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A NAC transcription factor gene of Chickpea (*Cicer arietinum*), *CarNAC3*, is involved in drought stress response and various developmental processes

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Summary

NAC transcription factors have been found to play important roles in plant development and responses to environmental stresses. Based on two cDNA libraries constructed from the PEG-treated and -nontreated seedling leaves of chickpea, a *NAC* gene, *CarNAC3*, was isolated and characterized. The results indicated that CarNAC3 contained 285 amino acids and had a conserved NAC domain. It was localized in the nucleus and possessed trans-activation activity in the C-terminus. Phylogenetic analysis showed that CarNAC3 belonged to the NAP (NAC-like, activated by APETALA3/PISTILLATA) subgroup of the NAC protein family. *CarNAC3* exhibited organ-specific expression and its induction was strongly dependent on leaf age. *CarNAC3* showed differential expression patterns during seed development and germination, and could be significantly induced by drought stress, abscisic acid (ABA), ethephon (Et) and indole-3-acetic acid (IAA), but was inhibited by N-6-benzyladenine (6-BA). Our data suggest that CarNAC3 may be a transcriptional activator involved in drought stress response and various developmental processes.

Abbreviations: ABA, abscisic acid; 6-BA, N-6-benzyl-adenine; CUC, cup-shaped cotyledon; DAF, days after flowering; EST, expressed sequence tag; Et, ethephon; GA3, gibberellin; GSP, gene-specific primer; HAI, hours after the initial imbibition; IAA, indole-3-acetic acid; MeJA, methyl jasmonate; NAM, no apical meristem; NAP, NAC-like, activated by APETALA3/PISTILLATA; NST, NAC secondary wall-thickening-promoting factor; ORF, open reading frame; RACE, rapid amplification of cDNA end; ROS, reactive oxygen specie; RT-PCR, reverse transcriptase polymerase chain reaction; SAM, shoot apical meristem; SA, salicylic acid; UTR, untranslated region.

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Introduction

The plant-specific *NAC* (for *NAM*, *ATAF1,2* and *CUC2*) genes exist widely in plants and comprise one of the largest transcription factor families (Duval et al., 2002; Ooka et al., 2003; Olsen et al., 2005). Since the first *NAC* gene denominated *NAM* (for "no apical meristem") was isolated from petunia (Souer et al., 1996), many NAC proteins have been reported to contribute to various developmental processes, such as shoot apical meristem (SAM) development (Souer et al., 1996), lateral root development (He et al., 2005), senescence (Guo and Gan, 2006; Uauy et al., 2006), flowering (Sablowski and Meyerowitz, 1998) and secondary wall formation (Mitsuda et al., 2007). Moreover, *NAC* genes are also involved in responses to biotic and abiotic stresses such as fungus infection, drought and high salinity (Hu et al., 2006; Zheng et al., 2009). However, previous research on NAC proteins has focused primarily on a few model plants such as *Arabidopsis* and rice (Olsen et al., 2005). Further investigation of the functions of *NAC* genes from other plant species is required and will aid in understanding the common and special physiological mechanisms of plant development and responses to stresses.

Chickpea is the third most important legume crop in terms of cultivation area, and is grown primarily in the arid and semi-arid regions of the world (FAOSTAT Database, http://www.faostat.fao.org/, 2006). Through long-term evolution and adaptation to extreme conditions, chickpea has been found to be rich in resistance genes for a range of abiotic stresses, including drought and cold, and has been suggested as a model plant for investigation of physiological mechanisms of plant development and responses to stresses (Singh et al., 1998). However, to the best of our knowledge, there has been no report about the identification and characterization of *NAC* genes from chickpea.

To identify the drought-responsive genes, we constructed two cDNA libraries using the PEG-treated and -nontreated seedling leaves of chickpea (Gao et al., 2008). Six expressed sequence tags (EST) induced by PEG treatment were found to show high similarity to *NAC* genes. Based on these, six *NAC* genes were isolated. Here, we report the identification and characterization of *CarNAC3* gene (GenBank accession NO. FJ356671).

Materials and methods

Plant growth and stress and chemical treatments

Chickpea plants (cv. *Xj-209*) prepared for tissue-specific expression analysis were field-grown under normal conditions. Young leaves, stems, and roots were collected from 2-week-old seedlings, while blooming flowers, developing seeds, pods, and leaves at different stages of senescence were sampled from mature plants. For germination assay, mature seeds were surface-sterilized with 1% hypochlorite solution for 10 min, followed by rinsing in sterile water. The seeds were then placed on a wet filter paper and incubated in darkness at 28 °C, and embryos were harvested at 2, 6, 12, 24, 36, and 48 h, respectively.

All stress and chemical treatments were applied to 2-week-old seedlings, which were grown in a chamber maintained at 25 °C, 60% RH and a 12 h/12 h (light/dark) cycle with white light illumination (110 μmol photons m^{-2} s^{-1}). Dehydration was induced by placing plants on a dry filter paper in air. Cold and heat treatments were applied by transferring plants to a growth chamber set to 4 and 37 °C, respectively. Salinity treatments were applied by submerging the roots of plants in 200 mM NaCl solution. Wounding was induced by scratching leaves using a bundle of needles. For chemical treatments, the roots of plants were submerged in an aqueous solution of abscisic acid (ABA, 100 μM), salicylic acid (SA, 100 μM), methyl jasmonate (MeJA, 100 μM), ethephon (Et, 200 μM), gibberellin (GA3, 100 μM), indole-3-acetic acid (IAA, 20 μM), N-6-benzyl-adenine (6-BA, 10 μM), and H_2O_2 (50 μM), respectively, with H_2O as control. The leaves were collected at 0, 1, 3, 5 (or 6), 12 and 24 h for each treatment. All tissues harvested were immediately frozen in liquid nitrogen and stored at −80 °C until use.

Isolation of *CarNAC3* gene

The EST chickpea.0679 in MH1 library (Gao et al., 2008) was found to have an intact 5′ end of a suspected *NAC* gene ORF (open reading frame) and was used as template to amplify the *CarNAC3* gene by the rapid amplification of 3′ cDNA end (RACE) technique. The 3′ cDNA end of the *CarNAC3* gene was cloned using the SMART™ RACE cDNA Amplification Kit (Clontech, USA). The gene-specific primer – 3′-GSP (5′GCCACCAAAGGGCCTCAAGACA GA3′) was used for the first round PCR, while another primer 3′-NGSP (5′TCCATGAGGCTAGATGAT TGGGTGTTG3′) was used for nested PCR. The PCR products were cloned into the pMD19-T vector (TakaRa, Japan) and sequenced (Invitrogen, USA). According to the 3′-RACE result, the primer pair, N3-1 (5′CAATGAATGGAAGAACAAGC3′, forward) and N3-2 (5′ATTGGTGAAGCTTATCGTCA3′, reverse), was used to obtain the full length cDNA and genomic