PCR products were stored at 4°C for electrophoretic analysis. During the RT-PCR procedure for the rice *CBF* genes, extra RT-PCR for *Rac1*, an actin gene with a constitutive expression, was concurrently performed to be used as the positive control showing the similar cDNA amount loaded in the reactions. The primer pair for *Rac1* was follows: 5′-CATGCTATCCCTC-GTCTCGACCT-3′ (forward) and 5′-CGCACTTCAT-GATGGAGTTGTAT-3′ (reverse).

3 Results

3.1 Identification and characterization of the putative nineteen rice *CBF* genes

For identification of the rice *CBF* genes, the Basic Local Alignment Search Tool (BLAST) program in National Center for Biotechnology Information

Table 1 The primer pairs used for detection of rice *CBF* (*OsCBF1* to *OsCBF12*) transcript abundance in roots and leaves under low temperature, salt stress, and drought conditions

gene name	primer	length of PCR product/bp
OsCBF1	forward: 5′-CCGGCGGGGAGGACCAAGTTCAGG reverse: 5′-GCCGCCGCCCATCCCGTCGTA	498
OsCBF2	forward: 5′-CGGGCGGGGAGGACCAAGTTCAAG reverse: 5′-ACCTCGCAGTCGTAGTCCTCCTC	527
OsCBF3	forward: 5′-GCGGGCGGGGAGGACCAAGTTCAA reverse: 5′-GCTCATCAGGGCTGGTTCGGTTCA	587
OsCBF4	forward: 5′-CCGCCGGCGAGGAGGAGAGCAG reverse: 5′-GCCGCGGCCGACGAGGTGGAC	436
OsCBF5	forward: 5′-GGCGGGCGGGGCGGAAGAAAT reverse: 5′-CCGGCGGCGTCAGCATCATC	480
OsCBF6	forward: 5′-CGGCGGGGCGGACCAAGTTCAG reverse: 5′-CGTCGGCGAGGATGGCGTCACC	588
OsCBF7	forward: 5′-GCCGCCATGCTCGCCTTGTGC reverse: 5′-CGTCCGCGCCTACCTCCTCCATTG	525
OsCBF8	forward: 5′-GCGGGGCGGTGGGTGTG reverse: 5′-CGCCGCCGCATCCTTCGTC	529
OsCBF9	forward: 5′-AGCCGTGCCGCCGCCTCTCG reverse: 5′-CGGTCGTGCTGCTGCTGCTGGTGA	486
OsCBF10	forward: 5′-ATGTGCGGGATCAAGCAGGAGAT reverse: 5′-ACTCAGGACGTCCAGTTCGAACG	594
OsCBF11	forward: 5′-GGACCAAGTTCAGGGAGACGAG reverse: 5′- CTGCGCCAAGCTCGCGTAGTAC	506
OsCBF12	forward: 5′-AGAGAGTCATCCATGGAGGTGGA reverse: 5′-GGAGAATCAAAAGGTGTCCACA	712

(NCBI) was performed in which the reported *CBF* genes in rice (*OsCBF13* to *OsCBF19*, GenBank accession number was AY785894, AY785895, AY785896, and AY785897 for *OsCBF13* to *OsCBF16*, and AF300970, AF300971 and AF300972 for *OsCBF17* to *OsCBF19*, respectively) were the queries. In the meantime, the rice genome sequences released in NCBI were searched for the identification of the *CBF* genes without redundancy with those identified by BLAST analysis. In total, twelve putatively novel rice *CBF* genes without redundancy were identified. The identified novel rice *CBF* genes and those reported previously were named separately as *OsCBF1* to *OsCBF19* in our study. The gene function, GenBank accession number, full length of cDNA, length of open reading frame (ORF) and translated amino acid number for the rice *CBF* genes were listed in Table 2. Among them, except for three (*OsCBF2*, *OsCBF15* and *OsCBF16*) with non-full length, the other sixteen had a full length cDNA, ranging from 711 bp in *OsCBF8* to 1375 bp in *OsCBF18*. The ORF length and deduced amino acids among the rice *CBF* genes changed from 645 bp (*OsCBF1*) to 825 bp (*OsCBF18*), and 214 aa (OsCBF1) to 274 aa (OsCBF18), respectively.

The conserved AP2 domain was located and the nuclear location signal (NLS) was analyzed based on online analysis. The 19 rice CBFs all contained the AP2 domain, with similar amino acid residue numbers, changing from 59 aa (OsCBF9) to 67 aa (OsCBF17). The AP2 domain positions varied in the proteins (Table 2). By mediating the protein into nucleus from the cytoplasm, the NLS is indispensable for transcription factors. In this study, the NLS was identified in all the rice CBFs, mostly with the conserved motif sequence (PKRR/PAGR), except for OsCBF4 and OsCBF18 which were with the distinct sequence RKRGDAGRHPSYRGVRR and GDCSVQVRKKRTRRK, respectively. This indicated that the 19 rice CBF genes could be the transcription factors functioning similarly as CBF members reported in *Arabidopsis* (Kaake et al., 2002; Liu et al., 1998) and barley (Skinner et al., 2005). Interestingly, it was found that the NLS motif and AP2 domain in OsCBF4 overlapped with the amino acid residues of YRGVRR (99 aa to 104 aa), different from others that had an internal spacer between the NLS and AP2 domain.

3.2 Phylogenetic analysis of the putative rice *CBF* genes

DNAStar software was used to construct the phylogenetic tree of the 19 rice CBFs. Based on the genetic distances and relationships, the rice CBFs could be classified into five subgroups. In subgroup I, II, III, IV and V, there were members of 4 (OsCBF11, OsCBF 12, OsCBF 13, and OsCBF 19), 3 (OsCBF 5, OsCBF 10, and OsCBF 17), 4 (OsCBF 7, OsCBF 8, OsCBF 9, and OsCBF 14), 4 (OsCBF 1, OsCBF 2, OsCBF 3, and OsCBF 15), and 4 (OsCBF 4, OsCBF 6, OsCBF 16, and OsCBF 18), respectively (Fig. 1). The five subgroups in the rice CBFs and the relative low identities between OsCBF1 and other OsCBFs at the amino acid level (changing from 18.7% with OsCBF18 to 46.3% with OsCBF11 and OsCBF13)

Table 2 Characterizations of the putative rice *CBF* genes *OsCBF1* to *OsCBF19*

gene name	gene function	GenBank accession No.	full length of cDNA/bp	length of ORF/bp	translated aa No.	length of AP2 domain (aa-aa)	NLS motif and position (aa-aa)	published or not
OsCBF1	putative DREB protein	AY327040	772	645	214	62 (32–93)	PKRPAGR (16–22)	no
OsCBF2	AP2 domain-containing CBF1-like protein	AY114110	No detected	660	219	61 (51–111)	PKRRAGR (36–42)	no
OsCBF3	AP2 domain-containing transcription factor	AY258283	737	660	219	61 (51–111)	PKRRAGR (36–42)	no
OsCBF4	putative DRE binding factor 2	XM_467125	864	864	287	61 (97–157)	RKRGDAGR-HPSYRGVRR (88–104)	no
OsCBF5	dehydration and cold-relative protein	AY345233	850	717	238	67 (49–115)	PKRPAGR (34–40)	no
OsCBF6	transcription factor RCBF2	AY345234	840	660	219	63 (45–107)	PKRRAGR (30–36)	no
OsCBF7	transcription factor RCBF4	AY345235	888	762	253	63 (38–100)	PKRPAGR (30–36)	no
OsCBF8	putative CRT/DRE binding factor	XM_483621	711	711	236	62 (44–105)	PKRPAGR (28–34)	no
OsCBF9	putative CRT/DRE binding factor	XM_483622	717	717	238	59 (41–99)	SKRPAGR (25–31)	no
OsCBF10	transcription factor	AF494422	717	717	238	64 (50–113)	PKRPAGR (34–40)	no
OsCBF11	putative DRE-binding protein 1B	AY166833	862	657	218	62 (32–93)	PKRPAGR (16–22)	no
OsCBF12	DRE-binding protein	AY319971	902	657	218	62 (32–93)	PKRPAGR (16–22)	no
OsCBF13	AP2 domain CBF protein	AY785894	925	657	218	62 (32–93)	PKRPAGR (16–22)	Skinner et al., (2005)
OsCBF14	AP2 domain CBF protein	AY785895	762	762	253	63 (38–100)	PKRPAGR (23–29)	Skinner et al., (2005)
OsCBF15	AP2 domain CBF protein	AY785896	No detected	660	219	61 (51–111)	PKRRAGR (36–42)	Skinner et al., (2005)
OsCBF16	AP2 domain CBF protein	AY785897	No detected	660	219	58 (46–103)	PKRRAGR (30–36)	Skinner et al., (2005)
OsCBF17	OsDREB1A	AF300970	895	717	238	67 (49–115)	PKRPAGR (34–40)	Dubouzet et al., (2003)
OsCBF18	DRE binding protein 2	AF300971	1375	825	274	61 (74–134)	GDCSVQVRK-KRTRRK (11–23)	Dubouzet et al., (2003)
OsCBF19	putative DRE-binding protein 1B	AF300972	897	657	218	62 (32–93)	PKRPAGR (16–22)	Dubouzet et al., (2003)

(Fig. 1) suggests the rice CBFs to be significantly divergent genetically during the evolution of rice.

The 19 rice CBFs were aligned based on ClustalW program in DNAStar software (Fig. 2). It could be observed that the NLS motifs and AP2 domains among the rice CBFs were much conserved, though with relatively low identities among them at the amino acid level.

3.3 Expression patterns of five novel rice *CBF* genes under low temperature, salt and drought conditions

3.3.1 Expression patterns of *CBF* genes under low temperature

Among the twelve novel *CBF* genes, only the transcripts of *OsCBF1*, *OsCBF2*, *OsCBF3*, *OsCBF8* and *OsCBF9* could be detected under the conditions of control (CK), low temperature, salt stress and drought stress. The non-transcripts detected for other rice *CBF* genes were probably due to no gene expression in the experimental conditions or the expression levels being lower than the detection scale in this study, or perhaps unsuitable designed primers used in the PCR reactions.

For the five *CBF* genes with detected transcripts, three of them (including *OsCBF2*, *OsCBF8* and *OsCBF9*) were to be induced in roots by low temperature. However, the patterns among them were not the same in a 3-h time course (Fig. 3(a)). The transcript abundance of *OsCBF2* in roots was elevated by 1-h at low temperature, then declined and was maintained at a level higher than that of CK at 3-h. *OsCBF8* and *OsCBF9*, especially *OsCBF8*, expressed an induction pattern, which shows a gradual increase with extended exposure to low temperature. The expression of *OsCBF1* and *OsCBF3* shows a pattern

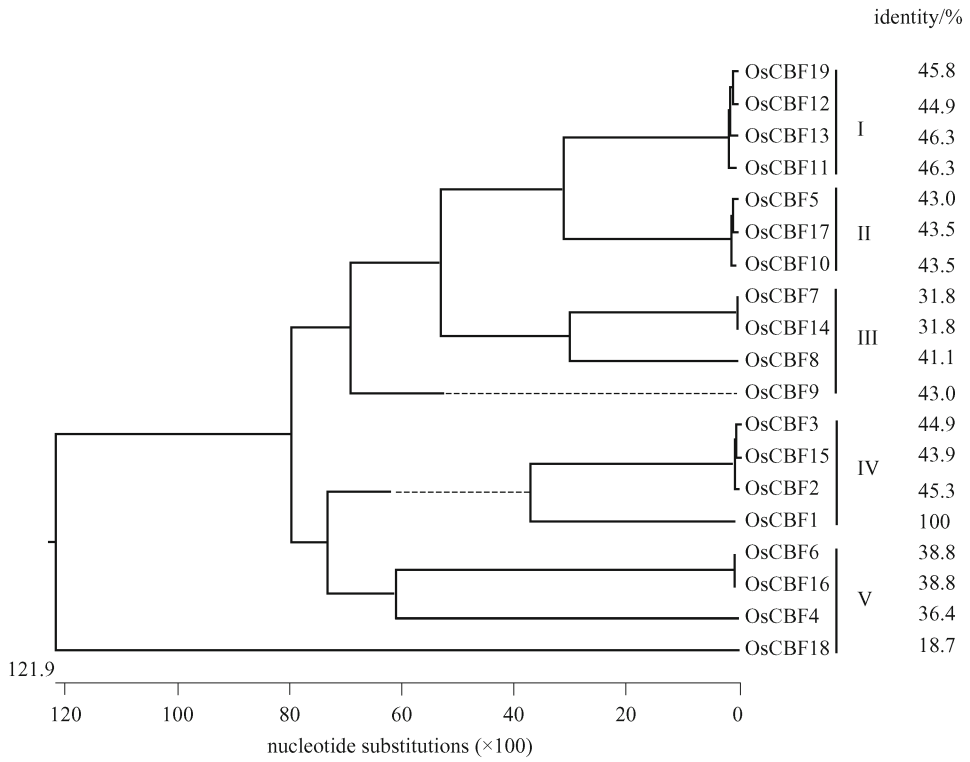

Fig. 1 The phylogenetic tree of the putative 19 rice CBFs

Note: The homologous identities are listed on the results between OsCBF1 and other rice CBFs. I, II, III, IV, and V represent Subgroup I, Subgroup II, Subgroup III, Subgroup IV, and Subgroup V, respectively. The accession numbers of the nineteen putative *CBF* genes in GenBank are *OsCBF1*, AY327040; *OsCBF2*, AY114110; *OsCBF3*, AY258283; *OsCBF4*, XM_467125; *OsCBF5*, AY345233; *OsCBF6*, AY345234; *OsCBF7*, AY345235; *OsCBF8*, XM_483621; *OsCBF9*, XM_483622; *OsCBF10*, AF494422; *OsCBF11*, AY166833; *OsCBF12*, AY319971; *OsCBF13*, AY785894; *OsCBF14*, AY785895; *OsCBF15*, AY785896; *OsCBF16*, AY785897; *OsCBF17*, AF300970; *OsCBF18*, AF300971; *OsCBF19*, AF300972.

as follows: high levels in CK, then declines after 1-h at low temperature and recovers to the CK level again after 3-h exposure to low temperature (Fig. 3(a)). The transcripts of the five *CBF* genes in the leaves show different patterns, including no transcripts detected in *OsCBF1* and *OsCBF2* and a pattern with high levels in CK, then declines after 1-h exposure to low temperature, and recovers to a similar level of CK again in *OsCBF3*, *OsCBF4*, and *OsCBF5*, similar to those of *OsCBF1* and *OsCBF3* in the roots mentioned above (Fig. 3(b)). Thus, *OsCBF2*, *OsCBF8*, and *OsCBF9*, with induced transcripts in the roots when exposed to low temperature, might be involved in mediating cold signaling transduction when plants are exposed to low temperature.

3.3.2 Expression patterns of novel rice *CBF* genes under salt stress

The transcripts of *OsCBF1*, *OsCBF2*, *OsCBF3*, and *OsCBF9* in roots were elevated after a 3-h exposure to 100 mmol·L^{-1} NaCl. But there were different patterns in the above *CBF* genes when the plants were exposed to 100 mmol·L^{-1} NaCl for a short period of time (1h), showing them to be at the middle levels of CK after a 3-h treatment, such as *OsCBF1* and *OsCBF3*, and to be lower than CK after a 3-h treatment, such as *OsCBF2* and *OsCBF9*, respectively. No transcripts of *OsCBF8* in the roots were detected at the two time points (Fig. 3(a)). The transcripts of *OsCBF1* in leaves were not detected in the CK and the salt treatment. Whereas the transcripts of *OsCBF2* in the leaves were significantly induced at the 1-h point and gradually declined at the 3-h point, but was kept higher than CK. Compared to CK, the transcripts of *OsCBF3*, *OsCBF8* and *OsCBF9* shows a decreasing tendency with the prolonging of salt stress (Fig. 3(b)). Together, the gradual increase of the transcripts of *OsCBF1* and *OsCBF3* in the roots and significant induction of *OsCBF2* expression in the leaves indicate that they play an important role in mediating the salt signal transduction when plants are exposed to salt stress.

3.3.3 Expression patterns of novel rice *CBF* genes under drought condition

The expression patterns of *OsCBF1*, *OsCBF2*, *OsCBF3*, *OsCBF8*, and *OsCBF9* under drought conditions were not the same as those at low temperature and salt stress.

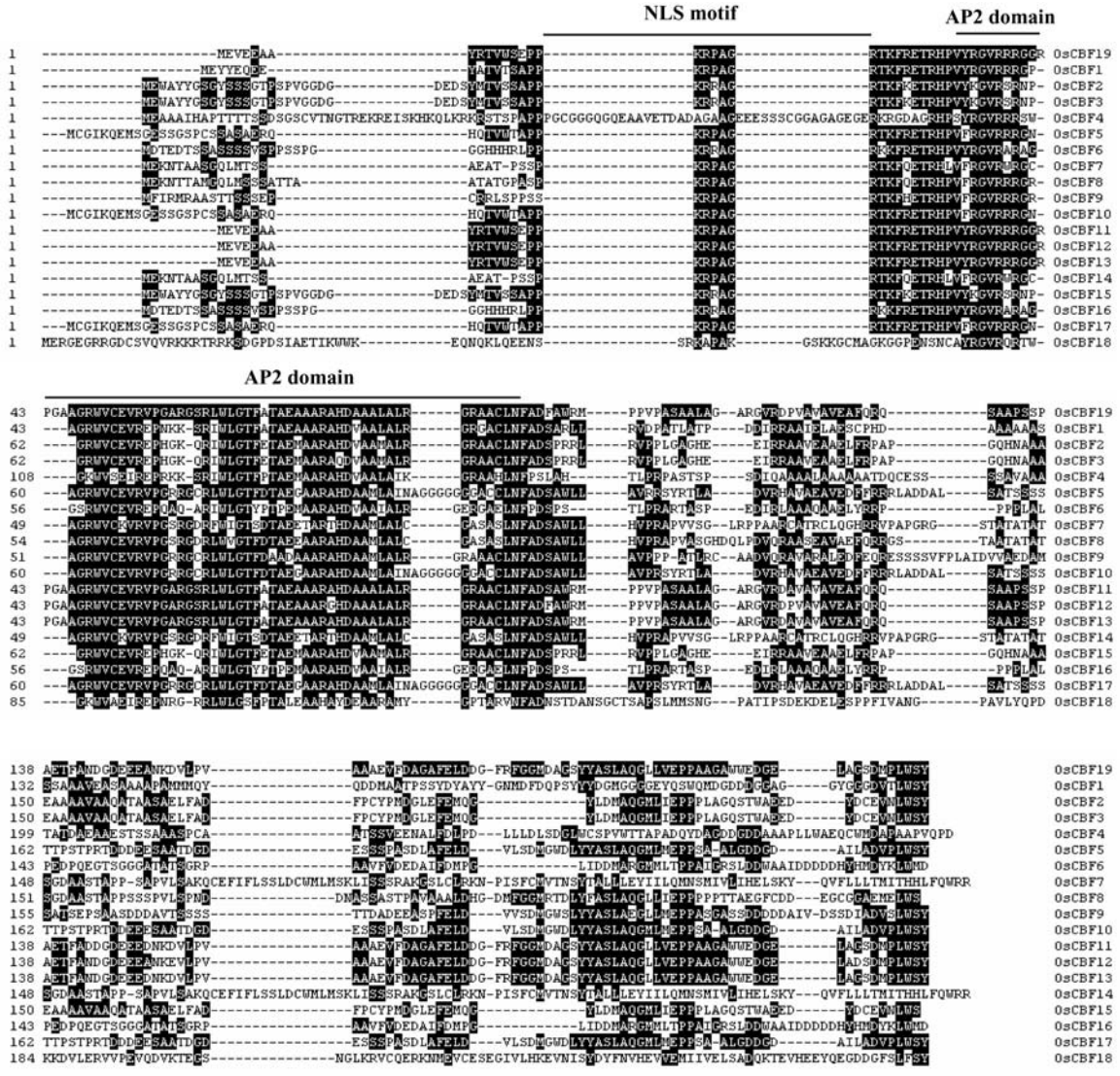

Fig. 2 The ClustalW analysis among the 19 rice CBFs (OsCBF1 to OsCBF19)

Note: The position of conserved AP2 domain for the putative rice CBFs is labeled by upline. So is the nuclear location signal (NLS) motif for most CBFs except OsCBF4 and OsCBF4 with their distinct NLSs.

Compared to CK, the transcript abundances of the above *CBF* genes in the roots were not varied under drought conditions. The expression levels of *OsCBF1*, *OsCBF2*, and *OsCBF8* in the leaves were also not changed compared to CK. However, 3 h of drought treatment dramatically reduced the transcripts of *OsCBF3* and *OsCBF9* in the leaves. Therefore, it seemed that *OsCBF3* and *OsCBF9* are involved in the drought signal transduction with a negative-regulation pathway.

4 Discussion

The transcription regulation of most eukaryotic genes is coordinated through sequence-specific binding of proteins to the promoter region located upstream of the gene. Many of conserved protein-binding sequences are found in a wide variety of organisms, among which the CCAAT-box element (Gelinas et al., 1985) is generally located between 80 and 300 bp 5′- from the transcription start site (Muro et al., 1992; Rieping and Schöffl, 1992). The proteins that bind to the CCAAT motif were first characterized in the yeast *Saccharomyces cerevisiae* through the analysis of mutants with reduced levels of expression of the *CYC1* gene (encoding iso-1-Cyt *c*) (Guarente et al., 1984; Hahn et al., 1988). It is observed that the *CYC1* promoter comprises two UAS, one of which (UAS2) contains an inverted CCAAT motif that is required for UAS2-directed transcription. Activation of transcription from UAS2 requires HAP2, HAP3, and HAP5 (Pinkham and Guarente, 1985; Pinkham et al., 1987; Hahn et al., 1988; McNabb et al., 1995), which may form a heterotri-

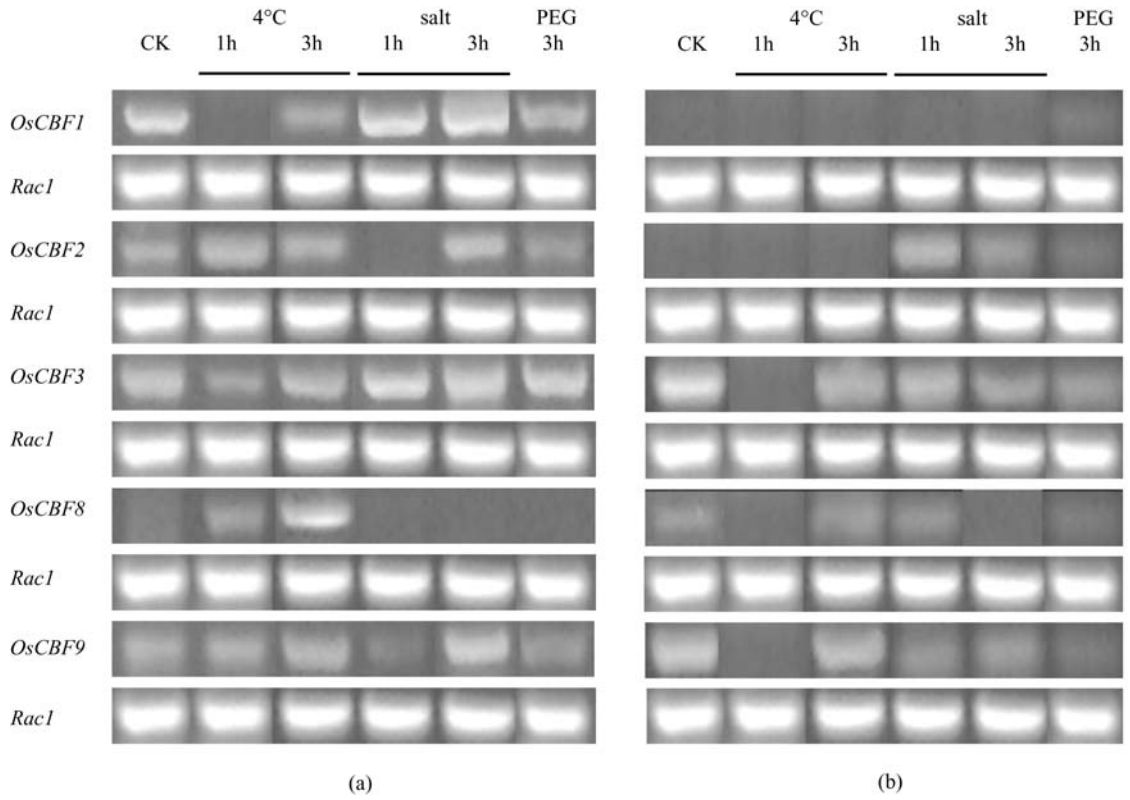

Fig. 3 Expression patterns of *OsCBF1*, *OsCBF2*, *OsCBF3*, *OsCBF8* and *OsCBF9* in roots and leaves under different abiotic stress treatments

Note: The rice actin gene *Rac1* was used as a positive control in the RT-PCR reactions. (a) represents transcript abundances of roots in CK and treatments of low temperature, salt stress and drought condition; (b) represents transcript abundances of leaves in CK and treatments of low temperature, salt stress and drought condition.

meric CCAAT-box-binding complex to initiate the gene transcription.

Except for yeast, the CCAAT-box-related motifs have also been identified in the promoters of a variety of genes in vertebrate and plant species. A range of transcription factors has been shown to bind to different CCAAT boxes with varying levels of specificity (Dorn et al., 1987; Raymondjean et al., 1988), and each is thought to play a distinct role in gene expression or DNA replication (Santoro et al., 1988). In *Arabidopsis*, there were about ten *CBF* genes, including *DREBc* (*CBF1*), *DREBb* (*CBF2*), *DREBa* (*CBF3*) (Medina et al., 1999) and *CBF4* (Haake et al., 2002) that have been identified. In rice, nowadays, there are seven *CBF* genes have been released or characterized (Skinner et al., 2005; Dubouzet et al., 2003). All of the CBFs in plant species contain the AP2 domain which interacts with the CCAAT boxes at the promoter region and a nuclear location signal (NLS) which direct the CBFs transporting to the nucleus after sorting.

In our study, nineteen rice *CBF* genes in total were identified, including seven released or were previously reported, and twelve uncharacterized novel ones based on BLAST analysis in the NCBI website and rice genome search. AP2 domain prediction and NLS detection by online analysis have figured out all of these in each of the rice CBFs. Relative low identities among these at the amino acid level indicates that the identified rice CBFs could function in regulating the gene transcription. Phylogenetic analysis indicated that the rice CBFs could be classified into five subgroups and were genetically divergent through the long history of evolution.

For *CBF* gene expression analysis, it is found some *CBF* genes in plant species could be induced by being short exposure to low temperatures, such as *CBF1*, *CBF2* and *CBF3* in *Arabidopsis*, and be induced very rapidly by a low-temperature treatment. Thus, *Arabidopsis CBF1*, *CBF2* and *CBF3* may function as transcriptional activators (Haake et al., 2002). In our study, five novel rice *CBF* genes show different expression patterns when the plants are exposed to low temperatures. The transcripts of *OsCBF2*, *OsCBF8* and *OsCBF9* in the roots were elevated when treated at low temperature for 1 h and 3 h. We also found that the cold responsive gene, *COR15*, shows a similar expression pattern when treated at the same low temperature (data not shown), indicating that *OsCBF2*, *OsCBF8* and *OsCBF9* were involved in low temperature signal transduction and plant acclimation to low temperature by modulation of their downstream genes. It is found that there were different expression patterns in

the roots and leaves when the plants were treated at low temperature, showing that the low temperature signal mediated by the roots and leaves might be through different pathways.

In plants, relative few studies on *CBF* gene expressions modulated by salt and dehydration were reported compared to those by low temperature. Haake et al. (2002) observed that the expression of *Arabidopsis CBF4* was up-regulated by drought stress, but not by low temperature. Overexpression of *CBF4* in transgenic *Arabidopsis* plants results in the activation of C-repeat/dehydration-responsive elements containing downstream genes that are involved in cold acclimation and drought adaptation (Haake et al., 2002). In our study, the transcripts of *OsCBF1*, *OsCBF2*, *OsCBF3* and *OsCBF9* in the roots were elevated after a 3-h exposure to 100 mmol·L^{-1} NaCl, though having two different patterns among them. The transcript abundances of *OsCBF2* in the leaves after 100 mmol·L^{-1} NaCl treatment for 1 h and 3 h were also obviously induced. These results suggest that the above rice *CBF* genes play an important role for plants to acclimate to the salt stress by regulating their downstream target genes. In this study, we found that the transcripts of *OsCBF3* and *OsCBF9* in the leaves under drought condition were also varied compared to CK but showed a reduced pattern with the extension of the dehydration process, indicating that the rice plants may be acclimated to drought with a negative-regulation pathway.

Transgenic plants overexpressing *Arabidopsis CBF1*, *CBF2* or *CBF3* constitutively express CBF-targeted cold-induced genes, the CBF regulon, and exhibit an increase in freezing tolerance independent of a cold stimulus (Jaglo-Ottosen et al., 1998; Liu et al., 1998). Overexpression of *CBF4* in transgenic *Arabidopsis* plants also results in the activation of C-repeat/dehydration-responsive element and improved the capabilities to cold acclimation and drought adaptation in plants (Haake et al., 2002). Therefore, some of the novel rice *CBF* genes identified in our study could possibly use the target genes to improve the plant resistances for abiotic stresses, such as low temperatures, osmotic and dehydration. It will be useful to evaluate the functions of the rice *CBF* genes, by which to generate transgenic crops with strong capabilities to be tolerant to the abiotic stresses in the future.

Acknowledgements This work was supported by the Natural Science Foundation of Hebei Province, China (No. 2006000434) and Doctorate Degree Unit Foundation of High School in China (No. 20060086001)

References

Artus N N, Uemura M, Steponkus P L, Gilmour S J, Lin C, Thomashow M F (1996). Constitutive expression of the cold-regulated *Arabidopsis thaliana COR15a* gene affects both chloroplast and protoplast freezing tolerance. Proc Natl Acad Sci USA, 93: 13404–13409

Baker S S, Wilhelm K S, Thomashow M F (1994). The 5′ region of *Arabidopsis thaliana COR15a* has cis-acting elements that confer cold-, drought- and ABA-regulated gene expression. Plant Mol Biol, 24: 701–713

Dorn A, Bollekens J, Staub A, Benoist C, Mathis D (1987). A multiplicity of CCAAT box-binding proteins. Cell, 50: 863–872

Drame K N, Clavel D, Repellin A, Passaquet C, Zuily-Fodil Y (2007). Water deficit induces variation in expression of stress-responsive genes in two peanut (*Arachis hypogaea* L.) cultivars with different tolerance to drought. Plant Physiol Biochem, 45: 236–243

Dubouzet J G, Sakuma Y, Ito Y, Kasuga M, Dubouzet E G, Miura S, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003). OsDREB genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression.Plant J, 33(4): 751–763

Gelinas R, Endlich B, Pfeiffer C, Yagi M, Stamatoyannopoulos G (1985). G-substitution to A-substitution in the distal CCAAT box of the gamma-globin gene in Greek hereditary persistence of fetal hemoglobin. Nature, 313: 323–325

Gilmour S J, Sebolt A M, Salazar M P, Everard J D, Thomashow M F (2000). Overexpression of the *Arabidopsis CBF3* transcriptional activator mimics multiple biochemical changes associated with cold acclimation. Plant Physiol, 124: 1854–1865

Gilmour S J, Zarka D G, Stockinger E J, Salazar M P, Houghton J M, Thomashow M F (1998). Low temperature regulation of the *Arabidopsis* CBF family of AP2 transcriptional activators as an early step in cold-induced *COR* gene expression. Plant J, 16: 433–442

Guarente L, Lalonde B, Gifford P, Alani E (1984). Distinctly regulated tandem upstream activation sites mediate catabolite repression of the *CYC1* gene of *S. cerevisiae*. Cell, 36: 503–511

Guy C L (1990). Cold acclimation and freezing stress tolerance: role of protein metabolism. Annu Rev Plant Physiol Plant Mol Biol, 41: 187–223

Haake V, Cook D, Riechmann J L, Pineda O, Thomashow M F, Zhang J Z (2002). Transcription factor CBF4 is a regulator of drought adaptation in *Arabidopsis*. Plant Physiol, 130: 639–648

Hahn S, Pinkham J, Wei R, Miller R, Guarente L (1988). The HAP3 regulatory locus of *Saccharomyces cerevisiae* encodes divergent overlapping transcripts. Mol Cell Biol, 8: 655–663

Jaglo-Ottosen K R, Gilmour S J, Zarka D G, Schabenberger O, Thomashow M F (1998). *Arabidopsis CBF1* overexpression induces *COR* genes and enhances freezing tolerance. Science, 280: 104–106

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1998). Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. Plant Cell, 10: 1391–1406

Ludlow M M, Muchow R C (1990). A critical evaluation of traits for improving crop yields in water-limited environments. Adv Agron, 43: 107–153

McNabb D S, Xing Y Y, Guarente L (1995). Cloning of yeast HAP5: a novel subunit of a heterotrimeric complex required for CCAAT binding. Genes Dev, 9: 47–58

Medina J, Bargues M, Terol J, Perez-Alonso M, Salinas J (1999). The *Arabidopsis CBF* gene family is composed of three genes encoding AP2 domain-containing proteins whose expression is regulated by low temperature but not by abscisic acid or dehydration. Plant Physiol, 119: 463–470

Muro A F, Bernath V A, Kornblihtt A R (1992). Interaction of the -170-cyclic AMP response element with the adjacent CCAAT box in the human fibronectin gene promoter. J Biol Chem, 267: 12767–12774

Nakashima K, Tran L S, Van Nguyen D, Fujita M, Maruyama K, Todaka D, Ito Y, Hayashi N, Shinozaki K, Yamaguchi-Shinozaki K (2007). Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress-responsive gene expression in rice. Plant J, 51: 617–630

Nanjo T, Kobayashi M, Yoshiba Y, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K (1999). Antisense suppression of proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana*. FEBS Lett, 461: 205–210

Pinkham J L, Guarente L (1985). Cloning and molecular analysis of the HAP2 locus: a global regulator of respiratory genes in *Saccharomyces cerevisiae*. Mol Cell Biol, 5: 3410–3416

Pinkham J L, Olesen J T, Guarente L P (1987). Sequence and nuclear localization of the *Saccharomyces cerevisiae* HAP2 protein, a transcriptional activator. Mol Cell Biol, 7: 578–585

Raymondjean M, Cereghini S, Yaniv M (1988). Several distinct CCAAT box binding-proteins coexist in eukaryotic cells. Proc Natl Acad Sci USA, 85: 757–761

Redondo-Gomez S, Mateos-Naranjo E, Davy A J, Fernandez-Muno F, Castellanos E M, Luque T, Figueroa M E (2007). Growth and photosynthetic responses to salinity of the salt-marsh shrub *Atriplex portulacoides*. Ann Bot, 100: 555–563

Riechmann J L, Meyerowitz E M (1998). The AP2/EREBP family of plant transcription factors. Biol Chem, 379: 633–646

Rieping M, Schöffl F (1992). Synergistic effect of upstream sequences, CCAAT box elements, and HSE sequences for enhanced expression of chimeric heat-shock genes in transgenic tobacco. Mol Gen Genet, 231: 226–232

Santoro C, Mermod N, Andrews P C, Tjian R (1988). A family of human CCAAT-box-binding proteins active in transcription and DNA replication: cloning and expression of multiple cDNAs. Nature, 334: 218–224

Skinner J S, von Zitzewitz J, Szucs P, Marquez-Cedillo L, Filichkin T, Amundsen K, Stockinger E J, Thomashow M F, Chen T H, Hayes P M (2005). Structural, functional, and phylogenetic characterization of a large *CBF* gene family in barley. Plant Mol Biol, 59(4): 533–551

Steponkus P L, Uemura M, Joseph R A, Gilmour S J, Thomashow M F (1998). Mode of action of the *COR15a* gene on the freezing tolerance of *Arabidopsis thaliana*. Proc Natl Acad Sci USA, 95: 14570–14575

Stockinger E J, Gilmour S J, Thomashow M F (1997). *Arabidopsis thaliana CBF1* encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. Proc Natl Acad Sci USA, 94: 1035–1040

Surjus A, Durand M (1996). Lipid changes in soybean root membranes in response to salt treatment. J Exp Bot, 47: 17–23

Taji T, Ohsumi C, Iuchi S, Seki M, Kasuga M, Kobayashi M, Yamaguchi-Shinozaki K, Shinozaki K (2002). Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*. Plant J, 29: 417–426

Thomashow M F (1999). Plant cold acclimation: Freezing tolerance genes and regulatory mechanisms. Annu Rev Plant Physiol Plant Mol Biol, 50: 571–599

Thomashow M F (2001). So what's new in the field of plant cold acclimation? Lots! Plant Physiol, 125: 89–93

Xiong L, Schumaker K S, Zhu J K (2002). Cell signaling during cold, drought, and salt stress. Plant Cell, 14(Suppl): 165–183

Yamaguchi-Shinozaki K, Shinozaki K (1994). A novel cis-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. Plant Cell, 6: 251–264