Transcription Regulation of Abiotic Stress Responses in Rice: A Combined Action of Transcription Factors and Epigenetic Mechanisms

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Abstract

Plant growth and crop production are highly reduced by adverse environmental conditions and rice is particularly sensitive to abiotic stresses. Plants have developed a number of different mechanisms to respond and try to adapt to abiotic stress. Plant response to stress such as drought, cold, and high salinity, implies rapid and coordinated changes at transcriptional level of entire gene networks. During the last decade many transcription factors, belonging to different families, have been shown to act as positive or negative regulators of stress responsive genes, thus playing an extremely important role in stress signaling. More recently, epigenetic mechanisms have been also involved in the regulation of the stress responsive genes. In this review, we have performed a comprehensive analysis of the rice transcription factors reported so far as being involved in abiotic stress responses. The impact of abiotic stresses on epigenomes is also addressed. Finally, we update the connections made so far between DNA-binding transcription factors (TFs), and epigenetic mechanisms (DNA methylation and histones methylation or acetylation) emphasizing an integrative view of transcription regulation.

Introduction

Rice production is severely affected by adverse environmental conditions, such as drought, submergence, and salinity, and the climate change is expected to raise carbon dioxide levels, temperatures, and to increase the frequency of flooding. Given that rice is one of the world’s most important staple food, providing 30% of the calories consumed in Asian countries, it is important to better understand the molecular mechanisms underlying tolerance to abiotic stresses. Plant response to environmental changes is associated with changes in signaling molecules [e.g., sugars, hormones, calcium, reactive oxygen species (ROS), nitrous oxide (NO)] (Tuteja and Sopory, 2008), but also with large-scale genomic restructuring (McClintock, 1984), including transposon activation (Cheng et al., 2006), and rapid changes on gene expression patterns (e.g., genes encoding transcription factors) (Wang et al., 2011a).

Many transcription factors (TFs) from different plants have been shown to play central roles in abiotic stress responses. The ability of TFs in acting as master regulators has been regarded as a sustainable approach to modify complex traits in crop plants (Century et al., 2008). In the previous decade, diverse TF families such as AP2/ERF, bZIP, Zn-finger, NAC, MYB, and WRKY have been described as playing a major role in abiotic stress tolerance (Century et al., 2008; Hirayama and Shinozaki, 2010; Saibo et al., 2009). In rice, three major strategies have been used to identify TFs associated with abiotic stress responses: comparative genomics—abiotic stress-responsive genes from Arabidopsis and maize were used to identify rice orthologs; forward genetics—genes related with traits, such as drought or hypoxia tolerance, were identified through association mapping; genome-wide expression profiles—transcriptome analysis using microarrays have been used to identify novel abiotic stress responsive genes.

Chromatin remodeling and nuclear organization has been also involved in plant response to stress (Kim et al., 2010). For example, in rice and wheat the salinity and heat-shock stresses caused decondensation of interphase ribosomal chromatin (Santos et al., 2011). Heterochromatin maintenance mechanisms may repress transcription under normal conditions but, under stress, those mechanisms may fail causing chromatin remodeling and novel patterns of gene expression (Arnholdt-Schmitt, 2004; Madlung and Comai, 2004).

Environmental epigenetics refers to the impact of stresses on epigenetic marks in the genome, with consequences on gene expression control (Chinnusamy and Zhu, 2009). An
epigenetic memory can be achieved through the interplay of several molecular mechanisms such as DNA methylation, posttranslational modifications of the N-terminal regions of nucleosome core histone proteins and chromatin remodeling (Zhang, 2008). Although many proteins have been shown to modulate the epigenetic response to environmental stresses, namely, TF-interacting proteins, only a few were already reported in rice. Rice epigenetic factors have been identified mainly through comparative genomics.

In this review we discuss the most recent findings on the transcriptional control of abiotic stress responses with special focus on rice. First, a comprehensive updated list of the rice TFs is described and then epigenetic changes occurring in response to abiotic stress are referred. The discussion section is mainly dedicated to the crosstalk between TFs and epigenetic mechanisms underlying regulation of gene expression in abiotic stress responses.

**Rice Transcription Factors Known To Be Involved in Abiotic Stress Responses**

Many rice TFs, belonging to different families, have been shown to be involved in abiotic stress (e.g., drought, salinity, cold, heat, and hypoxia) responses. Although numerous reports are only based on gene expression analysis (Supplementary Table S1), a number of abiotic stress-related TFs have already been functionally characterized through transgenic/mutant lines (Table 1). Although Supplementary Table S1 only includes validated gene expression data, our discussion will mainly consider Table 1. The total number of TF family members (in rice and other plant species with completed sequenced genomes) is shown in Supplementary Table S2.

**AP2/ERF family**

The AP2/ERF (APETALA2/ethylene response factor) family of transcription factors is characterized by the presence of the highly conserved AP2 DNA-binding domain (Dietz et al., 2010). This family was initially characterized as plant specific; nevertheless, there are evidences suggesting that a horizontal transfer of an HNH-AP2 endonuclease from bacteria or viruses may be related to the origin of this TF family (Magnani et al., 2004). Several AP2/ERF TFs have been isolated from various plants such as rice (Dubouzet et al., 2003), *Arabidopsis* (Sakuma et al., 2002), tobacco (Wu et al., 2007), wheat (Agarwal et al., 2006), and poplar (Dietz et al., 2010). Based on the similarity of the DNA binding domains, this family is divided in five subfamilies: AP2, RAV, ERF, DREB, and “others” (Sakuma et al., 2002). The AP2 subfamily contains two AP2 DNA-binding domains and is mainly involved in plant development (Dietz et al., 2010). Proteins containing a single AP2 domain and a B3 DNA binding domain are classified as members of the RAV subfamily. In *Arabidopsis*, members of this subfamily were shown to be mainly involved in plant development, but may also have a function in abiotic stress responses (Swaminathan et al., 2008; Woo et al., 2010). In rice, a RAV-like gene was identified, RAVL1, and shown to coordinate the brassinosteroid biosynthetic and signaling pathways, indicating a putative function in plant development (Je et al., 2010).

The DREB and ERF regulatory proteins contain a single AP2 DNA-binding domain, being characterized as regulators of biotic and/or abiotic stress responses. These subfamilies can be distinguished by their DNA binding specificity. DREB proteins interact with the DRE/CRT cis-element usually present in the promoter of genes involved in cold, drought, and high salinity responses. ERF proteins usually interact with the GCC box element present in several pathogenesis-related (PR) genes; nevertheless, other reports have suggested that this TFs can also bind to DRE elements (Sakuma et al., 2002; Xu et al., 2008b). In rice, most AP2/ERF proteins involved in biotic and abiotic stress responses belong to the ERF or DREB subfamilies. The ERF proteins were initially designated ethylene-responsive element binding proteins (EREBPs) due to their putative involvement in ethylene responses (Ohme-takagi and Shinshi, 1995). These proteins were able to bind to the GCC box element present in PR genes, suggesting a role in disease resistance responses (Gutterson and Reuber, 2004; Ohme-takagi and Shinshi, 1995; Zhou et al., 1997). Studies in *Arabidopsis*, tobacco, and tomato have shown that overexpression of ERF genes conferred resistance to fungal and bacterial pathogens (Xu et al., 2008b). Recent studies have also revealed the role of some ERF proteins in abiotic stress responses. *OsBIERF2* (*BENZOTHIAZIDIOLE-INDUCED ERF 2*) overexpression in rice enhanced tolerance to drought, high salinity, and low temperature (Oh et al., 2009). In field conditions, the same transgenic plants showed increased grain yield under drought stress conditions. ERF proteins identified in rice were shown to be involved in both biotic and abiotic stress responses. *OsBIERF1*, *OsBIERF3*, and *OsBIERF4* were induced by pathogen infection and also by cold, drought, and salt stress (Supplementary Table S1) (Cao et al., 2005, 2006). Moreover, overexpression of *OsBIERF3* enhanced disease resistance against viral and bacterial pathogens, and improved tolerance to drought and high salinity (Cao et al., 2005). Multifunctional ERF genes responding to biotic and abiotic stress stimuli were also identified in pepper, *Capsicum annuum* Pathogen and Freezing Tolerance-Related Protein (Xu et al., 2007), and wheat, *TaERF1* (Yi et al., 2004). The ERF subfamily also includes genes whose expression is induced by hypoxia. *SUB1A*, *SUB1B*, and *SUB1C* belong to the quantitative trait locus *Submergence-1* (*Sub1*) involved in rice submergence tolerance. *SUB1A* has two alleles and was shown to be present only in *indica* cultivars. The allele carried by the cultivar will define its susceptibility to submergence (Fukao et al., 2006). Introggression of *SUB1A* in *japonica* cultivars increased tolerance to submergence and also to drought stress (Fukao et al., 2011).

Transcription factors belonging to the DREB subfamily have been extensively studied in several plants, such as *Arabidopsis*, rice, wheat, tomato, and barley (Agarwal et al., 2006; Dietz et al., 2010; Yamaguchi-Shinozaki and Shinozaki, 2006). Based on studies in *Arabidopsis* this subfamily was further divided in two subclasses, DREB1/CBF and DREB2, according to their transcriptional response to abiotic stress conditions (Agarwal et al., 2006; Yamaguchi-Shinozaki and Shinozaki, 2006). The initially identified *DREB1/CBF* genes, *DREB1A*, *DREB1B*, and *DREB1C* were rapidly and transiently induced by cold, but not by drought or high salt stress, suggesting that in *Arabidopsis* they may be involved in cold stress responses. Contrastingly, *DREB2* genes, *DREB2A* and *DREB2B*, were induced by drought and high salt, but not by cold, indicating a putative function in the tolerance to drought and high salt stress (Agarwal et al., 2006; Nakashima et al., 2009). The identification of new members of the *DREB1/CBF*
Table 1. List of Rice Transcription Factors Functionally Characterized, Through Transgenic/Mutant Lines, Regarding Abiotic Stress Tolerance

<p>| Locus       | Gene     | TF Family | Cold | Drought | Salt | Heat | ABA | Hypoxia | Molecular studies                                                                 | Functional analysis                                                                 | Targets                                                                 | Crosstalk                                                                 | Reference                                                                 |
|-------------|----------|-----------|------|---------|------|------|-----|---------|----------------------------------------------------------------------------------|------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Os09g35030  | OsDREB1A | AP2/ERF   | +    | +       | +    | ND   | -   | /0      | ND Binds to DRE in vitro and activates transcription in rice protoplasts.         | OX in Arabidopsis enhances salt, drought and freezing tolerance. OX in rice improves | ND Possible role in biotic stress responses (wounding)                          | (Dubouzet et al., 2003)                                                                 |
| Os09g35030  | OsDREB1B | ND        | +    | +       | +    | 0    | ND  | ND      | GUS expression driven by OsDREB1B promoter in response to cold, ABA, drought, salt, methyl viologen, and salicylic acid. | OX in rice improves tolerance to salt, cold and drought. OX in Arabidopsis improves freezing and heat tolerance. OX in tobacco improves drought, salt, and freezing tolerance. | ND ND Involved in biotic stress responses and radical scavenging.              | (Ito et al., 2006; Fukao et al., 2011)                                        |
| Os06g06970  | OsDREB1D | ND        | 0    | 0       | 0    | ND   | 0   | ND      | ND Binds to DRE and LTRE and activates transcription in yeast.                   | OX in Arabidopsis improves cold and salt tolerance and decreases sensitivity to ABA. | ND ND Involved in abiotic stress responses.                                     | (Dubouzet et al., 2003; Ito et al., 2006; Qiu et al., 2007; Gosh and Reddy, 2008) |
| Os04g48330  | OsDREB1E | Os04g48330 | ND   | -       | ND   | ND   | -   | ND      | ND Involved in biotic stress responses (wounding).                               | ND Involved in abiotic stress responses.                                           | ND ND Involved in abiotic stress responses.                                     | (Chen et al., 2008; Wang et al., 2011)                                        |
| Os01g23070  | OsDREB1F | ND        | +    | +       | +    | ND   | ND  | ND      | ND Binds to DRE and activates transcription in yeast.                          | OX in Arabidopsis improves cold and salt tolerance.                                | ND ND Involved in abiotic stress responses.                                     | (Fukao et al., 2011)                                                        |
| Os08g43210  | OsDREB1G | ND        | ND   | ND      | ND   | ND   | ND  | ND      | ND Binds to DRE in vitro and activates transcription in rice protoplasts.       | OX in Arabidopsis improves cold and salt tolerance.                                | ND ND Involved in abiotic stress responses.                                     | (Chen et al., 2008)                                                          |
| Os01g07120  | OsDREB1A | Os04g43680 | OsMYB4 | MYB     | +    | 0    | 0   | 0       | OsMYB4 transactivates the PdAL2, Sd9 SAD cold inducible promoters.             | Overexpression in Arabidopsis and apple improves cold and drought tolerance. Overexpression in tomato improves drought tolerance. | ND Involved in abiotic stress responses.                                        | (Vannini et al., 2004; Battana et al., 2005; Vannini et al., 2006; Vannini et al., 2007; Pasquali et al., 2008; Agarwal and Jha, 2010) |</p>
<table>
<thead>
<tr>
<th>Locus</th>
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<th>TF Family</th>
<th>Cold</th>
<th>Drought</th>
<th>Salt</th>
<th>Heat</th>
<th>ABA</th>
<th>Hypoxia</th>
<th>Molecular studies</th>
<th>Functional analysis</th>
<th>Targets</th>
<th>Crosstalk</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Os01g62410</td>
<td>OsMYB3R-2</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Binds to the OsCycB1 promoter in vitro.</td>
<td>OX in rice improves cold tolerance. OX in Arabidopsis improves cold, drought and salt tolerance. Seed germination insensitive to ABA.</td>
<td>OsCycB1</td>
<td>Involved in cell-cycle regulation.</td>
<td>(Qi et al., 2007; Ma et al., 2009)</td>
</tr>
<tr>
<td>Os03g57240</td>
<td>DST</td>
<td>Zinc Finger - C2H2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Binds to conserved DNA motif and activates transcription.</td>
<td>Rice mutant is drought- and salt-tolerant, has thicker leaves, lower stomata density and closed stomata.</td>
<td></td>
<td></td>
<td>(Huang et al., 2008; Song and Matsuoka, 2009)</td>
</tr>
<tr>
<td>Os03g05600</td>
<td>ZFP182</td>
<td></td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>Constitutively expressed in leaves, culms, roots and spikes in adult plants.</td>
<td>OX in tobacco and rice improves salt tolerance.</td>
<td>Os01g62410</td>
<td>Involved in HDGe-induced stomata closure.</td>
<td>(Huang et al., 2007)</td>
</tr>
<tr>
<td>Os07g39670</td>
<td>ZFP245</td>
<td></td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Activates transcription.</td>
<td>OX in rice improves cold and drought tolerance and causes ABA-hypersensitivity.</td>
<td>Os01g62410</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Os12g39400</td>
<td>ZFP252</td>
<td></td>
<td>0</td>
<td>+</td>
<td>ND</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>OX in rice improves salt and drought tolerance.</td>
<td>Role in fertility: OX in rice increases sensitivity to salt. RNAi mutants are more sensitive to salt, drought, and dehydration.</td>
<td>Os01g64730</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Os01g64000</td>
<td>OsABE, OsbZIP-10</td>
<td>bZIP</td>
<td>+/−</td>
<td>+/−</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>Expressed in mature pollen. Two splice forms, with different transcription and DNA-binding activity. Binds to G-box. Both proteins interact with each other and with OsVP1.</td>
<td>OX in rice increases sensitivity to salt. RNAi lines have decreased fertility, increased salt tolerance and drought. OX in Arabidopsis results in ABA-hypersensitivity.</td>
<td>Os01g64000</td>
<td>ND</td>
<td>Role in fertility: Involved in pollen development.</td>
</tr>
<tr>
<td>Os01g64730</td>
<td>OsABF1, OsbZIP-12</td>
<td></td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Binds to ABRE and activates transcription.</td>
<td>Rice mutants are more sensitive to salinity and dehydration.</td>
<td>Os06g10880</td>
<td>ND</td>
<td>GA1 antagonists gene induction by ABA. Induced by ACC and MeJA.</td>
</tr>
<tr>
<td>Os02g52780</td>
<td>OsbZIP-23</td>
<td></td>
<td>+/0</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>Expressed mainly in leaves. Activates transcription.</td>
<td>OX in rice induces tolerance to drought and salt, hypersensitivity to ABA. Mutant has low tolerance to salt and drought, and decreased sensitivity to ABA.</td>
<td>Os01g64000</td>
<td>ND</td>
<td>Induced by ACC.</td>
</tr>
<tr>
<td>Os06g10880</td>
<td>OsABF3, OsABF2, OsbZIP-46</td>
<td></td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>Expressed in roots, shoots, stem, mature leaf, flag leaf, and panicle. Binds to ABRE and activates transcription.</td>
<td>Rice mutant is more sensitive to salt, drought and oxidative stress, and has reduced sensitivity to ABA.</td>
<td>Os01g64000</td>
<td>ND</td>
<td>Induced by ACC.</td>
</tr>
<tr>
<td>Os06g10880</td>
<td>ABL1</td>
<td></td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>Expressed mainly in coleoptiles and primary roots. Binds to ABRE and activates transcription.</td>
<td>Mutant is ABA-insensitive. OX in Arabidopsis rescues ethylene insensitivity phenotype.</td>
<td>Os01g64000</td>
<td>Os01g29000, Os01g29600, LEA3 and Rab16</td>
<td>Involved in auxin signalling. Induced by ACC and MeJA</td>
</tr>
<tr>
<td>Os09g28310</td>
<td>OsbZIP-2, OsbZIP-10</td>
<td>OsbZIP-46</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>/0</td>
<td>Expressed mainly in panicles under control conditions.</td>
<td>OX in rice with promHSP101- tolerance to heat and drought.</td>
<td>Os01g64000</td>
<td>Os01g29000, Os01g29600, LEA3 and Rab16</td>
<td>Involved in auxin signalling. Induced by ACC and MeJA</td>
</tr>
<tr>
<td>Os01g43650</td>
<td>OsWRKY11</td>
<td>WRKY</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Expressed mainly in panicles under control conditions.</td>
<td>OX in rice leads to higher sensitivity to salinity and cold. Enhanced resistance to biotic stress.</td>
<td>Os01g64000</td>
<td>Os01g29000, Os01g29600, LEA3 and Rab16</td>
<td>Involved in auxin signalling. Induced by ACC and MeJA</td>
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<tr>
<td>Os01g34600</td>
<td>OsWRKY13</td>
<td>OsWRKY13</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>OX in rice leads to higher sensitivity to salinity and cold. Enhanced resistance to biotic stress.</td>
<td>PR1, ACS5, LOX, OsWRKY24, 42, 45, 51, 74</td>
<td>Os01g64000</td>
<td>Os01g29000, Os01g29600, LEA3 and Rab16</td>
<td>Involved in auxin signalling. Induced by ACC and MeJA</td>
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<tr>
<td>Os05g20570</td>
<td>WRKY45</td>
<td>WRKY</td>
<td>+/−</td>
<td>+/0</td>
<td>+/0</td>
<td>0/−</td>
<td>+/−</td>
<td>+/−</td>
<td>Induced by benzothiadiazole (BTH).</td>
<td>OX in rice leads to enhanced resistance to blast infection via RTH-inducible responses. OX in Arabidopsis leads to enhanced tolerance to drought and high salinity. Decreased sensitivity to ABA in germination and seedling growth.</td>
<td>Os01g64000</td>
<td>Os01g29000, Os01g29600, LEA3 and Rab16</td>
<td>Involved in auxin signalling. Induced by ACC and MeJA</td>
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<tr>
<td>Os05g06610</td>
<td>OsWRKY10</td>
<td>OsWRKY10</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>OX in rice leads to higher sensitivity to salinity and cold. Enhanced resistance to biotic stress.</td>
<td>PR1, ACS5, LOX, OsWRKY24, 42, 45, 51, 74</td>
<td>Os01g64000</td>
<td>Os01g29000, Os01g29600, LEA3 and Rab16</td>
<td>Involved in auxin signalling. Induced by ACC and MeJA</td>
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<td>Os05g06610</td>
<td>OsWRKY80</td>
<td>OsWRKY80</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>OX in rice leads to enhanced resistance to biotic stress.</td>
<td>PR1, ACS5, LOX, OsWRKY24, 42, 45, 51, 74</td>
<td>Os01g64000</td>
<td>Os01g29000, Os01g29600, LEA3 and Rab16</td>
<td>Involved in auxin signalling. Induced by ACC and MeJA</td>
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### Table 1 (Continued)

<table>
<thead>
<tr>
<th>Locus</th>
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<th>Cold</th>
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<th>Heat</th>
<th>ABA</th>
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<tr>
<td>Os11g29870</td>
<td>OsWRKY72</td>
<td></td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>Expressed in aleurone cells. Promotes transcription.</td>
<td>Ox in <em>Arabidopsis</em> leads to enhanced sensitivity to ABA, salt and osmotic stresses and sugar starvation</td>
<td>ND</td>
<td>ND</td>
<td>(Xie et al., 2005; Yu et al., 2008a)</td>
</tr>
<tr>
<td>Os01g66120</td>
<td>SNAC2/ OsNAC6</td>
<td>NAC</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Induced by wounding. Tissue-specific expression according to stress. Transcriptional activator</td>
<td>Ox in rice: improved tolerance to cold, dehydration and salt. More sensitive to ABA.</td>
<td>ND</td>
<td>ND</td>
<td>(Hu et al., 2008a), (Takasaki et al., 2010), (Nakashima et al., 2007), (Orenishi et al., 2005)</td>
</tr>
<tr>
<td>Os03g60080</td>
<td>SNAC1/ OsNAC19</td>
<td></td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Particularly induced in stomata guard cells. Induced by blast, MeJA and ethylene. Activates transcription.</td>
<td>Ox in rice: improved tolerance to drought and salt. More sensitive to ABA with increased stomata closure.</td>
<td>ND</td>
<td>ND</td>
<td>(Hu et al., 2006), (Lin et al., 2007)</td>
</tr>
<tr>
<td>Os05g34300</td>
<td>OsNAC5/ ONAC088</td>
<td>ONAC063</td>
<td>0</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Increased expression mainly in roots. Activates transcription.</td>
<td>Ox in <em>Arabidopsis</em> the gene are hypersensitive to ABA and more tolerant to dehydration.</td>
<td>ND</td>
<td>ND</td>
<td>(Gao et al., 2010)</td>
</tr>
<tr>
<td>Os08g33910</td>
<td>ONAC10</td>
<td></td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>Expressed in root and panicles. RCC5:OsNAC10 plants showed enhanced drought tolerance at the reproductive stage due to differences in expression of OsNAC10-dependent target genes in roots and leaves. Activates transcription.</td>
<td>Ox in rice under GOS1 (constitutive promoter) and the RCC5 (root-specific) increased the plant tolerance to drought, high salinity, and low temperature at the vegetative stage.</td>
<td>ND</td>
<td>ND</td>
<td>(Yokotani et al., 2009)</td>
</tr>
<tr>
<td>Os11g33370</td>
<td>ONAC065</td>
<td></td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Expressed in roots and leaves. Activates transcription.</td>
<td>Ox in rice enhances tolerance to drought and high-salinity.</td>
<td>ND</td>
<td>ND</td>
<td>(Zheng et al., 2009)</td>
</tr>
<tr>
<td>Os11g38210</td>
<td>OsNAC5</td>
<td></td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Northern blot. Shows transcriptional activity in yeast but not in rice protoplasts</td>
<td>Ox in rice – tolerance to high salinity and drought.</td>
<td>ND</td>
<td>ND</td>
<td>(Fang et al., 2008)</td>
</tr>
<tr>
<td>Os03g06650</td>
<td>OsHSF2/ OsHSF2d</td>
<td>HSF</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Express in leaves &amp; flowers (qRT-PCR)</td>
<td>Ox in <em>Arabidopsis</em> with higher basal thermotolerance, not acquired.</td>
<td>ND</td>
<td>ND</td>
<td>(Basiwal et al., 2004, Yokotani et al., 2008, Liu et al., 2009, Mittal et al., 2009, Liu et al., 2010)</td>
</tr>
<tr>
<td>Os03g58160</td>
<td>OsHsfAl2</td>
<td></td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Activates transcription</td>
<td>Ox in <em>Arabidopsis</em> resulted in thermotolerance to transient heat and salt tolerance.</td>
<td>ND</td>
<td>ND</td>
<td>(Yokotani et al., 2008, Mittal et al., 2009, Liu et al., 2010)</td>
</tr>
<tr>
<td>Os11g32100</td>
<td>OsbHLH2/ OsICE1</td>
<td>HLH</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Stress-responsive genes DREB1A/ CBF3, RD29A, COR15A and KIN1 were up regulated in transgenic plants</td>
<td>Ox in <em>Arabidopsis</em> resulted in conformed increased tolerance to salt and osmotic stress.</td>
<td>ND</td>
<td>ND</td>
<td>(Zhou et al., 2009)</td>
</tr>
</tbody>
</table>

Plus, minus, and zero correspond to upregulation, downregulation and not regulated, respectively.

ND, not determined; OX, overexpressed.
subclass, DREB1D and DREB1F, which respond to drought and salt stress, respectively, may suggest a crosstalk between DREB1/CBF and DREB2 pathways in response to those abiotic stresses (Haake et al., 2002; Nakashima et al., 2009; Sakuma et al., 2002). In rice, several DREB1 and DREB2 orthologs were shown to play a role in abiotic stress responses. The rice DREB1A orthologs, OsDREB1A and OsDREB1B, were differently expressed in response to abiotic stress. Rice genes were induced by cold, but also by drought and high salt conditions (Dubouzet et al., 2003; Ito et al., 2006). OsDREB1B was also shown to respond to heat stress, suggesting a role in the response to this abiotic stress (Qin et al., 2007). Furthermore, the overexpression of OsDREB1A and OsDREB1B in rice resulted in tolerance phenotypes similarly to Arabidopsis plants overexpressing the DREB1A (Ito et al., 2006; Nakashima et al., 2009). Rice DREB2 genes, OsDREB2A and OsDREB2B, showed a similar response to drought and salt, but OsDREB2B was also induced by cold, suggesting a putative role of DREB2 proteins in cold responses (Matsukura et al., 2010). Furthermore, OsDREB2B was shown to be regulated at the posttranscriptional level by alternative splicing (Matsukura et al., 2010). A similar mechanism of regulation was also found for DREB2 proteins in barley, wheat, and maize (Nakashima et al., 2009). Most of the DREBs are involved in ABA-independent stress responses; however, some studies have reported DREBs that are responsive to ABA (Haake et al., 2002; Yamaguchi-Shinozaki and Shinozaki, 2006). For instance, OsDREB1F and ABA RESPONSIVE AP2-LIKE (ARAG1) gene expression was shown to be upregulated in response to ABA. Moreover, the ARAG1 knockdown line displayed hypersensitivity to ABA and drought, suggesting a role of this TF in the ABA-dependent drought stress response (Zhao et al., 2010). Interestingly, a possible role of rice DREB proteins in biotic stress responses was reported. OsDREB1B overexpression in tobacco improved plant resistance to virus infection and induced the expression of several PR genes (Gutha and Reddy, 2008). Only few DREB proteins were involved in biotic stress responses. In Arabidopsis, DREB2A gene expression was upregulated in ACTIVATED DISEASE RESISTANCE 1 (ADR1) overexpression lines (Agarwal et al., 2006).

**MYB family**

MYB transcription factors are characterized by the presence of MYB repeats (R) involved in DNA-binding and protein-protein interactions (Feller et al., 2011). MYB proteins were initially identified in animals, but similar proteins are reported in insects, plants (Supplementary Table S2) and fungi (Stracke et al., 2001). In plants, MYB proteins can be classified into three subfamilies, R-MYB, R2R3-MYB, and R1R2R3-MYB (MYB3R) depending on the presence of one, two, or three tandem MYB repeats, respectively (Feller et al., 2011). Several members of this family were identified in rice, Arabidopsis, maize, and soybean, and shown to be involved in a wide variety of cell processes, such as cell cycle and cell morphogenesis (Feller et al., 2011; Jin and Martin, 1999; Stracke et al., 2001). MYB proteins were also reported to be involved in biotic and abiotic stress responses. Studies in soybean revealed that GmMYB76, GmMYB92, and GmMYB177 are induced by several abiotic stress conditions and overexpression of these TFs improves tolerance to salt and freezing in Arabidopsis (Liao et al., 2008). In rice, only a few studies report the involvement of MYB proteins in abiotic and biotic stress responses. Response to cold seems to be a common feature among the best characterized rice MYB proteins.

Rice MYBS3 protein contains a single MYB repeat (MYB1R) and its gene expression is repressed by ABA and induced by cold and salt (Su et al., 2010). MYBS3 overexpression in rice significantly increases tolerance to cold and RNA interference lines displayed enhanced sensitivity to this abiotic stress suggesting a role of MYBS3 in rice cold responses. Moreover, microarray analysis and transactivation assays revealed that MYBS3 represses the cold signaling pathway dependent on the DREB1/CBF regulon (Su et al., 2010).

MYB3R subfamily proteins are known to be involved in cell-cycle regulation through the transcriptional control of cyclins. However, some studies revealed that TFs from this subfamily might play a role in abiotic stress responses (Feller et al., 2011). OsMYB3R-2 gene expression is induced by cold, drought, and high salinity and its overexpression in Arabidopsis has significantly enhanced cold tolerance (Dai et al., 2007). OsMYB3R-2 was also shown to target cyclin genes involved in the G2/M transition and thereby regulates the progress of the cell cycle during cold stress (Ma et al., 2009).

The R2R3-MYB proteins are the largest group of plant MYB proteins, including hundreds of members throughout the plant kingdom (Feller et al., 2011). The functions of these proteins are mainly related to regulation of plant morphology and metabolism, but a role in biotic and abiotic stress responses has also been reported. OsMYB4 was shown to be involved in the regulation of both types of stress responses and its overexpression confers varying levels of tolerance depending on the species (Agarwal and Jha, 2010). Its overexpression in Arabidopsis increases plant tolerance to cold and drought and the resistance to biotic stress (Mattana et al., 2005; Vannini et al., 2004, 2006). Tomato plants overexpressing this TF displayed a higher tolerance to drought and increased resistance to biotic stress, but no alteration on cold sensitivity (Vannini et al., 2007). In the apple, enhanced tolerance to drought and cold was reported in transgenic plants overexpressing OsMYB4 (Pasquali et al., 2008). Besides the different tolerance levels observed in different transgenic hosts, the increase in drought tolerance seems to be a common feature in all plants overexpressing OsMYB4.

Although several MYB proteins have been identified in rice, so far only a few OsMYBs have been functionally characterized for their role in abiotic stress responses (Table 1). The data already gathered suggest that rice MYBs play an important role in the cold stress response mechanisms.

**Zinc fingers**

The zinc-finger proteins play a major role in many cellular pathways and are present in all eukaryotic organisms. Zn finger TFs have been implicated in distinct pathways, such as nutrient homeostasis and root development (Devaih et al., 2007), flower development (Wu et al., 2008), carbohydrate metabolism (Tanaka et al., 2009) and light and hormonal signaling (Feurtado et al., 2011).

The C2H2-type Zn finger TFs are one of the most abundant Zn finger TFs and have been described as involved in the response of different plants to abiotic stress conditions (Mittler et al., 2006; Sakamoto et al., 2004; Vogel et al., 2005). These
TFs, also referred to as TFIIIA-type finger, are characterized by two cysteine and two histidine residues that bind to a zinc ion to form a structure that binds to the major groove of DNA (Pavletich and Fabo, 1991). The first of such TFs identified in plants was the petunia ZPT2-1, a zinc-finger protein TFIIIA-type (Takatsuji et al., 1992). In rice, despite the high number of genes encoding C2H2-type Zn finger TFs (Agarwal et al., 2007), only a few have been functionally characterized: ZINC-FINGER PROTEINS 182 (ZFP182), ZFP245, ZFP252 and DROUGHT SALT TOLERANCE (DST) (Huang et al., 2007, 2009a, 2009b; Xu et al., 2008a). The overexpression of the first three of these TFs in rice plants yielded similar phenotypes: increased tolerance to abiotic stress conditions (Huang et al., 2007, 2009a; Xu et al., 2008a). The DST protein, on the other hand, seems to have a different role in abiotic stress signaling, because rice plants harboring a mutation in its gene are more tolerant to abiotic stresses than the wild-type plants (Huang et al., 2009b). As shown in Table 1 and also in Supplementary Table S1, genes coding for different C2H2-type TFs in rice are differentially regulated by several abiotic stress conditions. This means that these TFs most likely have roles in different signaling pathways mediating abiotic stress responses.

There is much crosstalk between different families of TFs involved in abiotic stress signaling. C2H2-type TFs have been particularly described as interacting with the DREB1/CBF regulon. For example, Arabidopsis plants overexpressing DREB1A/CBF3 were found to have altered levels of ZAT10/STZ (Maruyama et al., 2004). This interaction is further supported by the finding that ZAT12, a transcriptional repressor also described as a regulator of several abiotic stress-responsive genes in Arabidopsis (Davletova et al., 2005), has a regulatory motif overlapping that of DREB1/CBF2 (Vogel et al., 2005). Moreover, ZAT12 was also described as a negative regulator of DREB1A/CBF3 and DREB1B/CBF1 (Vogel et al., 2005). In rice, plants overexpressing ZFP252 were reported to have altered levels of OsDREB1A (Xu et al., 2008a). C2H2-type TFs are therefore signaling components that can be located either up- or downstream the DREB1/CBF genes.

Other types of Zn fingers, such as the Zn finger Homeo-Domain TFs (ZF-HD), have been reported to have a role in abiotic stress signaling in plants. These proteins are characterized by the presence of Zn finger-like motifs upstream of a homeodomain (Windhovels et al., 2001). These TFs act in concert with NAC TFs in Arabidopsis, to regulate the expression of abiotic stress-related genes (Tran et al., 2006). In rice, however, the involvement of these TFs in stress signaling is yet to be described. Up to now, only microarray data is available on the transcriptional regulation of these genes in rice (Jain et al., 2008).

Leucine zippers

bZIP proteins are a large family of TFs, present in all eukaryotes, and that are characterized by the presence of a basic region, responsible for DNA-binding, and of a leucine zipper, involved in protein homo- and heterodimerization (jakoby et al., 2002). Many bZIP TFs have been linked to the ABA-dependent signaling pathway in several plant species, such as rice, Arabidopsis, and maize (Choi et al., 2000; Hobo et al., 1999a; Uno et al., 2000; Zhang et al., 2011). The role of bZIPs in this pathway was revealed by the reports that TFs belonging to this family bind to the ABA Responsive Element (ABRE; PyACGTGG/TC) (Choi et al., 2000; Guiltinan et al., 1990; Hobo et al., 1999a; Uno et al., 2000). This cis-acting element was initially identified in wheat (Guiltinan et al., 1990) and rice (Mundy et al., 1990) and was later shown to be present in the promoters of many ABA-responsive genes. The role of bZIP TFs in ABA-signaling was further supported by the findings that the overexpression of these TFs causes ABA-hypersensitivity in transgenic plants (Fujita et al., 2005; Kang et al., 2002; Zou et al., 2007; Xiang et al., 2008). In most cases, the overexpression of OsbZIPs in rice improves tolerance to abiotic stress conditions, namely, high salinity and drought, whereas its downregulation/knockout leads to higher sensitivity (see Table 1). Nevertheless, rice plants overexpressing OsAB15/OsbZIP10 are an exception, because they were reported to be more sensitive to salinity than the wild-type (Zou et al., 2008). It is also interesting to note that, most rice genes coding for bZIP TFs involved in abiotic stress signaling are induced by ABA and bind to the ABRE cis-element (Table 1). These results indicate that, in rice, abiotic stress signaling though bZIP TFs preferably occurs in an ABA-dependent manner. Moreover, the bZIP-mediated ABA signaling pathway seems to be conserved in several plant species, both monocots and dicots. For example, the homologous proteins TRAB1 (rice TRANSCRIPTION FACTOR RESPONSIBLE FOR ABA REGULATION 1), HvAB15 (barley ABA INSENSITIVE 5), and ABI5 (Arabidopsis ABA INSENSITIVE 5) interact respectively with their counterparts OsVP1 (rice VP1- VIVIPAROUS 1), HvVP1 (barley VP1), and AtABI3 (Arabidopsis ABA INSENSITIVE 3), which are themselves homologues, to modulate ABA-dependent gene expression (Casaretto and Ho, 2003; Hobo et al., 1999b; Nakamura et al., 2001).

Other than abiotic stress signaling, bZIP TFs have been described as involved in other signaling pathways, such as light signaling (Chattopadhyay et al., 1998), floral architecture (Maier et al., 2009), hormone signaling (Fukazawa et al., 2000), and pollen development (Iven et al., 2010). As can be seen in Table 1 and Supplementary Table S1, rice genes coding for bZIP TFs are regulated at the transcriptional level by several hormones, such as auxins (Yang et al., 2011), gibberellins (Shobbar et al., 2008), and ethylene (in the form of ACC) (Lu et al., 2009). This is indicative of a crosstalk between several signaling pathways, mediated by bZIP TFs.

Another group of bZIP TFs that has been implicated in abiotic stress responses are the HDZIP TFs. These proteins are characterized by the presence of a homeodomain directly before the bZIP domain (Ruberti et al., 1991). These HDZIP domains are responsible for homo- and heterodimerization of the TFs, and also for the DNA-binding specificity (Meijer et al., 2000). These proteins are plant-specific and do not occur in other Eukaryotes. Similar to bZIPs, HDZIP TFs have been implicated in several cellular processes, such as hormone (llegems et al., 2010) and light signaling (Wang et al., 2003), and also in plant development (Vernoud et al., 2009). Even though there are some reports on the involvement of HDZIPs in abiotic stress signaling, only a few of these TFs have so far been characterized. The heterologous expression of the flower gene HAB1-4 in Arabidopsis led to plants that were more tolerant to water stress (Dezar et al., 2005). Moreover, in the drought-resistant species Craterostigma plantagineum, all genes coding for the seven HDZIP TFs identified respond to drought stress at the transcriptional level (Deng et al., 2002;
Frank et al., 1998), which is a strong indication that these TFs play an important role in abiotic stress signaling. In rice, however, other than gene expression studies (Agalou et al., 2008; Jain et al., 2008), not much is known about the function of HDZIPs in the stress signaling pathways. Nevertheless, it is interesting to note that drought is the stress to which most of the genes coding for HDZIPs respond (Supplementary Table S1). This, together with previous data from other plant species (Deng et al., 2002; Dezar et al., 2005; Frank et al., 1998), seems to support a prominent role of these TFs in water stress signaling. It is also noteworthy that some, but not all of HDZIP-coding genes, respond to the hormone ABA (Deng et al., 2002; Dezar et al., 2005; Johannesson et al., 2003). This probably means that HDZIPs play a role in both ABA-dependent and independent signaling pathways.

WRKY family

The WRKY family of TF is one of the largest and oldest families of transcriptional regulators in the plant kingdom (Rushston et al., 2010). However, the first descriptions of this TF family came out on the mid-1990s (Ishiguro and Nakamura, 1994; Rushston et al., 1995). WRKY TFs are exclusive of plants and can be found from green algae to land plants. The WRKY TFs are characterized by a DNA-binding domain with approximately 60 a.a., the WRKY domain, which contains an almost invariant WRKY amino acid sequence at the N terminus and a zinc-finger motif in the C terminus (Rushston et al., 2010). The WRKY TF family is divided into three groups based on the number of WRKY domains (two domains in Group I and one in Groups II and III) and the structure of their zinc fingers (C2HC in Group III). The WRKY domain has been crystallized (Yamasaki et al., 2005) and the proposed structure is consistent with the cis-element where it should bind, the highly conserved W-box (TTGACC/T). Because this cis-element is conserved, the specificity for the different TFs must be obtained by the neighbouring areas of the W-box (Ciolkowksi et al., 2008). WRKY TFs have been described as having a role in the regulation of biotic and/or abiotic stress responses, germination, senescence, and developmental processes (Rushston et al., 2010). Research has however been focused on biotic stress responses (Rushston et al., 2010; Shimono et al., 2007; Tao et al., 2009). Although novel WRKY TFs have been described, clear phenotypes are difficult to obtain by reverse-genetics approaches (Berri et al., 2009), which may be due to regulatory redundancy among the members of this family.

More than 100 WRKY family members, belonging to five main phylogenetic groups, have been predicted described in rice (Berri et al., 2009). Despite their well-known response to biotic stress, some WRKY TFs have been reported as responding to abiotic stress (Table 1 and Supplementary Table S1). They are mainly upregulated in response to drought, salinity, and ABA, and downregulated in response to cold (Berri et al., 2009; Ramamoorthy et al., 2008). Nevertheless, despite the extensive list of rice WRKY TFs studied, only a few have been functionally characterized regarding their response to abiotic stress (Table 1). OsWRKY13 overexpression in rice increased sensitivity to salt and cold stress (Qiu et al., 2008), whereas OsWRKY45 enhanced tolerance to salt and drought in Arabidopsis (Qiu and Yu, 2009). The overexpression of OsWRKY11, known to be induced by drought and heat, enhanced heat, and drought tolerance after heat pretreatment as compared to wild-type plants (Wu et al., 2009). When OsWRKY08 was overexpressed in Arabidopsis, besides an improvement of primary root growth in the transgenic plants under osmotic stress, no clear phenotype regarding survival under abiotic stress was shown (Qiu et al., 2004; Song et al., 2009). OsWRKY72 was known to be involved in the ABA signaling (Xie et al., 2005) and its overexpression in Arabidopsis increased sensitivity to ABA, salt, and osmotic stress (Yu et al., 2010). Although the salt and osmotic stress phenotype was not obvious, there is a clear function of OsWRKY72 in ABA signaling and a crosstalk with the sugar metabolism and auxin transport pathways (Yu et al., 2010). OsWRKY13 (Qiu et al., 2007) and OsWRKY45 (Shimono et al., 2007; Qiu and Yu, 2009) were also shown to be involved in biotic stress resistance, whereas OsWRKY08, OsWRKY11, and OsWRKY72 were not investigated for their putative involvement in biotic stress response (Table 1).

Although few reports have clearly demonstrated the WRKY TFs function in abiotic stress in rice, they suggest that WRKY TFs are mainly involved in osmotic stress-related responses (Table 1). The overexpression of WRKY rice TFs can induce either improved tolerance or higher sensitivity to the stress, thus acting as positive or negative regulators in stress signalling pathways (Rushston et al., 2010).

NAC family

The NAC TF family is plant specific and one of the largest TF families. NAC proteins contain a highly conserved N-terminal DNA-binding domain (NAC domain) and a variable C-terminal domain that plays a major role in the regulation of transcription as an activator or repressor. This TF family was originally named from three consensus sequences identified from Petunia and Arabidopsis, both with similar DNA-binding domains, that is, NAC domain [NAM (no apical meristem), ATAF1-2 (Arabidopsis transcription activation factor), and CUC2 (cup-shaped cotyledon)] (Aida et al., 1997, 1999; Souer et al., 1996). NAC TFs have been involved in several aspects of plant development such as formation of boundary cells of the meristem, transition between cell division and cell expansion in stamen and petals, anther dehiscence, flowering, lateral root development, hormone signaling, senescence, and seed quality. Furthermore, this family was also found to participate in the responses to pathogens, viral infections, and environmental stimuli of different plant species (reviewed in Olsen et al., 2005; Tran et al., 2010).

Only a few members of this family were described in the last decade of the 20th century. The postgenomic era, with the availability of high-quality genome sequences of both Arabidopsis and rice, has offered an unprecedented opportunity to identify complete TF families and their networks. Comprehensive genome-wide analyses of the NAC TF family suggested that there are at least 117 and 151 putative members of the NAC TF family in Arabidopsis and rice, respectively (Nuruzzaman et al., 2010). In rice, sequence analysis of NAC TFs suggested that NAC family proteins could be classified into two major groups, A and B, further subdivided into seven and nine subgroups, respectively. Group A consists of 65 OsNAC proteins having low homology with the Arabidopsis NACs, whereas the 86 OsNAC genes assigned to group B showed high homology with the Arabidopsis and other NAC proteins (Nuruzzaman et al., 2010). The NAC TFs related to
TFs AND EPIGENETIC MECHANISMS IN ABIOTIC STRESS RESPONSES

development and stresses belong to group B. The OsNAC genes were distributed all over the 12 rice chromosomes and 36 OsNAC genes were observed in tandem duplication. This suggests that the NAC family increased rapidly during the course of evolution through tandem duplications of chromosomal regions (Fang et al., 2008; Nuruzzaman et al., 2010). Analysis of OsNAC gene promoter sequences revealed the presence of 17 cis-acting regulatory DNA elements known to be responsive to plant hormones (e.g., auxin, gibberellic acid, and salicylic acid) and environmental stimuli (light and cold) and related with seed specific expression (Fang et al., 2008).

Different authors have reported the differential expression of 151 NAC genes in microarray analyses of rice plants subjected to abiotic stress. According to the author, the number of differentially expressed genes was 15% (Fang et al., 2008) or 30% (Nuruzzaman et al., 2010), which may be due to the different varieties used in the study and the number of stresses analyzed. Among 20 OsNAC genes identified as responsive to abiotic stress, only 3 are induced by drought, salt, and cold, whereas the remaining genes were either induced by one or two stresses (Fang et al., 2008). On the basis of individual stress responses, 5 genes are induced by drought, 19 genes by salt, and 16 genes by cold (Supplementary Table S1). Moreover, all the 20 stress-inducible OsNAC genes were found to contain at least one of the stress-responsive cis-elements such as ABRE (ABA-responsive element), DRE (dehydration-responsive element), and LTRE (low temperature-responsive element) (Fang et al., 2008).

Despite all the research on rice NAC TF genes, so far only seven members of the family (OsNAC5, OsNAC6/SNAC2, OsNAC10, OsNAC19/SNAC1, ONAC045, OsNAC52, and ONAC063) have been functionally characterized for abiotic stress tolerance (Table 1). Apart from OsNAC063, which is not drought stress induced, all the other genes are induced under salt and drought stress. The overexpression of each of these genes in rice and/or Arabidopsis improved tolerance to drought and salt (except OsNAC52) stress in transgenic plants. Among these seven OsNAC genes, at least five are induced by ABA (Table 1). Furthermore, transgenic plants overexpressing OsNAC6/SNAC2, OsNAC19/SNAC1, and OsNAC52 were reported to be hypersensitive to exogenous ABA (Table 1), indicating that NAC TFs may be involved in the ABA signaling pathway.

The stress-inducible NAC TFs are promising candidates to improve tolerance to abiotic stress, particularly to salt and drought. However, so far only a few members of the NAC TF family have been overexpressed in rice.

Heat-shock factors

Heat-shock transcription factors (HSFs), although present in all living organisms known so far, are mostly represented in plants (Baniwal et al., 2004). These TFs are known to bind to heat-shock elements (HSEs) in the promoter regions of heat-shock proteins (HSPs). The HSEs are constituted by a palindromic sequence, the conserved 5′-GAAmTTCCn motif, to where HSFs can bind (Pelham, 1982). Plants HSFs are characterized by a DNA-binding domain on the N-terminal, oligomerization motif, nuclear localization signal, nuclear export signal, and an activation domain. Based on the oligomerization domain structure, HSFs are classified into three different classes, A, B, and C (Kotak et al., 2004). Although mostly described as involved in mechanisms related to heat stress (Charrng et al., 2007; Schramm et al., 2008), HSFs have also been related to other stress response networks, such as oxidative stress (Li et al., 2005), chilling stress (Li et al., 2003) and high-salinity stress (Yokotani et al., 2008).

Among the 26 HSFs predicted in rice, the members of the class A are the most abundant and the best studied so far. Recently, the gene expression of HSF family members has been studied under different stress conditions such as heat, oxidative stress, drought, cold, and high-salinity (Liu et al., 2010; Mittal et al., 2009). The members of this family are differentially expressed under abiotic stress conditions and are developmentally regulated in diverse tissue types under control conditions (Liu et al., 2010; Mittal et al., 2009). In addition, different HSF classes can be related to different stresses. For example, genes encoding HSF class C members seem to be more responsive to cold stress (Mittal et al., 2009) and class A members are more responsive to heat stress (Liu et al., 2010; Mittal et al., 2009). In fact, several members of the class A respond extremely fast to heat stress, eventually acting as heat sensors.

Many HSF genes are known to be differentially regulated by diverse abiotic stresses (Liu et al., 2010; Mittal et al., 2009), however, only two members of the HSF family (all from class A) have been functionally characterized in overexpression/mutant analyses (Table 1). The overexpression of OsHsfA2e in Arabidopsis greatly enhanced the thermotolerance of transgenic plants during germination, vegetative, and flowering stage (Yokotani et al., 2008). In addition, transgenic plants also showed improved tolerance to high-salinity both at seedling and vegetative stage. Another HSF, OsHsf7, was identified by Yeast-One hybrid screening using a dimeric HSE as bait (Liu et al., 2009). The overexpression of OsHsf7 in Arabidopsis did not lead to a higher level of HSFs (HSF targets) in transgenic plants. In addition, the transgenic plants showed a higher basal thermotolerance than wild-type plants. HSFs seem to play an important role in the response to high temperature and may be useful to improve heat tolerance in crop plants. It is known that a rise of +1°C could lead to a 10% decline in rice yield (Peng et al., 2004).

Epigenetic Mechanisms and Regulation of Gene Expression in Abiotic Stress Responses

Epigenetic mechanisms have a key role on plant plasticity responses to unpredictable abiotic stresses. This review is focused on DNA methylation and histone modification changes associated to abiotic stress responses reported in several plant species, including rice and Arabidopsis model plants.

DNA Methylation and Abiotic Stress

Cytosine methylation is a conserved epigenetic mark involved in genome defense against endogenous transposable elements or viral DNA and in regulation of gene expression throughout plant development. The addition of a methyl group to cytosine residues is catalyzed by methyltransferases (MTases) and in plants this may occur in both asymmetric (CHH) and symmetric (CG and CHG) contexts. Four main families of plant MTases have been identified so far, with distinct functions in de novo and/or maintenance methylation: DOMAINS REARRANGED METHYLTRANSFERASE
(DRM), METHYLASE 1 (MET1), CHROMOMETHYLTRANSFERASE (CMT), and DNA methyltransferase homolog 2 (Dnmt2) (reviewed by Zhang et al., 2010). The establishment of DNA methylation is counteracted by passive or active demethylation. While in the first, methylated cytosines are replaced with unmethylated ones during DNA replication, active demethylation involves a base excision repair mechanism, mediated by DNA glycosylases, without the occurrence of DNA replication (Ikeda and Kinoshita, 2009). Additionally, DNA methylation is modulated by other mechanisms, such as RNA-directed DNA methylation (RdDM) pathway, mediated by siRNAs (Chan et al., 2005) and chromatin remodeling factors (Meyer, 2010; Zhang et al., 2010). All together, these regulatory pathways provide a dynamic platform for establishment of DNA methylation patterns, which may be crucial to guarantee the epigenomic plasticity and enable an efficient response to developmental cues and environmental stress. The mechanistic effect of DNA methylation in transcription is still unclear and several lines of evidence suggest that the roles of cytosine methylation are equally diverse and likely individualized for different genes (Zhang et al., 2010). Methylated cytosines may attract methyl-binding proteins, which in turn recruit histone modifiers and chromatin remodeling proteins, forming a complex that can perturb the binding of transcription factors (Fransz and de Jong, 2002). High-resolution mapping of DNA methylation have uncovered some common aspects related to its distribution along plant genomes (Li et al., 2008; Yan et al., 2010; Zhang et al., 2006; Zilberman et al., 2007). Transcriptional inactive heterochromatic regions, which contain highly abundant transposable elements (TEs) and repetitive sequences, contain the highest density of methylated cytosines. In euchromatic regions, lower but still significant levels of cytosine methylation were also found. Surprisingly, in both Arabidopsis (Zhang et al., 2006; Zilberman et al., 2007) and rice (Li et al., 2008; Yan et al., 2010), DNA methylation related to active genes was more abundant in transcribed regions than in promoters. In Arabidopsis, the transcript elongation efficiency was negatively correlated to the extent of methylation within the gene body (Zilberman et al., 2007). One might expect that a similar mechanism also occurs in rice.

Abiotic stresses may induce changes in DNA methylation levels, which may be associated with chromatin remodeling and transcription regulation of stress responsive genes (Table 2).

<table>
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<td>−</td>
<td>(Kwon et al., 2009)</td>
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<td></td>
<td></td>
<td>H3ac</td>
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<td>(Pavangadkar et al., 2010)</td>
</tr>
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<td></td>
<td>Cell lines:</td>
<td>H3Ser10P</td>
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<td>(Sokol et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Nicotiana (By2)</td>
<td>H3Ser10PK14ac</td>
<td>+</td>
<td>(Wang et al., 2011b)</td>
</tr>
<tr>
<td></td>
<td>Arabidopsis thaliana</td>
<td>H4ac</td>
<td>+</td>
<td>(van Dijk et al., 2010)</td>
</tr>
<tr>
<td>Drought</td>
<td>Pisum sativum</td>
<td>5-mC</td>
<td>+</td>
<td>(Labra et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>Oryza sativa</td>
<td>5-mC</td>
<td>−</td>
<td>(Wang et al., 2011b)</td>
</tr>
<tr>
<td></td>
<td>Arabidopsis thaliana</td>
<td>H3K23ac</td>
<td>+</td>
<td>(Kim et al., 2008b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H3K27ac</td>
<td>+</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>H3K4me3</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arabidopsis thaliana</td>
<td>H3K4me3</td>
<td>+</td>
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<tr>
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<td>H3K4me2</td>
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<tr>
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</tr>
<tr>
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<td>(Chen et al., 2010)</td>
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<tr>
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<td>0</td>
<td>(Pecinka et al., 2010a)</td>
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<tr>
<td></td>
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<tr>
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<tr>
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<td>(Tsuji et al., 2006)</td>
</tr>
<tr>
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<td></td>
<td>H3ac</td>
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<td></td>
</tr>
<tr>
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<tr>
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<td>(Choi and Sano, 2007)</td>
</tr>
<tr>
<td>UV</td>
<td>Arabidopsis thaliana</td>
<td>H3ac</td>
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<td>(Lang-Mladek et al., 2010)</td>
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enzymes it is possible that a higher level of rearrangements may occur in other methylation sites. Transcriptional activation of specific loci associated with changes in DNA methylation have been identified for transposons and protein-coding sequences in response to abiotic stress (Choi and Sano, 2007; Hashida et al., 2006; Steward et al., 2002). For example, the aluminium and oxidative stresses were shown to induce DNA demethylation and transcription of a gene encoding a glycerophosphodiesterase-like protein (Choi and Sano, 2007). However, other studies reported transcriptional induction of silent loci after stress, without loss of DNA methylation, but instead a decrease in nucleosome occupancy (Lang-Mladek et al., 2010; Pecinka et al., 2010b; Tittel-Elmer et al., 2010). A decrease in DNA methylation in the Arabidopsis met1-3 mutant was also associated to higher expression of the AtHKT1 gene, which encodes for a vacuolar Na+/H+ transporter (Baek et al., 2011). The methylation pattern of a putative small RNA target region located in the AtHKT1 promoter is important for the differential expression of this gene in roots and leafs, which may affect salt response and sensitivity (Baek et al., 2011). Differences in global DNA methylation patterns were observed between two populations of Laguncularia racemosa that grow in salt marsh and riverside habitats (Lira-Medeiros et al., 2010) suggesting that epigenetic variation under natural conditions also play an important role in helping plants to adapt to different environments. In rice, several MTases have been identified, and microarray data suggested that some are preferentially upregulated during the initiation of floral organs (Sharma et al., 2009; Yamauchi et al., 2008). Additionally, higher levels of methylation in rice leaves (determined through MSAP) were detected at the booting and heading stages as compared to the tillering stage (Wang et al., 2011b). In turn, demethylation levels induced by drought were significantly higher at the tillering stage than at the booting and heading stages (Wang et al., 2011b). These studies suggest that changes in DNA methylation may also differentially modulate plant response to abiotic stress throughout development.

Histone Modifications and Abiostic Stress

In eukaryotic cells, nuclear DNA is packed and organized in association to a histone protein core forming nucleosomes, which are the structural units of chromatin. Changes in nucleosome structure through combinations of histone variants and covalent modifications of histone tails, by acetylation, methylation, phosphorylation, ubiquitination, biotinylation or SUMOylation, constitute an integrated histone code which has been correlated with regulation of gene expression (Bannister and Kouzarides, 2011; Jayani et al., 2010; Jenuwein and Allis, 2001; Strahl and Allis, 2000; Winter and Fischle, 2010; Zhou, 2009). The methylation of lysine residues on histone H3 has been correlated with either transcription activation or repression depending on which lysine is methylated and how many methyl groups are added (Chinnusamy and Zhu, 2009; Strahl and Allis, 2000; Zhang, 2008). For example, in maize, histone H3 lysine 4 trimethylation, (H3K4me3) has been associated to euchromatin and gene activation, being present in active gene sequences but absent in transposons (Wang et al., 2009). In Arabidopsis, histone H3 lysine 9 dimethylation (H3K9me2) has been regarded as a marker for heterochromatin and repressed transcription, thus associated with transposons (Zhang, 2008; Zhou, 2009). On the other hand, the H3K9me3 and H3K27me3 is enriched in genes of euchromatic regions (Zhang, 2008). Other histone modifications such as biotinylation and sumoylation have been associated to gene repression (Strahl and Allis, 2000). Regarding histone lysine acetylation, strong acetylation of histones has been associated to a more relaxed chromatin structure and thus to enhanced transcription, while weak acetylation has been related to compaction of chromatin and gene silencing (Berger, 2007; Zhu et al., 2008). Histone lysine acetylation is modulated by histone acetyltransferases (HAT) and histone deacetylases (HDAC). HDAC encoding genes are divided in four major classes (Pandey et al., 2002) being the HD2 class exclusive in plants (Lusser et al., 1997). In rice the OsHDT1 is involved in differential gene expression in hybrids (Li et al., 2011). Globally, the rice genome has at least 19 HDAC genes and most of them show to be differentially regulated by distinct abiotic stress conditions (Fu et al., 2007; Hu et al., 2009). Microarray data revealed that most rice HDAC genes were particularly responsive to drought or salt stresses, mainly through transcriptional repression (Hu et al., 2009). Thus, chromatin modifier enzymes themselves may be transcriptionally regulated by abiotic stresses and in this case the down regulation of HDAC could be necessary to allow the induction of stress responsive genes (Fu et al., 2007).

Histone modifications and DNA methylation crosstalks have been implicated on gene expression regulation. For example, in Arabidopsis, the abolishment of DNA methylation in the ddm1 mutants was correlated with lower levels of dimethylation of histone H3 at lysine 9 (H3K9me2) (Gendrel et al., 2002). An SUVH [Su(var)3–9 homologs] protein involved in H3K9 methylation was found to directly bind to methylated DNA, suggesting the occurrence of a self-reinforcing feedback loop for the maintenance of DNA and histone methylation in this species (Johnson et al., 2007). Several rice SUVH genes with a deduced role heterochromatin formation were recently identified (Qin et al., 2010). Some of these showed to mediate retrotransposon repression through DNA methylation and H3K9me3 (Ding et al., 2007; Qin et al., 2010). Moreover, in rice most protein-coding genes with methylated DNA are associated with H3K4me2 and/or H3K4me3 and in this case, the repressive effect of DNA methylation on gene expression was attenuated when H3K4me3 is also present (Li et al., 2008). The impact of abiotic stresses on genome-wide landscape of histone modifications starts to be deciphered (Table 2) mainly due to recent technical advances such as chromatin immunoprecipitation (ChiP) and genome-wide sequencing (ChiP-Seq). In rice, the submergence stress caused a decrease on H3K4me2 levels, an increase of H3K4me3 levels and a gradual increase of H3 acetylation at the 5¢- and 3¢-coding regions of the ALCOHOL DEHYDROGENASE 1 (ADH1) and PYRUVATE DECARBOXYLASE 1 (PDC1) genes (Tsuiji et al., 2006). These modifications were correlated with enhanced expression of the ADH1 and PDC1 observed in response to this stress (Tsuiji et al., 2006).

In Arabidopsis, the drought stress (Kim et al., 2008a) caused an increase of H3K4me3 and H3K9ac in the promoter region of stress responsive genes, while the coding region showed enrichment of H3K23ac and H3K27ac (Kim et al., 2008a). A genome-wide analysis of transcripts and H3K4 methylation patterns in dehydration stressed Arabidopsis plants revealed different dynamics on specific patterns of changes in H3K4me1, H3K4me2, and H3K4me3 profiles for upregulated,
Epigenetic Mechanisms and Transcription Factors Crosstalk in Abiotic Stress Responses

An intriguing question is how epigenetic mechanisms and TFs crosstalk to achieve a coordinated and specific gene regulation. In a simple view, a compact chromatin configuration can prevent the access of TFs and RNA polymerase to gene promoters and transcription start sites, while a more relaxed chromatin structure would facilitate the access to TFs enabling enhanced gene expression (Ahringer, 2000). TFs are essentially characterized by the presence of DNA binding domains with specificity to regulatory DNA sequences. Contrastingly, many other proteins also involved in the regulation of gene expression such as chromatin remodelers, histone acetylases, deacetylases, kinases, and methylases, do not have such DNA-binding domains. Thus, their inability to interact directly with target promoter genes implies the need of recruiting TFs. In *Brassica napus*, a novel protein, KID-CONTAINING PROTEIN (BnKCP1), which acts as a TF, contains a putative KID domain required for interaction with an HDAC (Gao et al., 2003). In *Arabidopsis*, it was also reported that the cold inducible TF CBF1 could interact with the GCN5 (Stockinger et al., 2001), an HAT required for acetylation of several H3 lysine residues (Servet et al., 2010). GCN5 is the component of chromatin modifying complexes related to transcriptional activation. Such interaction was also predicted for an additional putative TF AtEML protein, which is also regulated by cold stress (Gao et al., 2007). In a similar way, the *Arabidopsis ERF* genes, which are regulators of gene expression, were showed to interact with members of protein co-suppressor complexes associated to HDACs (Song et al., 2005; Song and Galbraith, 2006). In addition, the interaction of two WRKY TFs (WRKY38 and WRKY62) with an HDAC (HDA19) appeared to be determinant to fine-tune basal response to biotic stress in *Arabidopsis* (Kim et al., 2008a). Thus, TFs involved in stress response may be important players in the recruitment of histone modifying enzymes to target gene promoter regions, contributing for transcription modulation of TF-specific regulators (Gao et al., 2003, 2007; Song et al., 2005; Servet et al., 2010; Stockinger et al., 2001). Furthermore, in *Arabidopsis*, the WRKY70 TF was shown to be activated by the ATX1, which has a histone methyltransferase (HMT) activity with the ability to trimethylate H3K4 (Alvarez-Venegas et al., 2007). Thus, genes encoding TFs can be the primary targets of epigenetic factors while the entire networks of genes controlled by the TFs would be secondary targets also containing epigenetic marks that will facilitate transcription in case rapid changes are needed e.g. on stress response (Alvarez-Venegas et al., 2007). Taken together, these studies point out for a crucial role of TFs in the establishment of locus-specific chromatin remodeling, essential for transcriptional regulation, directly affecting nucleosome structure with consequences in gene activation/repression.

Conclusions and Perspectives

Plant response to abiotic stresses implies strict regulation of the transcriptional networks. However, little is known regarding regulation of gene expression under adverse environmental conditions. Crosstalk between TFs, DNA methylation, histone modifications, and chromatin remodeling enzymes have been involved in the transcriptional regulation of the stress responsive genes, pointing out for an integrated view of transcription regulation.

In rice, many TFs from different families (e.g., AP2/ERF, MYB, Zn finger, bZIP, NAC, WRKY, HSF) were already shown to play important roles in plant response to abiotic stress (Table 1), but much more are yet to be studied. Regarding the mechanisms underlying global epigenetic remodeling under environmental stress very little is known (Table 2). Figure 1 summarizes our present knowledge on the crosstalk between TFs and epigenetic mechanisms involved in abiotic stress responses in rice.
Based on the recent advances, investigation on plant responses to abiotic stress must follow several promising research lines, such as (1) charting gene regulatory networks, (2) deciphering genome-wide changes in the epigenome state, (3) characterizing protein/protein interactions involved in the transcription regulatory complexes, (4) integration of histone-modification pattern with transcriptomes and also with spatial nuclear architecture, (5) identification of TF targets and their interactions with chromatin regulators, (6) identification and functional studies on histone modification enzymes. These exciting issues count with technological advances, such as RNA-seq, chromatin immunoprecipitation (ChIP) coupled with next-generation sequencing technology (ChIP-seq), ChIP on chip, as well as shotgun proteomics. Nevertheless, advanced techniques to screen for proteins interactions need to be developed. The acquired knowledge on the molecular mechanisms underlying plant stress responses will be essential to generate transgenic crops or to unveil new allelic variations (e.g., EcoTILLING) in the natural population with enhanced tolerance to abiotic stresses. Definitely, a collaborative work between different researcher areas, for example, cell and molecular biology, genomics, transcriptomics, proteomics, and bioinformatics, will be needed.

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Author Disclosure Statement

No conflicting financial interests exist.

References


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