Plant Physiology and Biochemistry 105 (2016) 55–66

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

ZmNAC55, a maize stress-responsive NAC transcription factor, confers drought resistance in transgenic *Arabidopsis*

Hude Mao [a,*], Lijuan Yu [b], Ran Han [b], Zhanjie Li [b], Hui Liu [b]

[a] State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, People's Republic of China

[b] College of Life Sciences, Northwest A & F University, Yangling, Shaanxi 712100, People's Republic of China

ARTICLE INFO

Article history:
Received 27 February 2016
Received in revised form
9 April 2016
Accepted 9 April 2016
Available online 11 April 2016

Keywords:
Abiotic stress
NAC transcription factor
Transgenic plant
Drought resistance
Maize

ABSTRACT

Abiotic stress has been shown to significantly limit the growth and productivity of crops. NAC transcription factors play essential roles in response to various abiotic stresses. However, only little information regarding stress-related *NAC* genes is available in maize. Here, we cloned a maize NAC transcription factor ZmNAC55 and identified its function in drought stress. Transient expression and transactivation assay demonstrated that ZmNAC55 was localized in the nucleus and had transactivation activity. Expression analysis of *ZmNAC55* in maize showed that this gene was induced by drought, high salinity and cold stresses and by abscisic acid (ABA). Promoter analysis demonstrated that multiple stress-related *cis*-acting elements exist in promoter region of *ZmNAC55*. Overexpression of *ZmNAC55* in *Arabidopsis* led to hypersensitivity to ABA at the germination stage, but enhanced drought resistence compared to wild-type seedlings. Transcriptome analysis identified a number of differentially expressed genes between *35S::ZmNAC55* transgenic and wild-type plants, and many of which are involved in stress response, including twelve qRT–PCR confirmed well-known drought-responsive genes. These results highlight the important role of ZmNAC55 in positive regulates of drought resistence, and may have potential applications in transgenic breeding of drought-tolerant crops.

© 2016 Elsevier Masson SAS. All rights reserved.

1. Introduction

As sessile organisms, plants often encounter the adverse environment, such as drought, high salinity and extreme temperature, that frequently constrain their growth and development (Valliyodan and Nguyen, 2006). In order to combat and survive in these environmental adversities, plants have evolved a series mechanisms that involved in changes at whole-plant, tissue, cellular, physiological, and molecular levels (Shinozaki et al., 2003). The molecular mechanisms underlying plant adaptation to environmental stresses have been researched intensively, and transcriptional regulation of gene expression play an important role in this process. Transcription factors (TFs) and their corresponding *cis*-regulatory sequences act as molecular switches for gene expression, regulating their temporal and spatial expression (Badis et al., 2009). In plants, numerous transcription factors have been identified to involved in the regulation of plant stress responses, such as DREB, CBF, bZIP, zinc-finger, MYB, and NAC transcription factors (Shinozaki et al., 2003; Fujita et al., 2004).

NAC (NAM, ATAF1/2, CUC2) transcription factors family is a plant-specific transcription factor superfamily and is present in a broad diversity of plants (Olsen et al., 2005; Puranik et al., 2012). Consistent with their TF functions, members of the NAC family commonly harbor a highly conserved DNA-binding domain at their N termini, which comprise approximately 150 amino acid residues that are divided into five subdomains (A–E) (Olsen et al., 2005). However, the C-terminal regions of NAC family members are highly varied, suggesting that the C-terminal regions may have a role in determining specificity of binding to their respective target genes (Olsen et al., 2005). NAC TFs are associated with diverse biological processes, including cell division (Kim et al., 2006), shoot apical meristem development (Nikovics et al., 2006), secondary wall development (Mitsuda et al., 2005), lateral root development (Xie et al., 2000), leaf senescence (Guo and Gan, 2006; Kim et al.,

Abbreviations: TF, transcription factor; NAC, NAM, ATAF1/2 and CUC2; ABA, abscisic acid; ORF, open reading frame; GFP, green fluorescent protein; CaMV, cauliflower mosaic virus; qRT-PCR, quantitative reverse transcriptase PCR; WT, wild-type; ABRE, ABA-responsive element; DRE, dehydration-responsive element; CRT, C-repeat element; LTRE, low temperature responsive element; MYBRS, MYB recognition site; MYCRS, MYC recognition site.

* Corresponding author.
E-mail address: mhd163com@163.com (H. Mao).

http://dx.doi.org/10.1016/j.plaphy.2016.04.018
0981-9428/© 2016 Elsevier Masson SAS. All rights reserved.

2009; Kim et al., 2013), and response to many biotic and abiotic stresses (Tran et al., 2004; Puranik et al., 2012; Nakashima et al., 2012).

Recently, considerable attention has been focused on the role of NAC TFs in the drought stress response (Lee et al., 2012; Nakashima et al., 2012; Puranik et al., 2012; Xu et al., 2013; Sakuraba et al., 2015). Some members have been identified and studied in model plants such as *Arabidopsis*, and crops such as soybean, rice, wheat and maize, and found to play important roles in drought stress signaling pathways. Three *Arabidopsis* NAC proteins, ANAC019, ANAC055 and ANAC072 were identified as significantly improving drought resistance in transgenic plants (Tran et al., 2004). In rice, when the stress-responsive *NAC* gene *SNAC1* was introduced into rice, the transgenic plants displayed significantly enhanced tolerances to multiple abiotic stresses (Hu et al., 2006). Several ABA-dependent NAC TFs in rice, including OsNAC5, OsNAC6 and OsNAC10, enhance drought tolerance in transgenic plants by regulating distinct target genes successively (Puranik et al., 2012; Nakashima et al., 2012). Recently, three novel NAC members from wheat, TaNAC2, TaNAC67, and TaNAC29 were characterized for their enhanced multi-abiotic stress tolerance in *Arabidopsis* (Mao et al., 2012, 2014; Huang et al., 2015). For soybean, the drought-inducible gene *GmNAC2* functions as a negative regulator in abiotic stress, whereas *GmNAC20* overexpression in plants induces enhanced salt and freezing tolerance (Hao et al., 2011; Jin et al., 2012). A maize NAC gene, *ZmSNAC1*, is strongly induced by drought, cold, high salinity, and abscisic acid (ABA) treatments. Overexpression of *ZmSNAC1* in *Arabidopsis* induces enhanced drought tolerance (Lu et al., 2012). Recently, *ZmNAC111* has been identified as important in maize drought tolerance; increasing *ZmNAC111* expression in transgenic maize improves water use efficiency and upregulation of drought-responsive genes under water stress (Mao et al., 2015). These studies indicate that NAC TFs have important roles in response to drought stress and that their overexpression can improve the drought tolerance of transgenic plants.

Although maize is one of the most planted crops world-wide, its productivity is frequently hampered by water scarcity. Thus, increasing drought tolerance is a priority target in maize breeding programs. In this study, a novel NAC transcription factor gene *ZmNAC55* was cloned from maize. Gene expression analysis demonstrated that *ZmNAC55* was upregulated by drought, high salinity and cold stresses and by ABA. Furthermore, ZmNAC55 is localized in the nucleus and functions as a transcriptional activator. *ZmNAC55*-overexpressing transgenic *Arabidopsis* showed enhanced sensitivity to ABA in the germination stage, and exhibited enhanced drought resistence through regulating many stress-responsive genes. Our results suggest that *ZmNAC55* play an important role in drought stress response and is expected to be used in genetically modified crops.

2. Materials and methods

2.1. Plant materials and stress treatments

Seeds of the maize inbred line B73 (*Zea mays* L. cv B73, from Northwest A & F University, China) were surface-sterilized in 1‰ (v/v) Topsin-M (Rotam Crop Sciences Ltd.) for 10 min. Then they were washed in deionized water and germinated on wet filter paper at 28 °C for 3 days. The germinated seeds were placed in a nutrient solution (0.75 mM K_2SO_4, 0.1 mM KCl, 0.25 mM KH_2PO_4, 0.65 mM $MgSO_4$, 0.1 mM EDTA-Fe, 2.0 mM $Ca(NO_3)_2$, 1.0 mM $MnSO_4$, 1.0 mM $ZnSO_4$, 0.1 mM $CuSO_4$, 0.005 mM $(NH_4)_6Mo_7O_{24}$) for hydroponic cultivation with a 16-h light/8-h dark cycle at 28 °C. Four kinds of treatments: drought, high salinity, cold and abscisic acid (ABA) were separately applied to three-leaf stage B73 seedlings. The high salinity and ABA treatments were conducted by culturing the seedlings in 200 mmol/L of NaCl and 100 μmol/L of ABA culture solutions, respectively. For drought treatment, the seedlings were placed on a clean bench and subjected to dehydration (28 °C, relative humidity of 40–60%). For cold treatment, seedlings were transferred to a growth chamber at a temperature of 4 °C. Leaves from a minimum of three seedlings were collected after 0, 5, 10 and 24 h for drought, high salinity and cold treatments; 0, 2, 5 and 10 h for ABA treatment. Field grown maize plants (*Zea mays* L. cv B73, from Northwest A & F University, China) were used for measuring organ-specific expression patterns of *ZmNAC55*. Young root, stem, and leaf (at the three-leaf stage), mature root, stem, and leaf (at anthesis stage), tassel, anther and ear were collected. All collected samples were immediately frozen into liquid nitrogen and stored at −80 °C for RNA extraction.

2.2. RNA extraction and quantitative real-time PCR

Total RNA was isolated using TRIZOL reagent (Biotopped, China) according to the manufacturer's instructions. RNA was treated with RNase-free DNase I (TaKaRa, China) to remove any genomic DNA contamination. First-strand cDNA was synthesized from 1 μg of total RNA using Recombinant M-MLV reverse transcriptase (Promega, USA). Quantitative real time-PCR (qRT-PCR) was performed in optical 48-well plates using an ABI7300 Thermo-cycler (Applied Biosystems, USA). Reactions were carried out in 10 μl volume, containing 1 μl diluted cDNA, 200 nM gene-specific primers, and 5 μl SYBR Premix Ex Taq II (TaKaRa) with the following conditions: 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 30 s at 60 °C. The specificity of the amplicon for each primer pair was verified by melting curve analysis. The expression of *ZmUbi-2* (UniProtKB/TrEMBL; ACC:Q42415) was used as an internal control. The $2^{-\Delta\Delta Ct}$ quantification method (Livak and Schmittgen, 2001) was used, with the variation in expression being estimated from three biological replicates. The primer pairs used for qRT-PCR analysis are listed in Supplemental Table 1.

2.3. ZmNAC55 isolation and sequence analysis

The gene sequence of *ZmNAC55* was downloaded from the 5b.60 version of the maize genome sequence database (http://www.maizegdb.org/). To obtain the full-length open reading frame (ORF) by the reverse transcription polymerase chain reaction (RT-PCR) approach, two primers were used (sense: 5′-ATGGGTCCTGAATCAGCT-3′ and antisense: 5′-TCAGAAGGGGCCCAACCCC-3′). The PCR conditions for amplifying *ZmNAC55* were as follows: 5 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 1.5 min at 72 °C; and then 10 min at 72 °C. The PCR products were purified and cloned into the pBluescript SK (pSK, Clontech) cloning vector for sequencing.

Multiple alignment of amino acid sequences was constructed using ClustalW (Chenna et al., 2003), using the Gonnet protein weight matrix, with multiple alignment gap opening/extension penalties of 10/0.5 and pairwise gap opening/extension penalties of 10/0.1. Phylogenetic tree was constructed with MEGA 5.0 software (Tamura et al., 2011) using the neighbor-joining (NJ) method. Bootstrapping was carried out on 1000 replicates with the pairwise deletion option.

2.4. In silico promoter sequence analysis

To identify putative *cis*-acting regulatory elements in the promoter region of *ZmNAC55*, the 1500 bp upstream of the coding region of *ZmNAC55* was selected as promoter sequence, and downloaded from the 5b.60 version of the maize genome sequence

Download English Version:

https://daneshyari.com/en/article/2015521

Download Persian Version:

https://daneshyari.com/article/2015521

Daneshyari.com