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Genome-wide identification and expression pattern of drought-responsive members of the NAC family in maize

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ABSTRACT

NAC proteins are plant-specific transcription factors (TFs). Although they play a pivotal role in regulating distinct biological processes, TFs in maize are yet to be investigated comprehensively. Within the maize genome, we identified 152 putative NAC domain-encoding genes (*ZmNACs*), including eight membrane-bound members, by systematic sequence analysis and physically mapped them onto ten chromosomes of maize. In silico analysis of the *ZmNACs* and comparison with similar genes in other plants such as *Arabidopsis*, rice, and soybean, revealed a similar NAC sequence architecture. Phylogenetically, the *ZmNACs* were arranged into six distinct subgroups (I–VI) possessing conserved motifs. Phylogenetic analysis using stress-related NAC TFs from *Arabidopsis*, rice, and soybean as seeding sequences identified 24 of the 152 *ZmNACs* (all from Group II) as putative stress-responsive genes, including one dehydration-responsive *ZmSNAC1* gene reported earlier. One drought-tolerant genotype (HKI577) and one susceptible genotype (PC13T-3) were used for studying the expression pattern of the *NAC* genes during drought stress. qRT-PCR based expression profiles of 11 genes predicted to be related to stress confirmed strong differential gene expression during drought stress. Phylogenetic analyses revealed that *ZmNAC18*, *ZmNAC51*, *ZmNAC145*, and *ZmNAC72*, which were up-regulated in the tolerant genotype and down-regulated in the susceptible genotype, belonged to the same group to which

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also belong other drought-responsive genes, namely *SNAC1*, *OsNAC6*, *ANAC019*, and *ANAC055*, which act as a transcriptional activator and are strongly induced under stress from various abiotic sources. Differentially expressed *ZmNAC* genes, alone or in combination with each other or with other type(s) of TFs, may control the general cellular machinery and regulate stress-responsive downstream genes. Alternatively, they may serve as a platform to regulate a broad set of genes, which are subsequently fine-tuned by specific regulators. This genome-wide identification and expression profiling opens new avenues for systematic functional analysis of new members of the *NAC* gene family, which may be exploited in developing lines that are better adapted to drought.

Introduction

Drought, cold, and salinity are major forms of stress from abiotic sources that adversely affect plant growth and productivity (Nakashima et al., 2012), of which drought is considered as the most devastating. Water is one of the significant limiting factors and affects maize at all stages of its growth. Maize is especially sensitive to drought at the reproductive stage, particularly between tassel emergence and early grain-filling (Grant et al., 1989). Drought stress during this period reduces kernel size and thus lowers grain yield significantly (Bolanos and Edmeades, 1993a, b). Plants adapt to drought stress at physiological, biochemical, and molecular levels by activating a number of defense mechanisms that increase the plant's tolerance to water deficit.

Transcription factors (TFs) are key proteins that regulate gene expression at the transcription level by interacting with promoter elements of stress genes resulting in over-expression of many functional genes. Many TFs have been characterized as drought-responsive TFs and include WRKY (Rushton et al., 2012), zinc finger (Huang et al., 2009), AP2/ERF2 (Sakuma et al., 2002), MYB (Abe et al., 1997), ZmDREB2A (Qin et al., 2007), and NAC (Tran et al., 2004).

NAM, ATAF, and CUC (NAC) proteins belong to a plant-specific TF superfamily, possess a highly conserved N-terminal DNA binding domain (NAC) and a variable C-terminal transcription regulation region (TRR), and can activate or suppress the transcription of multiple target genes. The C-terminal regions of some NAC TFs also contain transmembrane motifs (TMs), which anchor to the plasma membrane (Tran et al., 2004; Nakashima et al., 2007). Several *NAC* genes have been identified in different species: 117 non-redundant putative *NAC* genes in *Arabidopsis* (Nuruzzaman et al., 2010), 151 putative *NAC* or *NAC* like genes in rice (Nuruzzaman et al., 2010), and 152 non-redundant genes in soybean (Le et al., 2011). In *Arabidopsis ANAC019*, *ANAC055*, and *ANAC072* were induced by drought, salinity and low temperature (Tran et al., 2004), and *OsNAC10* and *OsNAC6* have been reported as responsive to drought stress in rice (Ohnishi et al., 2005; Jeong et al., 2010).

Research in *Arabidopsis* indicates that NAC TFs recognize the CACG core motif that acts as a drought-responsive NAC recognition sequence (NACRS). Wu et al. (2009) reported that *Arabidopsis* plants over-expressing the abiotic stress-responsive gene *ATAF1*, which is homologous to *RD26*, showed greater drought tolerance. These reports indicate that NAC TFs are important in stress tolerance and that their over-expression has a potential biotechnological application in improving stress tolerance in plants.

However, only a few NAC members have been characterized in maize. *ZmSNAC1* has shown drought tolerance in transgenic *Arabidopsis* (Zimmama and Veer, 2005; Lu at el., 2012). In maize, 56 transcription families were predicted, of which the plant-specific NAC transcription family consists of more than 190 putative members. The importance of NAC TF family proteins in drought tolerance prompted us to carry out a genome-wide systematic analysis of the ZmNAC (*Zea mays* NAC) TF family for (a) identifying complete NAC domain containing gene sequences in silico and identifying gene orthologs and drought responsive genes by phylogenetic analysis of the *NAC* genes and (b) exploring the expression pattern of *NAC* genes using a set of contrasting maize genotypes under drought stress.