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Research Article

Molecular characterization of *BrRZFPs* genes encoding C3HC4 type RING zinc finger protein under abiotic stress from Chinese cabbage (*Brassica rapa* L.)

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Abstract The novel *BrRZFPs* genes encoding C3HC4-type RING zinc finger protein were identified from FOX (full length cDNA over-expressing) library of *Brassica rapa*. Ten full-length cDNAs obtained from the library encode zinc-finger protein containing 346 amino acids, designated *BrRZFPs*. These genes were classified into four groups by phylogenic analysis showing conserved protein sequences at both termini. The tissue distribution of *BrRZFPs* transcription was examined by qRT-PCR revealing ubiquitous expression pattern. However, each gene was strongly expressed in the specific tissue. Transcriptional analysis showed that those acquired 10 genes were inducible under abiotic stresses. Likewise, the transcript of *BrRZFP3* was strongly induced (~12-folds) by exogenous abscisic acid, whereas the transcripts of *BrRZFP1, BrRZFP2* and *BrRZFP3* were (> 9-folds) induced by cold. We suggest that these *BrRZFPs* that function as signal or response to abiotic stress are useful for crop improvement.

Keywords abiotic stress, *Brassica rapa*, RING zinc finger protein

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Introduction

Transcription factors regulate plant cellular and physiological responses to environmental stimuli (Xiong et al. 2002; Zheng et al. 2009). There are several transcription factor families in *Arabidopsis* that have more than 100 members each. The four largest families are the MYB super family, AP2/EREBP, basic helix loop helix (bHLH), and C2H2 zinc finger family. Several transcription factor families are found only in plants, some of which have been greatly amplified during evolution. These include the AP2/EREBP, NAC and WRKY families (Riechmann and Meyerowitz 1998; Eulgem et al. 2000). This includes several families of transcription factors that are specifically important regulators of environmental stresses, such as dehydration responsive element binding factors (DREBs), C-repeat binding factors (CBFs), bZIPs and some zinc finger proteins (Xiong et al. 2002; Tran et al. 2007; Cong et al. 2008; Zheng et al. 2009; Golldack et al. 2011).

Zinc finger proteins (ZFPs) are one of the best studied transcription factor families, playing important roles in various cellular functions, including transcriptional activation, regulation of apoptosis, and protein folding and assembly (Laity et al. 2001). Zinc finger protein such as C_2HC_5 (LIM finger) and C_3HC_4 (RING finger), are mostly implicated in protein-protein interactions. Many LIM - containing proteins have been implicated in the transcriptional regulation of cell differentiation and growth regulation (Takatsuji 1998). One group of ZFPs contain the RING-finger motif, which consists of 40-60AA residues that bind two atoms of zinc, and can be further divided into two general classes: RING-H2 (C3H2C3) and RING-HC (C3HC4) ZFPs (Freemont et al. 1991; Freemont 1993). The consensus sequence of C3HC4-type RING finger can be described as Cys-X2-Cys-X(9-39)-Cys-X(1-3)-His-X(2-3)-Cys-X2-Cys-

X(4-48)-Cys-X2-Cys, where phenylalanine and proline residues are highly conserved but not invariable, and the loops vary in length. The C3HC4-type RING finger proteins have been studied on a genomic scale in *Arabidopsis* and nucleotide sequence from ends of several BAC clones of *Brassica rapa* have been found as similar to *Arabidopsis* genomic sequence yet there has been no examination of these genes in this species (Ma et al. 2009). Additionally, ZFPs are known to function in the formation, development or signaling processes linked to stress processes, including the light perception, peroxisome formation, and during seed and root development (Xu and Li 2003; Wang et al. 2006; Pepper and Chory 1997; Chen and Ni 2006; Prestele et al. 2010).

In this study we describe the isolation and characterization of ten genes encoding C3HC4-type RING zinc finger proteins from *B. rapa*. These genes were identified in the phylogenetic relationship, motif compositions, and possible *cis*-elements. Also, the expression profiles of these genes in the development stage and under different stress treatment conditions were also analyzed using data from qRT-PCR and real-time PCR. Such a comprehensive analysis of these ten genes may provide important clues for understanding their diverse roles in the growth and development of the Chinese cabbage plant.

Materials and Methods

Plant materials

Chinese cabbage (*B. rapa* cv. Sosongchae) plants were grown in a greenhouse at Hankyong National University, Anseong, Korea. Different parts of the plant, i.e. young (first to third from the shoot apex), mature (fourth to sixth from shoot apex) and old (eighth and ninth from shoot apex) leaves, intermodal segments, flower buds, open flowers, pods and roots (branched side roots) from 2-month-old nursery grown plants were used as plant materials.

Stress treatments

Chinese cabbage seeds were aseptically grown on half-strength MS (HMS) agar medium in a culture room under a 16 h light photoperiod at 25°C. After two weeks of growth, the seedlings were transferred to fresh liquid HMS (without sucrose) medium containing 250 mM NaCl for salt stress, 100 μM abscisic acid (ABA) stress treatments, 3% Hydrogen peroxide (H_2O_2) for oxidative stress, 20% PEG6000 for osmotic stress and water stress for 4 hours. In addition, drought stress treatment was applied by keeping the seedling on filter paper for 4 hours. Induction of cold stress was examined on seedlings grown as above for other treatment conditions 4 hours after transfer to 8°C.

Database search

The protein family was isolated for obtaining sequences of the C3HC4-type RING finger family genes from full length cDNA over-expresser (FOX) library in *B. rapa* according to Lee et al. (2010). All the corresponding protein sequences of the putative C3HC4-type RING finger family members were downloaded and confirmed with the Pfam database (http://www.sanger.ac.uk/Software/Pfam/search.shtml). Information about the amino acid (aa) length and full-length cDNA accessions for each gene was obtained from NCBI (http://www.n cbi.nlm.nih.gov/).

RNA isolation and Real-time PCR

Total RNA from various Chinese cabbage tissues were extracted using the RNeasy mini kit (Qiagen, USA), after which it was treated with RNase-free DNase (Promega,

Table 1 The primers used in real-time PCR analysis for expression level of C3HC4 type zinc finger protein genes

Primer name	Primer pair (5`-3`)	Amplicon size
BrRZFP1	F: ACAACAATCAGACACCAACC R:TTGGACGATGAAGGTAAGAG	128 bp
BrRZFP2	F: CGGCTATAGTCTTGGACGTG R: ACGATCCAGAACCTAAGAGG	112 bp
BrRZFP3	F: CGGTTATAGTCTTGGACGTG R: ACGATCCAGAACCTAAGAGG	112 bp
BrRZFP4	F: GCAACATCTGCTTCGAGTTA R: CATTCTTGGGAGTGAGAGTG	112 bp
BrRZFP5	F: GGTTGCTGTTGTTGTTCTTC R: CACCAGAGGAGAGTGTTTGA	121 bp
BrRZFP6	F: TTCTTCTCCTCCTTCTCAGG R: TTCAGACTCAGGTTCCTCAA	129 bp
BrRZFP7	F: TGCCTAGGTCCATTAGCATA R: TGGAAATGAAAGGAGTGAGA	121 bp
BrRZFP8	F: TCTCTGTTGCTGTATCCATT R: GAACATGAGCCATATGAGGA	123 bp
BrRZFP9	F: TGCCTAGGTCCATTAGCATA R: TGGAAATGAAAGGAGTGAGA	121 bp
BrRZFP10	F: CTATGCATATGGGTTGCTTC R: CCCTTATCTCGGCATCTAAT	120 bp
BrActin	F: CAACCAATCGTCTGTGACAA R: ATGTCTTGGCCTACCAACAA	106 bp

USA) to remove genomic DNA contaminants.

Real-time PCR was performed using a Bio-RAD I Cycler IQ5 machine as previously described using RT pre-mix (TOYOBO Co., Japan) (Ali-Benali et al. 2005). The threshold cycle (Ct) values of PCR reactions from three independent biological replicates were averaged and the relative quantification of the expression levels was performed using the comparative Ct method for all experiments (Livak et al. 2001). The fold change in total RNA of a target gene relative to the reference gene (actin gene) was determined by the following formula: fold change = $2^{-\triangle\triangle Ct}$, where $\triangle\triangle Ct = (Ct_{\text{target gene}} - Ct_{\text{actin gene}})$ target genes - $(Ct_{\text{target gene}} - Ct_{\text{actin gene}})$ reference gene.

First-strand cDNA was generated by using Superscript™ III Reverse Transcriptase according to manufacturer's instructions (Invitrogen, USA). This cDNA (1 μl) was used as template for amplification of *BrRZFP* genes using primer pairs (Table 1). The PCR amplification program consisted of an initial step at 94°C for 5 min followed by 30 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 2 min, and a final step at 72°C for 10 min. Means were separated using Duncan's multiple range test ($P = 0.05$) in the SAS (2010) program.

Multiple sequence alignment and phylogenetic analysis

Multiple sequence alignment of C3HC4-type zinc finger protein was performed by using ClustalX version 1.83 (Thompson et al. 1997). A phylogenetic tree was constructed using DDBJ (http://clustal w.ddbj.nig.ac.jp/top-e.html), and bootstrap testing was performed with 1,000 resamplings. An alignment search was conducted using NCBI

Table 2 RING zinc finger proteins in *Brassica rapa, Arabidopsis thaliana, Oryza sativa* and other plant species

No	Plant species	Gene name	Fl-cDNA accession no.	Amino acid identity to BrRZFP1 (%)	Amino acid Residues	Annotation
1	*Brassica rapa*	BrRZFP1	HM579864	100	346	C3HC4 type RING zinc finger
2	*Brassica rapa*	BrRZFP2	HM579867	13.4	313	C3HC4 type RING zinc finger
3	*Brassica rapa*	BrRZFP3	HM579892	15.3	363	C3HC4 type RING zinc finger
4	*Brassica rapa*	BrRZFP4	HM579873	11	227	C3HC4 type RING zinc finger
5	*Brassica rapa*	BrRZFP5	HM579874	16.6	205	C3HC4 type RING zinc finger
6	*Brassica rapa*	BrRZFP6	HM579878	13	388	C3HC4 type RING zinc finger
7	*Brassica rapa*	BrRZFP7	HM579879	11.3	677	C3HC4 type RING zinc finger
8	*Brassica rapa*	BrRZFP8	HM579882	12.4	355	C3HC4 type RING zinc finger
9	*Brassica rapa*	BrRZFP9	HM579883	12.4	293	C3HC4 type RING zinc finger
10	*Brassica rapa*	BrRZFP10	HM579885	12.4	193	C3HC4 type RING zinc finger
11	*A. thaliana*	RHG1a	AAF97276	40.5	383	RING zinc finger protein
12	*A. thaliana*	ATL6	NM_111393	11.3	398	RING zinc finger protein
13	*A. thaliana*	AtCOP1	NP_180854	11.3	675	C3HC4 type RING zinc finger
14	*A. thaliana*	AtPEX2	Q9XIB6	11	847	C3HC4 type RING zinc finger
15	*A. thaliana*	AtPEX10	NP_565621	12.1	381	C3HC4 type RING zinc finger
16	*A. desertorum*	AdZFP1	AAY17949	11	445	RING zinc finger protein
17	*C. annuum*	CaRZFP1	ACN63363	13.7	219	RING zinc finger protein
18	*Oryza sativa*	OsRHC1	AK065293	10.4	473	C3HC4 type RING zinc finger /Plasma membranes
19	*Oryza sativa*	OsRHC4	AK073728	20.5	501	C3HC4 type RING zinc finger/Nucleus
20	*Oryza sativa*	OsRHC7	AK106014	10.4	446	C3HC4 type RING zinc finger/Cytoplasm
21	*Oryza sativa*	OsRHC12	AK120632	10.4	531	C3HC4 type RING zinc finger/ Mitochondrial matrix space
22	*Oryza sativa*	OsRHC13	AK071071	10.7	365	C3HC4 type RING zinc finger /Chloroplast stroma
23	*Oryza sativa*	OsRHC15	AK242034	11.6	376	C3HC4 type RING zinc finger/Nucleus
24	*Oryza sativa*	OsRHC17	AK102413	10.7	244	C3HC4 type RING zinc finger/Nucleus
25	*Oryza sativa*	OsRHC29	AK101391	14	171	C3HC4 type RING zinc finger/Nucleus

BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) and the programBLASTp, with the "nr" database. Accession numbers for the clones are as follows Table 2.

Expression profile analysis

Expression profile data were obtained by *Brassica* GeneChip microarray. In this study we singled out the expression signal values of *Brassica* C3HC4-type RING finger genes from the database for 10 tissue or organs and abiotic stresses. A gene was regarded as expressed in a tissue if its average expression signal value from the microarray database was greater than 30. To identify the tissue-preferential expresses genes, we selected one tissue and then compared it with all other 10 tissue by performing Student's *t*-test in each genotype separately. A gene expression value in a tissue in three genotype with *P* value less than 0.05 and expression values more than two fold higher than in all other tissue were considered to be expressed preferentially.

Results and Discussion

C3HC4-type RING finger family members

To identify the C3HC4-type RING finger genes, the full-length cDNA of *BrRZFPs* was isolated to FOX cDNA library according to Lee et al. (2010). The cDNA of *BrRZFPs* was identified by a database search of the dbEST division of GenBank with zinc finger protein motif as the probe sequence. Search of the BLAST analysis obtained significant matches with nine genes models encoding putative C3HC4 type RING finger proteins in *B. rapa* genome. 10 *BrRZFP*s had a typical C3HC4-type RING finger domain and various cDNA full-length (Table 3). Sequence analysis of *BrRZFP1* exhibited high identity with *RHG1a* (97%), *OsRHC1* (43%), and *OsRHC4* (52%). A comparative analysis of the protein sequence of *BrRZFP1* with known zinc finger protein sequences (ZFP) in *Arabidopsis* and rice (Yang et al., 2008; Ma et al., 2009; Zeba et al. 2009) showed that the presence of zinc motif C-X2-C-X(9-39)-C-X(1-3)-H-X(2-3)-(N/C/H)-X2-C-X(4-48)-C-X2-C was con- served (data not shown), where phenylalanine and proline residues are highly conserved but not invariable, and the loops vary in length. Whereas other characterized RING domains contain a His at metal ligand position 4, the C3HC4 type differs with the presence of a Cys residue at metal ligand position 5 (Freemont 1993; Lovering et al. 1993). Different subclasses of the RING finger domain determine specificity toward different E2 ubiquitin-conjugating enzymes (Huibregtse et al. 1995).

Until now, C3HC4-type RING finger proteins have been studied on a genomic scale in *Arabidopsis*, rice and maize. Biological function of these proteins were known with photomorphogenesis (von Arnim and Deng 1993; Hardtke et al. 2002), *AtTED3* (light signaling) (Pepper and Chory 1997), *AtRMA1* (secretory pathway) (Matsuda et al. 2001), *AtPEX10* and *AtPEX12* (peroxisome biogenesis) (Schumann et al. 2003; Fan et al. 2005), *AtPRT1* (N-end rule pathway) (Potuschak et al. 1998; Stary et al. 2003), *AtXB3* (root development) (Wang et al. 2006), *AtHUB1* and *AtHUB2* (chromatin modifications) (Liu et al. 2007b), and *AtSDIR1* (stress tolerance) (Zhang et al. 2007). Table 2 showed that 10 *BrRZFPs* genes were given systematic names from *BrRZFP1* to *OsRHC29*. Search of the KOME database obtained significant matches with 10 full-length cDNA. From the results, *BrRZFP5* and *BrRZFP10* showed localization in the nucleus by a PSORT analysis (http://psort.nibb.ac.jp/), whereas *BrRZFP2*, *BrRZFP3*, *BrRZFP6*, *BrRZFP7* and *BrRZFP8* proteins were located on plasma membranes.

Table 3 General information about C3HC4-type RING zinc finger protein-encoding genes in *Brassica rapa* L

Group	Gene name	Accession numbers	Full length (bp)	5´-UTR (bp)	ORF (bp)	3´-UTR (bp)
Group I	BrRZFP2	HM579867	1,391	164	942	285
	BrRZFP3	HM579892	1,450	74	1,092	284
	BrRZFP5	HM579874	994	162	618	214
Group II	BrRZFP1	HM579864	1,304	92	1,041	171
	BrRZFP8	HM579882	1,448	207	1,068	173
Group III	BrRZFP7	HM579879	2,536	122	2,034	380
	BrRZFP9	HM579883	2,530	117	882	1,531
Group IV	BrRZFP4	HM579873	1,075	159	684	232
	BrRZFP6	HM579878	1,355	71	1,167	117
	BrRZFP10	HM579885	1,307	382	582	343

And the remaining 2 proteins showed localization on two different organelles: one on the mitochondrial matrix space (*BrRZFP1*), and one on chloroplast stroma (*BrRZFP4*).

Phylogenetic relationship analysis

The C3HC4-type RING finger family members in Chinese cabbage (10 genes), *Arabidopsis* (5 genes), *Oryza sativa* (8 genes) and other plant species were used to construct the joint unrooted phylogenetic tree (Fig. 1).The 15 published proteins, such as *AtCOP1*, *OsRHC* family and *AtPEX* family, were included as reference sequences. Ten Chinese cabbage, rice and *Arabidopsis* C3HC4 RING finger gene pairs with very close phylogenetic relationships were found in the phylogenetic tree. *Brassica* and rice C3HC4 RING finger gene pairs with close phylogenetic relationships were found in the phylogenetic tree, *BrRZFP* genes (*BrRZFP6* and *BrRZFP10*) and *OsRHC* genes (*OsRHC15*, *OsRHC17*, and *OsRHC29*). Also, *RHG1a* of *Arabidopsis* C3HC4 RING finger gene was very closely related with *BrRZFP1*, and *CaRZFP1* of *Capsicum annuum* C3HC4 RING finger gene has very close phylogenetic relationship with *BrRZFP*4 (Fig. 1). Our results suggested that the C3HC4-type RING finger family proteins in *B. rapa* may be classified into four major groups (I, II, III and IV) with well supported bootstrap values. Group I contained 3 genes (*BrRZFP2*, *BrRZFP3* and *BrRZFP5*), group II contained 2 genes (*BrRZFP1* and *BrRZFP8*), group III contained 2 genes (*BrRZFP7* and *BrRZFP9*) and group IV contained 3 genes (*BrRZFP4*, *BrRZFP6* and *BrRZFP10*) (Fig. 2A).

Motif analysis

To identify plant genes that encode RING zinc finger sequences the GenBank database was screened using the Basic Local Alignment Search Tool (BLAST) search algorithm. Sequence comparison of *BrRZFP* to the existing data base entries indicated that *BrRZFP* genes contain a RING zinc finger region that is homologous to the RING zinc finger region of other plant species. The similarity of the motifs observed in C3HC4 motifs of the RING zinc finger protein family suggested that the *BrRZFP* genes may be transcription factors. A C-X2-C-X(9-39)-C-X(1-3)-H-X(2-3)-(N/C/H)-X2-C-X(4-48)-C-X2-C sequence (x representing any amino acid) has been defined as the DNA binding domain of the RING zinc finger family. In this study, we analyzed 25 members of the RING zinc finger family from *Brassica rapa, Arabidopsis thaliana, Oryza sativa* and other plant species. The conserved motifs were identified in the C3HC4-type RING finger family members based on

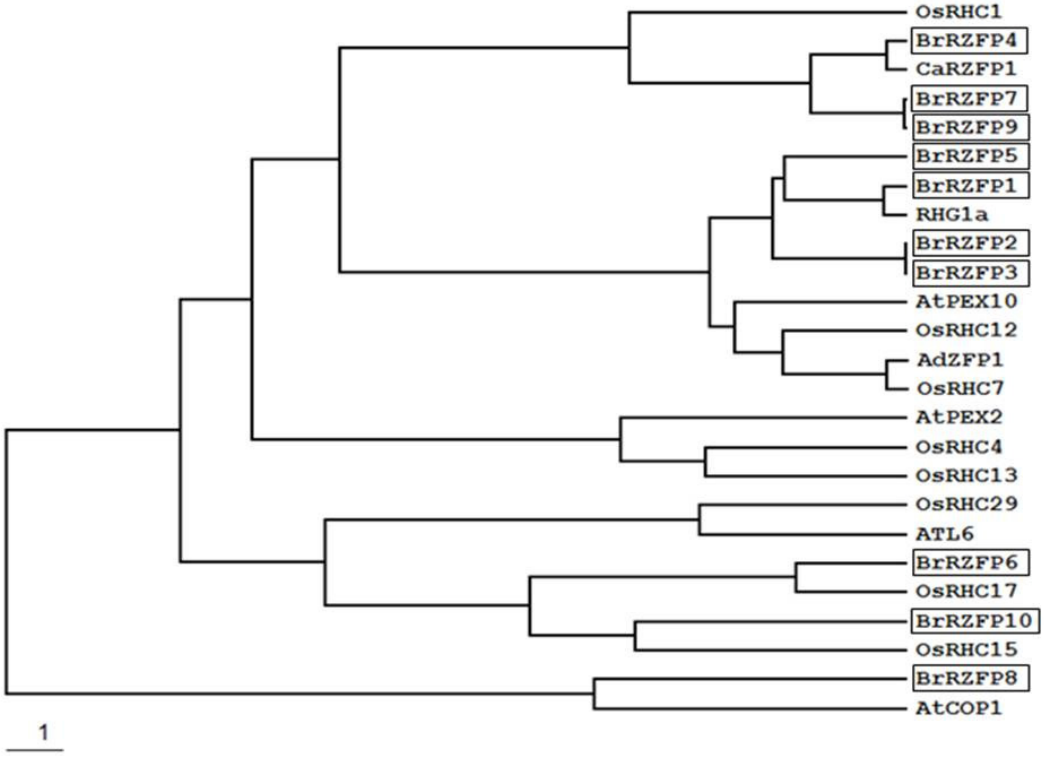

Fig. 1 The phylogenetic tree has been constructed to compare the sequence relationship of RING zinc finger domains in *Brassica, Arabidopsis,* rice and other plant species. The 25 genes deduced from amino acid sequences of the RING zinc finger region

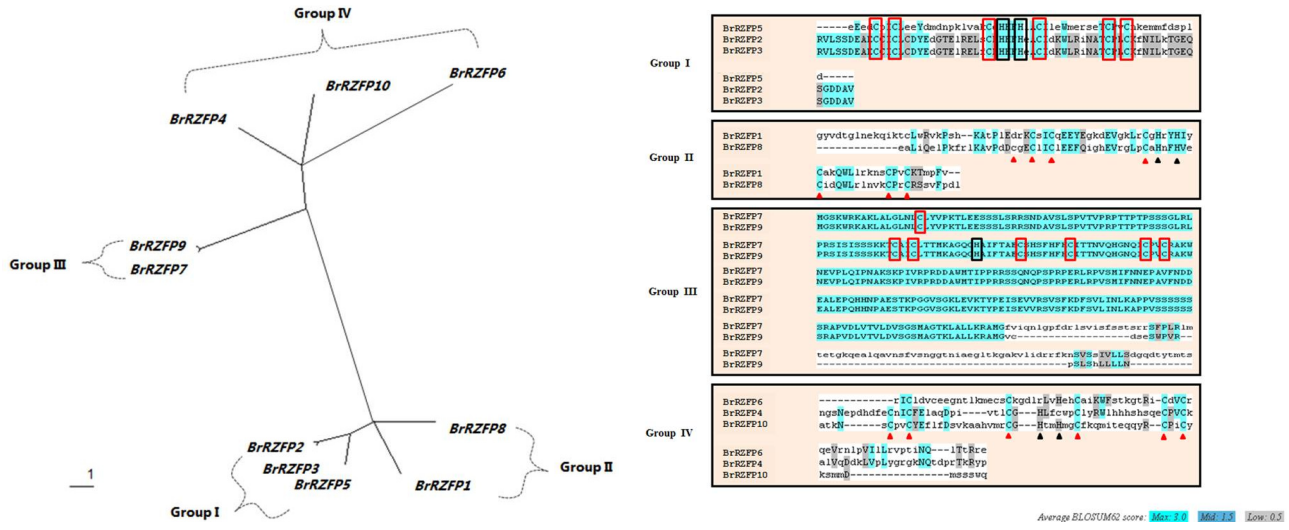

Fig. 2(A) Fig. 2(B)

Fig. 2 The phylogenetic relationships of 10 *BrRZFP* genes were analyzed using the ClustalW program. They were separated 4 groups. Group I and III were conserved in C-terminus and group II and IV were conserved In N-terminus. The bootstrap values for the respective branches are shown. The scale bar indicates 0.1 substitutions per site

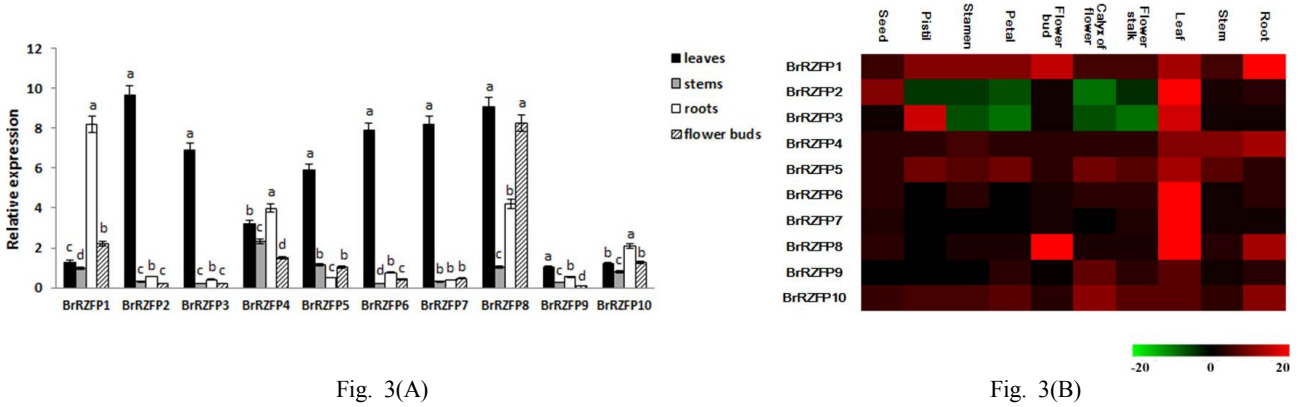

Fig. 3(A) Fig. 3(B)

Fig. 3 (A) The gene expressions of 10 *BrRZFP* genes in different organs in *B. rapa* plants were determined by qRT-PCR in leaves, stem, roots and flower buds *B. rapa* plants. Bractin were used as internal control. Different letters above bars represent significant differences at $P < 0.05$. The experiments were repeated 3 times. (B) Expression pattern of C3HC4 type zinc finger protein genes in tissues collected from *B. rapa.* The patterns showed that red color indicates up-regulation and green color indicates down-regulation

the protein sequence alignment using the Multiple Sequence Alignment program (http://align.genome.jp/) (Fig. 2B).

Expression profiles of C3HC4-type RING finger genes in different tissues and organs

Expression of the *BrRZFPs* genes was detected in various organs. Total RNA was isolated from seeds and different tissues, including vegetative tissue (leaf, stem, and root) and reproductive tissues (stamen, pistil, petal, flower bud, calyx of flower, and flower stalk), respectively, of Chinese cabbage (Fig. 3B). Real-time PCR analysis using *BrRZFPs* primers indicated that the gene was expressed in all the tested tissues except seed tissue. The primers sequences are shown in Table 1. After real-time PCR analysis, the result showed that levels of *BrRZFPs* were very various. *BrRZFP1* was preferentially expressed in the roots and *BrRZFP2, BrRZFP3, BrRZFP5, BrRZFP6, BrRZFP7* and *BrRZFP8* were expressed in the leaves. Also, *BrRZFP8* was highly expressed in flower buds (Fig. 3A).

Effect of abiotic stress on *BrRZFP* genes transcript level

Transcription factors have been shown to play important roles in signal transduction and gene expression under plant stress responses including salt, cold and drought (Chen et

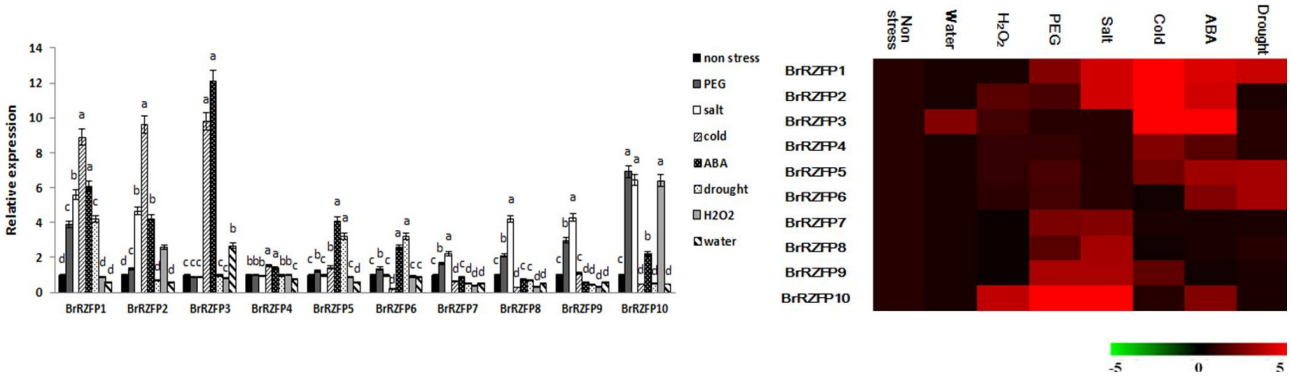

Fig. 4(A) Fig. 4(B)

Fig. 4 (A) Real-time qPCR analysis of 10 *BrRZFP* genes after application under various stresses in *B. rapa*. (B) Expression pattern of C3HC4 type zinc finger protein genes under abiotic stresses from *B. rapa*. The patterns showed that red color indicates up-regulation and green color indicates down-regulation

al. 2002; Shinozaki et al. 2003; Bartels and Sunkar 2005; Yang et al. 2008). The C3HC4-type RING zinc finger proteins have been shown to play important roles in a variety of plant processes including the regulation of growth and development, protein-protein interactions, and signalling networks (Freemont 1993; Borden et al. 1995; Tsuge et al. 2001; Wang et al. 2006). In addition, these transcription factors have been linked to abiotic stress processes of cold and salt (Lee et al. 2001; Xiong et al. 2002; Mukhopadhyay et al. 2004). Plants alter their structure and physiology to enhance their tolerance to stressful conditions. Stress-regulated gene expression plays an important role in stress acclimation and tolerance establishment when plants are exposed to unfavourable environmental changes. Many groups of genes induced by environmental stresses have been cloned and characterized, including those responsive to desiccation (RD) (Yamaguchi-Shinozaki et al. 1992), low temperature (LTI) (Nordin et al. 1993; Welin et al. 1995), cold (COR) (Horvath et al. 1993) and others. A MYC-related DNA-binding transcription factor is induced early after dehydration, salt stress, and ABA. The dehydration-responsive element-binding transcription factor DREB2 is induced within 10 min after dehydration and salt stress, whereas a subset of *Arabidopsis* Cys_2/His_2-type zinc-finger transcription factors are induced early after desiccation, salt, or ABA treatment.

BrRZFPs genes were expressed under various abiotic stresses during 4 hours (water, 3% H_2O_2, 20% PEG6000, 250mM NaCl, 8°C cold, 100 μM ABA and drought stresses) (Fig. 4B). The peaks of transcript levels showed that very various from C3HC4 type RING zinc finger protein, respectively. The qRT-PCR analysis showed that the steady-state level of *BrRZFP1*, *BrRZFP2* and *BrRZFP3* mRNA were significantly (> 9-folds) induced by cold treatment than in the non-stress control, and *BrRZFP3* was strongly induced (~12-folds) by exogenous ABA (Fig. 4A). qRT-PCR analysis showed that the expression of *BrRZFP2* could obviously be induced early by additional ABA, which indicated that *BrRZFP2* might play a role in an ABA-dependent signal pathway. In case of the *BrRZFP1, BrRZFP2 and BrRZFP3* by cold stress experiment, the expressed levels of stressed transgenic plants were much higher compared with WT plants. *BrRZFP10* was strongly induced (~7-folds) by PEG, salt and H_2O_2 except for cold, drought and water stress compared with non-stress (Fig. 4A). This result is supported by previous research that has indicated that most of the drought-inducible genes are induced by high salinity and 10% of the drought-inducible genes are induced by cold stress (Shinozaki et al. 2003).

These results suggest that the RING zinc finger protein gene *BrRZFP* genes may be of importance in plant responses to cold and salt stresses. Furthermore, this study aids in better understanding the molecular mechanisms of plants under environmental stress and in response to environmental factors, as well as the role of the RING zinc finger in the signal transduction pathway.

In conclusion, this study has characterized *BrRZFP* genes from Chinese cabbage and unraveled a determinant of abiotic stress tolerance that may be used to engineer stress tolerance in other crop plants.

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References

Ali-Benali MA, Alary R, Joudrier P, Gautier M (2005) Com-parative expression of five LEA genes during wheat seed development and in response to abiotic stresses by real-time quantitative RT-PCR. Biochimica et Biophysica Acta 1730:56-65

Bartels D, Sunkar R (2005) Drought and salt tolerance in plants. Critical Reviews in Plant Sciences 24:23-58

Borden KL. Boddy MN, Lally J, O'Reilly NJ, Martin S, Howe K, Solomon E, Freemont PS (1995) The solution structure of the RING finger domain from the acute promyelocytic leukaemia proto-oncoprotein PML. The EMBO Journal 14:1532-1541

Chen M and Ni M (2006) RFI2, a RING-domain zinc finger protein, negatively regulates CONSTANS expression and photoperiodic flowering. The Plant Journal 46:823-833

Chen W, Provart NJ, Glazebrook J, Katagiri F, Chang HS, Eulgem T, Mauch F, Luan S, Zou G, Whitham SA, Budworth PR, Tao Y, Xie Z, Chen X, Lam S, Kreps JA, Harper JF, Si-Ammour A, Mauch-Mani B, Heinlein M, Kobayashi K, Hohn T, Dangl JL, Wang X, Zhu T (2002) Expression profile matrix of *Arabidopsis* transcription factor genes suggests their putative functions in response to environmental stress. The Plant Cell 14:559-574

Cong L, Chai TY, Zhang YX (2008) Characterization of the novel gene BjDREB1B encoding a DRE-binding transcription factor from Brassica juncea L. Biochemical and Biophysical Research Communications 371:702-706

Eulgem T, Rushton PJ, Robatzek S, Somssich IE (2000) The WRKY superfamily of plant transcription factors. Trends Plant Sci 5:199-206

Fan J, Quan S, Orth T, Awai C, Chory J, Hu J (2005) The *Arabidopsis* PEX12 gene is required for peroxisome biogenesis and is essential for development. Plant Physiol 139:231-239

Freemont PS (1993) The RING finger: a novel protein sequence motif related to the zinc finger. Annals of the New York Academy of Sciences 684:174-192

Freemont PS, Hanson IM, Trowsdale J (1991) A novel cysteine-rich sequence motif. Cell 64:483-484

Golldack D, Lüking I, Yang O (2011) Plant tolerance to drought and salinity: stress regulating transcription factors and their functional significance in the cellular transcriptional network. Plant Cell Rep 30:1383-1391

Hardtke CS, Okamoto H, Stoop-Myer C, Deng XW (2002) Biochemical evidence for ubiquitin ligase activity of the *Arabidopsis* COP1 interacting protein 8 (CIP8). Plant J 30: 385-394.

Horvath DP, McLarney BK, Thomashow MF (1993) Regulation of *Arabidopsis thaliana* L. (Heyn) *cor78* in response to low temperature. Plant Physiol 103:1047-1053

Huibregtse JM, Scheffner M, Beaudenon S, Howley PM (1995) A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase. Proc. Natl. Acad. Sci. U. S. A. 92:5249

Laity JH, Lee BM, Wright PE (2001) Zinc finger proteins: new insights into structural and functional diversity. Current Opinions in Structural Biology 11:39-46

Lee H, Xiong L, Gong Z, Ishitani M, Stevenson B, Zhu JK (2001) The *Arabidopsis* HOS1 gene negatively regulates cold signal transduction and encodes a RING finger protein that displays cold-regulated nucleo-cytoplasmic partitioning. Genes & Development 15:912-924

Lee IH, Jung YJ, Park JI, Nou IS, Kang KK (2010) Systematic approaches to identify functional genes using the FOX-hunting system in Chinese cabbage. J. Plant Biotechnol. 37:175-186

Liu Y, Koornneef M, SoppeWJ (2007b) The absence of histone H2B monoubiquitination in the *Arabidopsis* hub1 (rdo4) mutant reveals a role for chromatin remodeling in seed dormancy. Plant Cell 19:433-444

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\triangle \triangle Ct}$ method. Methods 25:402-408

Lovering R, Hanson IM, Borden KLB, Martin S, Reilly NJO, Evan GI, Rahman D, Pappin DJC, Trowsdale J, Freemont PS (1993) Identification and preliminary characterization of a protein motif related to the zinc finger. Proc Natl Acad Sci U S A 90:2112-2116

Ma K, Xiao J, Li X, Zhang Q, Lian X (2009) Sequence and expression analysis of the C3HC4-type RING finger gene family in rice. Gene 444:33-45

Matsuda N, Suzuki T, Tanaka K, Nakano A (2001) Rma1, a novel type of RING finger protein conserved from *Arabidopsis* to human, is a membrane-bound ubiquitin ligase. J Cell Sci 114:1949-1957

Mukhopadhyay A, Vij S, Tyagi AK (2004) Overexpression of a zinc-finger protein gene from rice confers tolerance to cold, dehydration, and salt stress in transgenic tobacco. Proceedings of the National Academy of Sciences 101:6309-6314

Nordin K, Vahala T, Palva ET (1993) Differential expression of two related, low-temperature-induced genes in *Arabidopsis thaliana* (L.) Heynh. Plant MOI Biol 21:641-653

Pepper AE, Chory J (1997) Extragenic suppressors of the *Arabidopsis* det1 mutant identify elements of flowering-time and light-response regulatory pathways. Genetics 145:1125-1137

Potuschak T, Stary S, Schlogelhofer P, Becker F, Nejinskaia V, Bachmair A (1998) PRT1 of *Arabidopsis* thaliana encodes a component of the plant N-end rule pathway. Proc Natl Acad Sci USA 95:7904-7908

Prestele J, Hierl G, Scherling C, Hetkamp S, Schwechheimer C, Isono E, Weckwerth W, Wanner G, Gietl C (2010) Different functions of the C3HC4 zinc RING finger peroxins PEX10, PEX2, and PEX12 in peroxisome formation and matrix protein import. Proceedings of the National Academy of Sciences 107:14915-14920

Riechmann JL, Meyerowitz EM (1998) The AP2/EREBP family of plant transcription factors. Biol Chem 379:633-646

Schumann U, Wanner G, Veenhuis M, Schmid M, Gietl C (2003) AthPEX10, a nuclear gene essential for peroxisome and stor-

age organelle formation during *Arabidopsis* embryogenesis. Proc Natl Acad Sci USA 100:9626-9631

Shinozaki K, Yamaguchi-Shinozaki K, Seki M (2003) Regulatory network of gene expression in the drought and cold stress responses. Current Opinions in Plant Biology 6:410-417

Stary S, Yin XJ, Potuschak T, Schlogelhofer P, Nizhynska V, Bachmair A (2003) PRT1 of *Arabidopsis* is a ubiquitin protein ligase of the plant N-end rule pathway with specificity for aromatic amino-terminal residues. Plant Physiol 133:1360-1366

Takatsuji H (1998) Zinc finger transcription factor in plants. Celluler and Molecular Life Sciences 54:582-596

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25:4876-4882

Tran LS, Nakashima K, Shinozaki K, Yamaguchi-Shinozaki K (2007) Plant gene networks in osmotic stress response: from genes to regulatory networks. Methods in Enzymology 428: 109-128

Tsuge T, Inagaki N, Yoshizumi T, Shimada H, Kawamoto T, Matsuki R, Yamamoto N, Matsui M (2001) Phytochrome-mediated control of COP1 gene expression in rice plants. Molecular Genetics & Genomics 265:43-50

von Arnim AG, Deng XW (1993) Ring finger motif of *Arabidopsis* thaliana COP1 defines a new class of zinc-binding domain. J Biol Chem 268:19626-19631

Wang YS, Pi LY, Chen X, Chakrabarty PK, Jiang J, De Leon AL, Liu GZ, Li L, Benny U, Oard J, Ronald PC, Song WY (2006) Rice XA21 binding protein 3 is a ubiquitin ligase required for full Xa21-mediated disease resistance. The Plant Cell 18: 3635-3646

Welin BV, Olson A, Palva ET (1995) Structure and organization of wocloselyrelated low-temprature-induced *dhn/lea/rab*-like genes in *Arabidopsis* thaliana L. Heynh. Plant Mol Boil 29:391-395

Xiong L, Schumaker KS, Zhu JK (2002) Cell signaling during cold, drought, and salt stress. The Plant Cell 14:165-183

Xu R, Li QQ (2003) A RING-H2 zinc-finger protein gene RIE1 is essential for seed development in *Arabidopsis.* Plant Molecular Biology 53:37-50

Yamaguchi-Shinozaki K, Koizumi M, Urao S, Shinozaki K (1992) Molecular cloning and characterization of 9 cDNAs for genes that are responsive to desiccation in *Arabidopsis* thaliana: Sequence analysis of one cDNA clone that encodes a putative transmembrane channel protein. Plant Cell Physiol 33:217-224

Yang X, Sun C, Hu Y, Zhongping L (2008) Molecular cloning and characterization of a gene encoding RING zinc finger ankyrin protein from drought-tolerant *Artemisia desertorum.* Journal of Bioscience 33:103-112

Zeba N, Isbat M, Kwon NJ, Lee MO, Kim SR, Hong CB (2009) Heat-inducible C3HC4 type RING zinc finger protein gene from Capsicum annuum enhances growth of transgenic tobacco. Planta 229:861-871

Zhang Y, Yang C, Li Y, Zheng N, Chen H, Zhao Q, Gao T, Guo H, Xie Q (2007) SDIR1 is a RING finger E3 ligase that positively regulates stress responsive abscisic acid signaling in *Arabidopsis.* Plant Cell 19:1912-1929

Zheng X, Chen B, Han B (2009) Overexpression of a NAC transcription factor enhances rice drought and salt tolerance. Biochemical and Biophysical Research Communications 379:985-989