

Mini Review

Roles of the *bZIP* gene family in rice

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ABSTRACT. The basic leucine zipper (*bZIP*) genes encode transcription factors involved in the regulation of various biological processes. Similar to WRKY, basic helix-loop-helix, and several other groups of proteins, the *bZIP* proteins form a superfamily of transcription factors that mediate plant stress responses. In this review, we present the roles of *bZIP* proteins in multiple biological processes that include pathogen defense; responses to abiotic stresses; seed development and germination; senescence; and responses to salicylic, jasmonic, and abscisic acids in rice. We also examined the characteristics of the *bZIP* proteins and their genetic composition. To ascertain the evolutionary changes in and functions of this supergene family, we performed an exhaustive comparison among the 89 rice *bZIP* genes that were previously described and those more recently listed in the MSU Rice Genome Annotation Project Database using a Hidden Markov Model. We excluded 3 genes from the list, resulting in a total of 86 *bZIP* genes in *japonica* rice.

Key words: *bZIP*; *Oryza sativa*; Rice; Transcription factor;
Stress response

INTRODUCTION

Biotic stresses such as fungal, bacterial, and viral invasions; and abiotic stresses such as drought, low temperature, and high salinity greatly influence plant growth and development, usually resulting in decreased crop yield and quality (Cramer et al., 2011). Throughout the course of evolution, plants have gained the ability to tolerate or adapt to these unfavorable environmental conditions via transcription factor families such as the basic leucine zipper (bZIP) proteins, which have uniquely expanded and evolved to play critical roles in plant growth and development.

bZIP proteins are defined by the conserved bZIP domain (Landschulz et al., 1988; Nijhawan et al., 2008). The bZIP domain is 60 to 80 amino acids in length with 2 structures, including a highly conserved DNA binding basic region and a more diversified leucine zipper dimerization region. The basic region has ~16 amino acid residues. The most striking characteristic of this basic region is the presence of an invariant N-x7-R/K motif (Figure 1). In contrast, the Leu zipper is composed of heptad repeats of Leu, a motif consisting of a repeating pattern of several amino acids or other hydrophobic amino acids. The Leu zipper is located exactly 9 amino acids from the C terminus region. Due to its unique amino acid composition, the leucine zipper tends to form an amphipathic-helix with 2 helical turns in each heptad (Landschulz et al., 1988; Nijhawan et al., 2008).

HMM	ecelkrerrkqk N ReAArrsRq R KkaeieeL
OsbZIP05	DKESKRERRK Q S N RESARRSRL R KQAETEEL
OsbZIP38	GADERKRKRMLS N RESARRSRA R KQQRLEEL
OsbZIP60	DDEAKRRARLVR N RESAHQS R Q R KKQYVEEL
OsbZIP52	STETKRIRRMSV N RESARRSRR R KQAQLSEL
OsbZIP19	IADPKRVKRILAN R QSAQRSRV R KLQYISEL
OsbZIP49	TPDPKTLRRLA Q NREAARKSRL R KKAYIQQL
OsbZIP66	KVVERRQRRMIK N RESAARSRA R KQAYTMEL
OsbZIP44	KEDLTKTRRPLG N REAVRKYREKKKAHAFL
OsbZIP75	LVDPKRAKRIWAN R QSAARSKER R KMRYIAEL
OsbZIP21	-----EKSLKRRSGQRSRV R KLQYIADL
OsbZIP82	-----EADTKRAKQQYAQRSRV R KLQYIAEL
OsbZIP85	-----NKLLPTTHFRQYAQRSRV R KLQYIAEL

Figure 1. Invariant N-x7-R/K motif. The basic region of bZIP proteins is highly conserved, composed of ~16 amino acid residues characterized by the presence of an invariant N-x7-R/K motif. Ten typical bZIP proteins with the N-x7-R/K motif are shown. *OsbZIP21/LOC_Os02g33560* and *OsbZIP82/LOC_Os11g11100* lack this motif.

The mechanism of transcriptional regulation by bZIP proteins has been studied in detail. Most bZIP proteins show high binding affinity for the ACGT motifs, which include CACGTG (G box), GACGTC (C box), TACGTA (A box), AACGTT (T box), and a GCN4 motif, namely TGA(G/C)TCA (Landschulz et al., 1988; Nijhawan et al., 2008). A small num-

ber of bZIP factors such as OsOBF1 can also recognize palindromic sequences (Shimizu et al., 2005). However, the others, including LIP19, OsZIP-2a, and OsZIP-2b, do not bind to DNA sequences. Instead, these bZIP proteins form heterodimers with other bZIPS to regulate transcriptional activities (Nantel and Quatrano, 1996; Shimizu et al., 2005).

Genetics of the *bZIP* gene family in rice

bZIP proteins have been identified in all eukaryotes, including yeasts, vertebrates, and plants (Deppmann et al., 2006). The bZIP superfamily transcription factors have been well studied in many species such as humans and rice. Humans have 56 bZIP proteins (Deppmann et al., 2006), whereas there are a total of 89 *bZIP* genes in rice (Nijhawan et al., 2008; Ji et al., 2009). More recent updates on rice genome sequences from next generation sequencing datasets have allowed us and others to use PF00170, PF07716, and PF12498, referred to as the *bZIP*-gene family accessions in the Pfam database (<http://pfam.sanger.ac.uk/>), as query models to blast the Rice Genome Annotation Project Database to examine these 89 bZIP proteins. After eliminating the redundant and alternative splicing sequences, we obtained 86 rice *bZIP* genes with typical bZIP domains and the conserved N-x7-R/K motif. We excluded 3 genes, *OsbZIP21/LOC_Os02g33560*, *OsbZIP82/LOC_Os11g11100*, and *OsbZIP85/LOC_Os12g09270*, from the *bZIP* gene family (Table 1; **Table S1**) because these 3 genes lack the invariant N-x7-R/K motif of a bZIP protein (Figure 1).

Gene structures of the *bZIP* genes have been previously described (Nijhawan et al., 2008). In this review, the numbers and positions of exons and introns of each *bZIP* gene were further defined through the RGAP database. Similar to previous reports, we found that introns were absent in 16 coding sequences of 86 *OsbZIP* genes, and the number of introns in other coding sequences ranged from 1 to 14 (Table 1; **Table S1**). The putative transmembrane regions that were predicted by the TMHMM Server v2.0 (<http://www.cbs.dtu.dk/services/TMHMM/>) were present in *OsbZIP39* and *OsbZIP60* but not the other *OsbZIP* proteins.

The *OsbZIP* genes are scattered across all 12 rice chromosomes (Nijhawan et al., 2008). However, their distribution pattern indicates that the densities of the *OsbZIP* genes vary from chromosome to chromosome. For example, more than 10 *OsbZIP* genes each are present on chromosomes 1, 2, and 6, whereas small amounts of bZIP members are located on chromosomes 4 (N = 3) and 10 (N = 4) (Table 1; **Table S1**). Chromosome 4 is similar in size to chromosome 6, suggesting that the *bZIP* genes are unevenly distributed on rice chromosomes. However, the significance of this even distribution pattern is unclear.

Phylogenetics and expression of the *OsbZIP* genes

Many of the 86 *OsbZIPs* are evolutionarily related. To estimate their relationships, phylogenetic analysis was performed based on alignment of the bZIP proteins (Nijhawan et al., 2008). The results are illustrated in Figure 2A. Additionally, a number of motifs that are shared by different groups of *OsbZIPs* have been identified and described using the MEME motif search tool (Nijhawan et al., 2008). To examine their potential associations, the distributions of these motifs in *OsbZIPs* were analyzed (Figure 2B). It is interesting to point out that several motifs were widespread among a number of *OsbZIPs*.

Table 1. The 86 *bZIP* genes and 12 chromosomes in rice.

Chr.	No. <i>bZIP</i> genes	MSU locus (Loc.)	Locus name	Genes with no introns
Chr1	12	Os01g07880; Os01g11350; Os01g17260; Os01g36220; Os01g46970; Os01g55150; Os01g58760; Os01g59350; Os01g9760; Os01g64000; Os01g64020; Os01g64730; Os02g03580; Os02g03960; Os02g07840; Os02g09830; Os02g1040; Os02g10860; Os02g14910; Os02g16680; Os02g19950; Os02g32780; Os02g58670; Os03g20310; Os03g3550; Os03g13614; Os03g19370; Os03g20310; Os03g20650; Os03g21800; Os03g21800; Os03g56010; Os03g58250; Os03g59460	Os ZIP01 ; Os ZIP02 ; Os ZIP03 ; Os ZIP04 ; Os ZIP05 ; Os ZIP06 ; Os ZIP07 ; Os ZIP08 ; Os ZIP09 ; Os ZIP10 ; Os ZIP11 ; Os ZIP12 ; Os ZIP13 ; Os ZIP14 ; Os ZIP15 ; Os ZIP16 ; Os ZIP17 ; Os ZIP18 ; Os ZIP19 ; Os ZIP20 ; Os ZIP22 ; Os ZIP23 ; Os ZIP24 ; Os ZIP25 ; Os ZIP26 ; Os ZIP27 ; Os ZIP28 ; Os ZIP29 ; Os ZIP30 ; Os ZIP31 ; Os ZIP32 ; Os ZIP33 ; Os ZIP34 ; Os ZIP35 ; Os ZIP36 ; Os ZIP37	2
Chr2	11	Os01g059760; Os01g64000; Os02g03960; Os02g07840; Os02g1040; Os02g14910; Os02g16680; Os02g19950; Os02g32780; Os02g58670; Os03g20310; Os03g3550; Os03g13614; Os03g19370; Os03g20310; Os03g21800; Os03g21800; Os03g56010; Os04g10260; Os04g141820; Os04g54474	Os ZIP38 ; Os ZIP39 ; Os ZIP40 ; Os ZIP41 ; Os ZIP42 ; Os ZIP43 ; Os ZIP44 ; Os ZIP45	2
Chr3	10	Os01g059760; Os01g64000; Os02g03960; Os02g07840; Os02g1040; Os02g14910; Os02g16680; Os02g19950; Os02g32780; Os02g58670; Os03g20310; Os03g3550; Os03g13614; Os03g19370; Os03g20310; Os03g21800; Os03g21800; Os03g56010; Os04g10260; Os04g141820; Os04g54474	Os ZIP46 ; Os ZIP47 ; Os ZIP48 ; Os ZIP49 ; Os ZIP50 ; Os ZIP51 ; Os ZIP52 ; Os ZIP53 ; Os ZIP54 ; Os ZIP55 ; Os ZIP56 ; Os ZIP57 ; Os ZIP58 ; Os ZIP59 ; Os ZIP60 ; Os ZIP61 ; Os ZIP62 ; Os ZIP63 ; Os ZIP64 ; Os ZIP65 ; Os ZIP66 ; Os ZIP67 ; Os ZIP68 ; Os ZIP69	1
Chr4	3	Os01g059760; Os01g64000; Os02g03960; Os02g07840; Os02g1040; Os02g14910; Os02g16680; Os02g19950; Os02g32780; Os02g58670; Os03g20310; Os03g3550; Os03g13614; Os03g19370; Os03g20310; Os03g21800; Os03g21800; Os03g56010; Os04g10260; Os04g141820; Os04g54474	Os ZIP70 ; Os ZIP71 ; Os ZIP72 ; Os ZIP73 ; Os ZIP74 ; Os ZIP75 ; Os ZIP76 ; Os ZIP77	0
Chr5	8	Os01g059760; Os01g64000; Os02g03960; Os02g07840; Os02g1040; Os02g14910; Os02g16680; Os02g19950; Os02g32780; Os02g58670; Os03g20310; Os03g3550; Os03g13614; Os03g19370; Os03g20310; Os03g21800; Os03g21800; Os03g56010; Os04g10260; Os04g141820; Os04g54474	Os ZIP78 ; Os ZIP79 ; Os ZIP80 ; Os ZIP81 ; Os ZIP82 ; Os ZIP83 ; Os ZIP84 ; Os ZIP85 ; Os ZIP86 ; Os ZIP87 ; Os ZIP88 ; Os ZIP89	2
Chr6	11	Os01g059760; Os01g64000; Os02g03960; Os02g07840; Os02g1040; Os02g14910; Os02g16680; Os02g19950; Os02g32780; Os02g58670; Os03g20310; Os03g3550; Os03g13614; Os03g19370; Os03g20310; Os03g21800; Os03g21800; Os03g56010; Os04g10260; Os04g141820; Os04g54474		
Chr7	7	Os01g059760; Os01g64000; Os02g03960; Os02g07840; Os02g1040; Os02g14910; Os02g16680; Os02g19950; Os02g32780; Os02g58670; Os03g20310; Os03g3550; Os03g13614; Os03g19370; Os03g20310; Os03g21800; Os03g21800; Os03g56010; Os04g10260; Os04g141820; Os04g54474		
Chr8	6	Os01g059760; Os01g64000; Os02g03960; Os02g07840; Os02g1040; Os02g14910; Os02g16680; Os02g19950; Os02g32780; Os02g58670; Os03g20310; Os03g3550; Os03g13614; Os03g19370; Os03g20310; Os03g21800; Os03g21800; Os03g56010; Os04g10260; Os04g141820; Os04g54474		
Chr9	8	Os01g059760; Os01g64000; Os02g03960; Os02g07840; Os02g1040; Os02g14910; Os02g16680; Os02g19950; Os02g32780; Os02g58670; Os03g20310; Os03g3550; Os03g13614; Os03g19370; Os03g20310; Os03g21800; Os03g21800; Os03g56010; Os04g10260; Os04g141820; Os04g54474		
Chr10	1	Os01g059760; Os01g64000; Os02g03960; Os02g07840; Os02g1040; Os02g14910; Os02g16680; Os02g19950; Os02g32780; Os02g58670; Os03g20310; Os03g3550; Os03g13614; Os03g19370; Os03g20310; Os03g21800; Os03g21800; Os03g56010; Os04g10260; Os04g141820; Os04g54474		
Chr11	3	Os01g059760; Os01g64000; Os02g03960; Os02g07840; Os02g1040; Os02g14910; Os02g16680; Os02g19950; Os02g32780; Os02g58670; Os03g20310; Os03g3550; Os03g13614; Os03g19370; Os03g20310; Os03g21800; Os03g21800; Os03g56010; Os04g10260; Os04g141820; Os04g54474		0
Chr12	6	Os01g059760; Os01g64000; Os02g03960; Os02g07840; Os02g1040; Os02g14910; Os02g16680; Os02g19950; Os02g32780; Os02g58670; Os03g20310; Os03g3550; Os03g13614; Os03g19370; Os03g20310; Os03g21800; Os03g21800; Os03g56010; Os04g10260; Os04g141820; Os04g54474		2

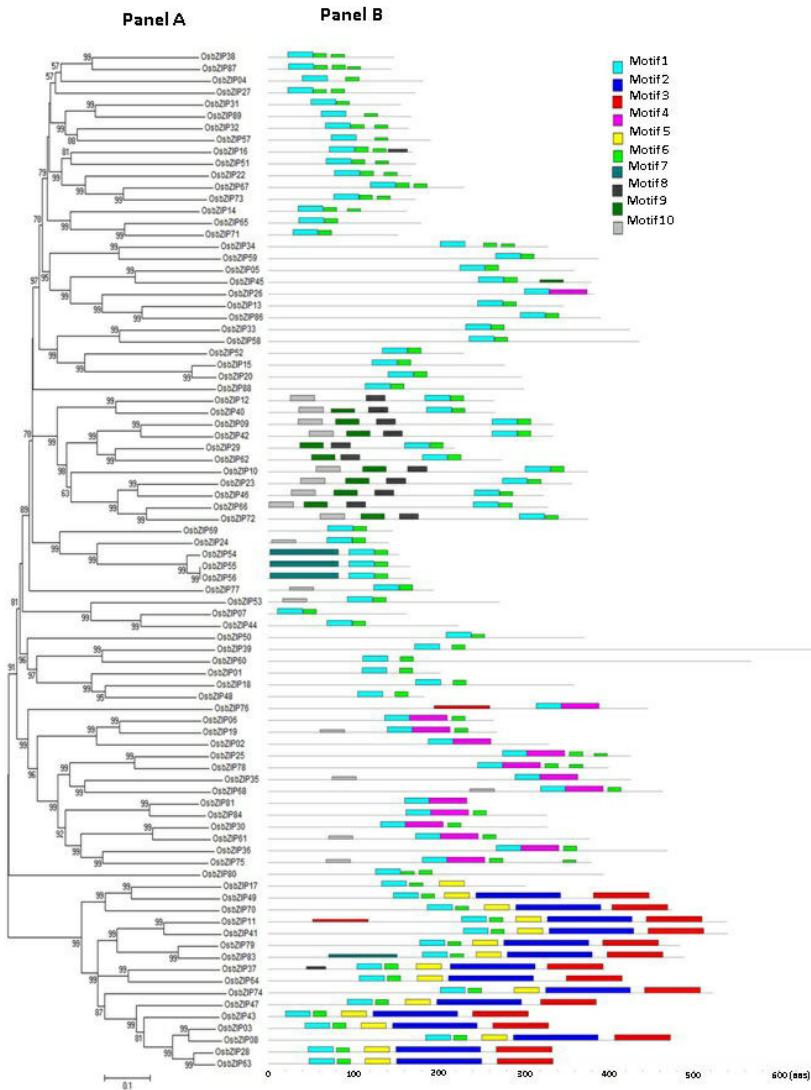


Figure 2. Panel A = phylogenetic analysis of OsbZIPs; Panel B = domain alignment in OsbZIP proteins.

We utilized full-length complementary DNAs (cDNAs) or expressed sequence tags (ESTs) from the UniGene database on the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov/unigene/>) to examine expression of the *bZIP* genes. We found that 72 of the 86 (83.53%) *OsbZIP* genes contained at least one corresponding full-length cDNA (FL-cDNA) or EST sequence ([Table S2](#)), indicating that most of the *bZIP* genes are expressed under certain conditions including cold, heat, water deprivation, salt stress, oxidative stress, and so on. For the remaining 14 *bZIP* genes, neither an FL-cDNA or EST sequence were found in the databases, suggesting that these genes are not expressed, have

very low expression levels, or are expressed only in specific tissues/cells or under specific conditions that have yet to be characterized. Various *OsbZIP* genes show high expression levels in the root, stem, leaf, panicle, and seed. Several of the *OsbZIP* genes have tissue-specific or abundant expression patterns, including *OsbZIP02* in flowers; *OsbZIP78* in leaves; *OsbZIP16*, *OsbZIP47*, *OsbZIP67*, *OsbZIP69*, and *OsbZIP77* in panicles; *OsbZIP04* and *OsbZIP27* in roots; *OsbZIP48* in stems; and *OsbZIP87* in all tissues (**Table S2**). With the accumulation of next generation sequencing datasets, we believe that the expression of *bZIP* genes in the different tissues of rice during different developmental stages and under different conditions will be further clarified in the near future.

Biological functions of the *bZIP* proteins in rice

Humans have fewer *bZIP* proteins (i.e., 56) (Deppmann et al., 2006) than rice (Nijhawan et al., 2008; Ji et al., 2009). However, similar to rice, *bZIP* proteins in humans are involved in many biological processes. One might wonder how 56 *bZIP* proteins can exert so many different functions in the complex processes of humans. One explanation by Deppmann et al. (2006) is that most *bZIP* proteins in humans can form both homodimers and heterodimers. A combination of possible homodimers and heterodimers of the 56 *bZIP* proteins in humans can yield 340 diversified unique dimers (Deppmann et al., 2006), significantly expanding the diverse roles of *bZIP* proteins. In contrast, plants have more *bZIP* proteins, but only a handful of *bZIP* proteins in plants can form heterodimers. Therefore, fewer *bZIP* dimers can be produced in plants. For example, only 175 unique dimers are predicted in *Arabidopsis thaliana*. Nevertheless, *bZIP* proteins play important roles in many processes in plants, including rice (as described below).

Roles in responses to biotic stresses and salicylic acid (SA)

Systemic acquired resistance (SAR) induced by SA confers broad-spectrum resistance to pathogens in plants. rTGA2.1, belonging to the TGA class of *bZIP* proteins, can regulate SAR by interacting with OsNPR1 to alter pathogenesis-related (*PR*) gene expression in rice. Transgenic rice suppressing *rTGA2.1* displays increased tolerance to *Xanthomonas oryzae* pv. *oryzae* (*xoo*) and altered accumulation of the *PR* genes (Fitzgerald et al., 2005). Therefore, it was concluded that wild-type rTGA2.1 has a negative impact on rice basal defense responses to bacterial pathogens. In contrast, it was shown that *OsbZIP1* expression can be rapidly induced in leaves treated with *Magnaporthe grisea* or SA, suggesting that *OsbZIP1* may play a positive role in the SA-dependent signal transduction pathway for the defense of rice against pathogens (Meng et al., 2005).

The role of *bZIPs* in response to biotic stresses and SA has also been demonstrated in many other studies. For example, rice tungro disease (RTD), a severe limiting factor for rice production in South and Southeast Asia, is primarily caused by rice tungro bacilliform virus (RTBV). It has been shown that 2 *bZIP* proteins, RF2a and RF2b, are transcriptional activators of the phloem-specific RTBV promoter, suggesting their involvement in RTD (Yin et al., 1997; Petrusselli et al., 2001; Zhu et al., 2002; Dai et al., 2003, 2004, 2008; Ordiz et al., 2010). Further studies have demonstrated that a subdomain of RF2a is comprised of 56-84 amino acids, which is essential and sufficient for its transcriptional activity (Ordiz et al., 2010). RF2a

interacts with RF2b or itself and forms a RF2a/RF2b heterodimer or homodimer. The dimers can regulate expression of RTBV by specifically binding to Box II, an essential *cis* element of the RTBV promoter, leading to symptomatic development of RTD (Yin et al., 1997; Dai et al., 2003). In addition, it seems that RF2a and RF2b suppress RTBV replication as evidenced by a report that transgenic plants overexpressing RF2a and RF2b are tolerant to RTD and exhibit reduced accumulation of RTBV viral DNA (Dai et al., 2008). In light of the importance of the bZIP proteins, a strategy against RTD and other viral diseases can be developed in rice by up-regulating the expression of host regulators such as the *bZIP* genes.

Another good example of the involvement of bZIPs in biotic stresses is the *OsTGAPI* gene. Studies on the knockout mutant and overexpressing lines for *OsTGAPI* have shown that OsTGAPI functions as a key regulator of the coordinated transcription of genes involved in inductive diterpenoid phytoalexin production in rice, and OsTGAPI plays an essential role in expression of the clustered genes for momilactone biosynthesis (Okada et al., 2009). It is well known that momilactones and phytocassanes, major diterpenoid phytoalexins, are defense-related compounds that are induced upon recognition of pathogenic invasions in rice. Therefore, we conclude that *OsTGAPI* plays an important role in response to biotic stresses such as pathogen infection.

Roles in response to abiotic stresses and abscisic acid (ABA)

The phytohormone ABA in plants plays important roles in various response processes to environmental stresses such as drought, high salinity, and low temperature. In general, these processes are associated with expression of genes induced by ABA. A number of bZIP proteins such as OsbZIP52/RISBZ5 (Liu et al., 2012), OsAREB1/OsABF2 (Hossain et al., 2010; Jin et al., 2010; Yang et al., 2001, 2011), OsABI5/OREB1 (Zou et al., 2007, 2008; Hong et al., 2011), and OSBZ8 (Nakagawa et al., 1996; Mukherjee et al., 2006) have been shown to bind to ABA-responsive elements (ABRE) and transcriptionally regulate ABA-induced ABRE-containing genes. The *OsbZIP52* gene encodes a transcriptional activator that is strongly induced by low temperature stress (4°C). The importance of *OsbZIP52* in response to cold conditions has been demonstrated in *OsbZIP52* overexpressed lines, for which several abiotic stress-related genes, such as *OsLEA3*, *OsTPP1*, and *Rab25*, are downregulated, thus leading to a significant decrease in the tolerance to cold and drought stress in rice. These data suggest that OsbZIP52 functions as a negative regulator in cold and drought stress environments (Liu et al., 2012). Analogously, *OsABI5*, which has 2 splicing variants (Zou et al., 2007), is induced by high salinity, indicating that *OsABI5* also has a negative impact on response to salt stress (Zou et al., 2008).

However, unlike OsbZIP52 and OsABI5, it has been shown that OsbZIP46 functions as a positive regulator of abiotic stress through an ABA-dependent pathway (Hossain et al., 2010; Jin et al., 2010; Tang et al., 2012). *OsbZIP46* as well as *OsABF2*, *OsAREB1*, and *ABF1* are located at the same gene locus on chromosome 6 (Hossain et al., 2010; Jin et al., 2010; Yang et al., 2011; Tang et al., 2012). OsbZIP46 is an ABRE-binding protein that can be induced by multiple abiotic stresses such as drought, salinity, cold, and oxidative stress. In addition, OsbZIP46 exhibits auxin responses, indicating a possible role of OsbZIP46 in mediating the cross talk between indole-3-acetic acid and ABA (Tang et al., 2012). Consistent with these studies, the ectopic expression of *OsbZIP46* in *Arabidopsis* (Jin et al., 2010) validated the role of Os-

bZIP46 in response to abiotic stresses in rice. Similar to OsbZIP46 (Tang et al., 2012), several other bZIP proteins, including OsABF1(Amir et al., 2010), OsbZIP72 (Lu et al., 2009), OsbZIP23 (Xiang et al., 2008), and OsbZIP16 (Chen et al., 2012), are also ABRE-binding factors and function as positive transactivators in response to drought tolerance in rice. Additionally, it has been shown that OsABF1 and OsbZIP72 are involved in salinity tolerance (Xiang et al., 2008; Lu et al., 2009; Amir et al., 2010; Chen et al., 2012). OSBZ8, another bZIP protein, is rapidly induced by ABA (Nakagawa et al., 1996). A correlation between the expression level of *OSBZ8* and salt tolerance in rice cultivars (Mukherjee et al., 2006) suggests that *OSBZ8* plays a key role in the transcriptional regulation of vegetative tissue in rice. Furthermore, several studies have demonstrated that OSBZ8 can be phosphorylated, and phosphorylation of OSBZ8 is mediated by spermidine and protein kinase OsPDK (Gupta et al., 2012). With regard to the role of phosphorylation in bZIP proteins, it was found that OREB1 possesses multiple highly-conserved phosphorylation domains (C1, C2, C3, and C4) and 2 kinase recognition motifs, RXXS/T and S/TXXE/D, within different functional domains (Shimizu et al., 2005), suggesting that phosphorylation regulates OREB1 and perhaps other bZIP proteins.

Two additional bZIP proteins, OsOBF1 and LIP19, are involved in the response to cold stress (Aguan et al., 1991, 1993; Shimizu et al., 2005). OsOBF1 homodimerizes and binds to the ACGTCA motif. In contrast, LIP19 lacks the ability to homodimerize and bind to DNA. However, LIP19 can interact with OsOBF1. In fact, the interaction of heterocombination between OsOBF1 and LIP19 is stronger than that of the homocombination of OsOBF1. At room temperature, OsOBF1 is highly expressed and forms homodimers. Lower temperatures induce a robust expression of LIP19, leading to interactions between LIP19 and OsOBF1. The resulting heterodimers bind to the C/G hybrid sequence, but not the ACGTCA motif, and activates cold-tolerant genes. Therefore, it is concluded that LIP19 plays a molecular switch role in cold signaling in rice (Shimizu et al., 2005).

Roles in endoplasmic reticulum (ER) stress response

The accumulation of unfolded proteins in the ER lumen in plants that causes ER stress is usually triggered by biotic and abiotic stresses such as pathogen attack, drought, heat, and salinity. In response, eukaryotes activate specific signal transduction pathways to maintain ER homeostasis. These pathways are designated as the ER stress response pathways.

In rice, 3 bZIP transcription factors that contain a putative transmembrane domain (TMD) in their C-terminal regions, namely OsbZIP39, OsbZIP50, and OsbZIP60, are characterized as candidates of the ER stress sensor transducer (Takahashi et al., 2012). Among these, 3 bZIP proteins, OsbZIP50, and OsbZIP39 have been more intensively studied. OsbZIP50 and OsbZIP39 are transcription factors that are involved in the activation of numerous chaperone genes (Hu et al., 2011; Wakasa et al., 2011; Hayashi et al., 2012; Lu et al., 2012; Takahashi et al., 2012). Under normal conditions, OsbZIP50 and OsbZIP39 are ER membrane-integrated proteins with no transcriptional activation activity. When plants are exposed to ER stress-inducing agents, OsbZIP50 and OsbZIP39 can be converted into truncated forms lacking TMD, which are subsequently translocated to the nucleus and awaken several chaperone genes, including *Bip1* (Lu et al., 2012; Takahashi et al., 2012). However, the mechanisms that generate active truncated forms of OsbZIP50 and OsbZIP39 are different. OsbZIP50 is activated by IRE1-mediated cytoplasmic splicing, which splices out the conserved double stem-loop

structures of OsbZIP50 mRNA (Hayashi et al., 2012; Lu et al., 2012); whereas OsbZIP39 is activated by proteolytic cleavage that removes the TMD of the OsbZIP39 protein (Takahashi et al., 2012). In other words, activation of OsbZIP50 and OsbZIP39 are post-transcriptionally and translationally regulated. We deduce from these studies that during the ER stress response, active OsbZIP39 may be generated more quickly than active OsbZIP50, but active OsbZIP50 functions for a longer period of time than active OsbZIP39.

While OsbZIP60 is also affected by ER stress (Oono et al., 2010; Yu et al., 2011), the roles of OsbZIP60 in the ER stress response are not as well documented as those of OsbZIP50 and OsbZIP39. Several studies have predicted that OsbZIP60 has similar functions to that of OsbZIP50 in the response to ER stress (Oono et al., 2010; Yu et al., 2011). In one report, OsbZIP60 was shown to be involved in heat and drought tolerance (Yu et al., 2011). In another study, OsbZIP60 was demonstrated to interact with Bphi008a, a brown planthopper-induced protein that is generated in response to brown planthopper feeding (Hu et al., 2011).

Roles in seed development

ABA is not only associated with abiotic stress responses but also with seed development (Romagosa et al., 2001). One would expect that the bZIP proteins can also regulate seed development by transcriptional regulation of ABRE-containing genes, a notion that is supported by multiple studies. For example, the transcription factor VP1 can activate ABA-responsive genes, leading to maturation and dormancy in rice seeds. However, it is well known that VP1 does not specifically bind to ABREs alone. Rather, TRAB1, a bZIP protein that binds to ABREs, leads to an indirect association between VP1 and ABREs (Hobo et al., 1999), suggesting that the association among VP1, TRAB1, and ABRE regulates maturation and dormancy in plant embryos through VP1-dependent, ABA-inducible transcription. Moreover, it has been shown that the bZIP transcriptional activator RITA-1 is highly expressed during seed development, and RITA-1 is able to bind the A-, C-, and G-boxes but not T-box elements, indicating that RITA-1 plays a role in the regulation of rice seed development (Izawa et al., 1994).

Another example of the involvement of bZIPs in seed development is the binding of RISBZ1, a bZIP transcriptional factor, GCN4 motif (Onodera et al., 2001), and highly conserved *cis*-element that plays a key role in controlling endosperm-specific expression of cereal seed storage protein genes. Reduction in RISBZ1 results in an increase in free lysine content and storage lipid accumulation in rice grain. Moreover, it has been shown that the interaction and compensation between the RISBZ1 protein and the prolamin box binding factor (RPBF) play critical roles during grain filling. The double knockdown mutant of RISBZ1 and RPBF triggers a significant reduction in seed starch accumulation (Yamamoto et al., 2006; Kawakatsu et al., 2009; Kawakatsu and Takaiwa 2010; Takahashi et al., 2012). In addition to RISBZ1, REB, an endosperm bZIP transcriptional activator, can also bind to the GCN4 motif, in particular in the α -globulin, *sbe1*, and *waxy* genes, thus resulting in accumulation of the total soluble protein in mature seeds (Nakase et al., 1997; Yang et al., 2001; Cai et al., 2002; Cheng et al., 2002). Together, these data reveal an essential role of the *bZIP* genes in seed development.

Roles in other developmental processes

The *bZIP* genes are implicated in other plant developmental processes. For instance,

in addition to their responses to adaptive stresses, OsABI5 can also regulate rice fertility. Previous studies have demonstrated that repression of *OsABI5* resulted in low fertility of rice. Based on these analyses, it appears that OsABI5 plays a negative role in abiotic stress tolerance but has a positive influence on rice fertility (Zou et al., 2008).

Another example illustrating the role of bZIP proteins in other plant developmental processes is OsAREB1. OsAREB1 functions as a positive regulator in drought/heat stress responses (Jin et al., 2010). However when OsAREB1 is expressed ectopically in *Arabidopsis* (Jin et al., 2010), expression of numerous flowering-related genes, such as *FT*, *SOC1*, *LFY*, and *API*, is downregulated, leading to delayed plant flowering. Although additional studies are needed to determine the mechanisms of OsAREB1 activity, we speculate that OsAREB1 plays a similar role in flowering in rice.

Perspectives and opportunities

The *bZIP* transcriptional factors are indispensable in plant growth and development. The expression of the *bZIP* genes can be induced in response to biotic and abiotic stresses. Increasing evidence indicates that bZIP proteins modulate the signaling networks for a number of hormones and regulate the biosynthesis of starches, storage proteins, lipids, and phytoalexins in rice. Different bZIP proteins may have different roles in various biological processes, including stress responses. Therefore, investigations on the bZIP transcription factor family will provide a better understanding of the biological networks that are mediated by the *bZIP* genes in these biological processes and present us with potential opportunities for additional research to increase agricultural productivity by conferring rice resistance to stresses through modulating the expression of specific bZIP proteins.

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Supplementary material

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