Journal of Integrative Agriculture 2018, 17(3): 530–538

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RESEARCH ARTICLE

GmNAC15 overexpression in hairy roots enhances salt tolerance in soybean

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Abstract

The NAC (NAM, ATAF1/2 and CUC2) transcription factor family plays a key role in plant development and responses to abiotic stress. *GmNAC15* (*Glyma15g40510.1*), a member of the NAC transcription factor family in soybean, was functionally characterized, especially with regard to its role in salt tolerance. In the present study, qRT-PCR (quantitative reverse transcription PCR) analysis indicated that *GmNAC15* was induced by salt, drought, low temperature stress, and ABA treatment in roots and leaves. *GmNAC15* overexpression in soybean (*Glycine max*) hairy roots enhanced salt tolerance. Transgenic hairy roots improved the survival of wild leaves; however, overexpression of *GmNAC15* in hairy root couldn't influnce the expression level of *GmNAC15* in leaf. *GmNAC15* regulates the expression levels of genes responsive to salt stress. Altogether, these results provide experimental evidence of the positive effect of *GmNAC15* on salt tolerance in soybean and the potential application of genetic manipulation to enhance the salt tolerance of important crops.

Keywords: NAC, salt tolerance, soybean, hairy roots

1. Introduction

Soybean is an important commercial crop and is the primary source of oil and protein for humans. China is one of the largest soybean producers in the world, with more than 1 600 000 tons of soybean harvested from more than 300 000 ha each year. However, abiotic stressors, such as salinity, drought, and low temperatures, pose a serious threat to soybean development and yield, especially high salt levels. High salt concentrations seriously limit the production of soybean through negative effects on the cellular, organ, and whole-plant levels (Phang *et al.* 2008). Salt stress is a complicated process that causes water deficits. A lack of water leads to the accumulation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\cdot-}$). High concentrations of cytotoxic oxygen can disturb normal cell metabolism through the destruction of the cell membrane structure, which ultimately leads to cell death (Parida *et al.* 2005).

In plants, NAC is a large gene family. NAC comprises three subfamilies: no apical meristem (NAM), *Arabidopsis* transcription activation factor (ATAF), and cup-shaped cotyledon (CUC). Several NAC family members in model plants have been identified and characterized, such as

Received 24 February, 2017 Accepted 31 May, 2017
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doi: 10.1016/S2095-3119(17)61721-0

151 in rice (*Oryza sativa*), 117 in *Arabidopsis* (Nuruzzaman *et al*. 2010), and 152 in tobacco (*Nicotiana tabacum*) (Rushton *et al.* 2008). In soybean, 152 NAC genes have been identified (Le *et al.* 2011). The NAC protein consists of two parts: highly conserved N-terminal NAC-binding domain (DB) (150–160 amino acids) and highly variable C-terminal transcription regulatory (TR) region (Puranik *et al.* 2012). The N-terminal plays a role in protein-binding and dimerization, and the C-terminal is responsible for activator or repressor function. The N-terminal of NAC contains five motifs (A–E). A participates in dimer formation, B and E may be involved in different functions of NAC, and C and D are positively charged and responsible for DNA recombination (Ernst *et al.* 2004; Jensen *et al.* 2010).

Many members of the NAC family are relevant to plant development (Feng *et al.* 2014). *GmNAC20* promotes lateral root formation by regulating auxin-related genes (Hao *et al.* 2011). *CUC1* of *Arabidopsis* regulates the formation of the shoot apical meristem (Takada *et al*. 2001). *CUC1*, *CUC2*, and *CUC3* make significant contributions to postembryonic development (Hibara *et al.* 2006). In addition, the NAC family plays important roles in response to abiotic stressors such as salt, drought, and cold. *ANAC002*, *ANAC019*, *ANAC055*, and *ANAC72* enhance the drought tolerance of *Arabidopsis* (Huang *et al.* 2016). Similarly, overexpression of *SINAC1*, *TaNAC29*, and *ONAC045* in tomato, *Arabidopsis*, and rice facilitates increased resistance to salt stress (Zheng *et al.* 2009; Yang *et al.* 2011; Huang *et al.* 2015).

The root is the first plant organ damaged by salt in the soil, so it is useful for studying the salt tolerance of plants. The transgenic soybean has been cultivated on a large scale in the world; however, there are many unsolved problems in the *Agrobacterium*-mediated transformation system, such as the low transformation efficiency and complex procedure. In addition, this method is susceptible to pollution and is time-consuming. These problems can be solved by using *Agrobacterium rhizogenes*, which infects wound sites and transfers T-DNA from the bacterial cell to the plant cell. K599, one strain of the *A. rhizogenes*, can be easily injected into the cotyledonary node and effectively induce hairy roots at the infection site to form composite soybean plants that comprise both wild-type shoots and transgenic hairy roots. The *A. rhizogenes*-mediated transformation system is fast, simple, and highly efficient (Estrada-Navarrete *et al.* 2006; Kereszt *et al.* 2007). In this study, we elucidated the role of *GmNAC15* in salt tolerance using the *A. rhizogenes*-mediated hairy root system.

2. Materials and methods

2.1. Plant materials, growth conditions, and treatments

The soybean cultivar Williams 82 was used to isolate the *GmNAC15* gene and to analyze tissue-specific expression. RNA was isolated from the roots, stems, leaves, and cotyledons of 20-day-old seedlings and flowers from mature plants. Seeds were germinated in pots containing vermiculite, and 20-day-old seedlings were subjected to salt, dehydration, abscisic acid (ABA), and cold treatment. The roots of the seedlings were immersed in Hoagland solutions containing 250 mmol L^{-1} NaCl, 20% polyethylene glycol (PEG), and 100 μmol L^{-1} ABA for different periods of time. For the cold treatment, the seedlings were maintained at 4°C for the indicated time period.

2.2. Construction of plant expression vector

Total RNA was isolated from Williams 82 using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). Genomic DNA was removed with DNaseI (Thermo Scientific, Vilnius, Lithuania) according to the manufacturer's instructions. First-strand complementary DNA (cDNA) synthesis was performed with the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). The full-length cDNA of *GmNAC15* was obtained by PCR with *Pfu* DNA polymerase (TransGen Biotech, Beijing, China) and a pair of primers (forward primer: 5′-ATGAGCAACATAAGCATGGTA-3′; reverse primer: 5′-TCAGTAATTGTTCCACATGTG-3′). The amplified products were purified and cloned into the Blunt vector with pEASY-Blunt Simple Cloning Kit (TransGen Biotech). *GmNAC15* was cloned into pCAMBIA3301 with the Quick-Fusion Cloning Kit (TransGen Biotech) and a pair of primers (forward primer: 5′-GAACACGGGGGACTCTTGACA TGAGCAACATAAGCATGGTA-3′; reverse primer: 5′-TTACCCTCAGATCTACCATGTCAGTAATTGTTCCACA TGTG-3′).

2.3. *Agrobacterium rhizogenes*-mediated transformation and salt treatment

The seeds were placed into identical pots containing mixed soil (humus:vermiculite=2:1) at a depth of 1–2 cm. Then, the potted seeds were placed in a greenhouse at 28°C and watered daily. Six-day-old seedlings with folded cotyledons were infected by *A. rhizogenes* K599 with the vector control (pCAMBIA3301) and overexpression vector around the cotyledonary node area using a syringe needle. The soybean plants were maintained under a 12 h light/12 h dark cycle at 28°C. After the initiation of hairy root formation from the infection site, the hairy roots were covered with vermiculite to maintain high humidity. A month later, once the hairy roots were 5–10 cm in length and could support the growth of the plant, the main roots were removed. The plants with hairy roots were transferred into mixed soil (humus:vermiculite=2:1) and watered every 3 days. The