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Heterologous expression of betaine aldehyde dehydrogenase gene from *Ammopiptanthus nanus* confers high salt and heat tolerance to *Escherichia coli*

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ABSTRACT

Betaine aldehyde dehydrogenase (BADH) catalyzes the synthesis of glycine betaine, a regulator of osmosis, and therefore BADH is considered to play a significant role in response of plants to abiotic stresses. Here, based on the conserved residues of the deduced amino acid sequences of the homologous *BADH* genes, we cloned the *AnBADH* gene from the xerophytic leguminous plant *Ammopiptanthus nanus* by using reverse transcription PCR and rapid amplification of cDNA ends. The full-length cDNA is 1868 bp long without intron, and contains an open reading frame of 1512 bp, and 3′- and 5′-untranslated regions of 294 and 62 bp. It encodes a 54.71 kDa protein of 503 amino acids. The deduced amino acid sequence shares high homology, conserved amino acid residues and sequence motifs crucial for the function with the BADHs in other leguminous species. The sequence of the open reading frame was used to construct a prokaryotic expression vector pET32a-*AnBADH*, and transform *Escherichia coli*. The transformants expressed the heterologous *AnBADH* gene under the induction of isopropyl β-D-thiogalactopyranoside, and demonstrated significant enhancement of salt and heat tolerance under the stress conditions of 700 mmol L^{-1} NaCl and 55 °C high temperature. This result suggests that the *AnBADH* gene might play a crucial role in adaption of *A. nanus* to the abiotic stresses, and have the potential to be applied to transgenic operations of commercially important crops for improvement of abiotic tolerance.

1. Introduction

Drought, salinity, extreme temperatures and other abiotic stresses significantly restrict productivity and quality of crops (Hu and Xiong, 2014; Roy et al., 2014; Tambo and Abdoulaye, 2012), which adapt themselves to these abiotic stresses through a series of mechanisms (Dong et al., 2014; Neumann, 2008; Zheng et al., 2010). Compatible solutes, such as proline, glycine betaine and soluble sugars play a crucial role in the process counteracting stress via balancing osmotic potential (Chen and Murata, 2011; Dong et al., 2014; Silvente et al., 2012). Glycine betaine is an important osmoprotectant that can stabilize the structure and function of biomembrane system, enzymes, photosystem II complexes, ribulose-1,5-bisphosphate carboxylase/oxygenase, and many other functional proteins (Bao et al., 2011; Carillo et al., 2008, 2011; Chen and Murata, 2008; Gill et al., 2014; Prasad and Saradhi, 2004). In plants, the biosynthesis of glycine betaine consists of two steps: choline oxidation to betaine aldehyde catalyzed by choline monooxygenase, and betaine aldehyde to glycine betaine via deoxygenation catalyzed by betaine aldehyde dehydrogenase (BADH) (Rasheed et al., 2011). The heterologous overexpression of betaine aldehyde dehydrogenase genes increased betaine aldehyde accumulation, and improved tolerance of the transgenic plants to drought, salinity, extreme temperatures and other abiotic stresses (Fan et al., 2012; Karabudak et al., 2014; Li et al., 2011, 2014; Wu et al., 2008; Zhang et al., 2012; Zhou et al., 2008). However, the different improved phenotypes were obtained from these transgenic events. Except for the differentiation of gene expression, the activities of the BADHs encoded by the transformed exogenous genes themselves were probably one of the major reasons. Therefore, cloning and function evaluation of new betaine aldehyde dehydrogenase genes, particularly from abiotic stress-tolerant plant species, become helpful for transgenic operation of commercially important crops for improvement of abiotic tolerance.

Ammopiptanthus nanus, one of the two relict species of the *Ammopiptanthus genus* (*Leguminosae*), is the unique evergreen broadleaf bush in the plateau desert from the west edge of the Tarim Basin to the border between China and Kyrgyzstan (Cheng, 1959). It is endemic to the harsh environments of arid climate (annual precipitation

Abbreviations: RACE, rapid amplification of cDNA ends; BADH, betaine aldehyde dehydrogenase; ORF, open reading frame; CTAB, hexadecyltrimethylammonium bromide; UTR, untranslated region; IPTG, isopropyl β-D-thiogalactopyranoside; LB, Luria–Bertani; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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< 200 mm), extreme temperatures (from −30 °C to 40 °C), dryness (dryness usually more than 4), poor soil quality and high salinity (the habitats are usually stony and/or sandy slopes) (Pan et al., 1992; Wang, 2005; Wang et al., 2007; Yan et al., 2000). According to the criteria that 80%–100% of leaves are severely affected (Miller, 1963), the critical high temperature of *A. nanus* is 65 °C, significantly higher than other sandy plants (Liu and Qiu, 1982; Liu et al., 1995). A germination test showed that the critical concentration of NaCl that permits the germination of *A. nanus* seeds was 1.38% (Wang and Yin, 1991). Another study of our research team has demonstrated that the antifreeze protein gene of *A. nanus* has stronger function than those cloned from other plants (Deng et al., 2014). Therefore, it is promising to clone and evaluate other stress-related genes from this tenacious species for understanding the molecular mechanism of tolerance to abiotic stress and probable application in transgenic operation.

This paper reports the cloning of the *AnBADH* gene from *A. nanus* and the function evaluation by heterologous expression in *Escherichia coli*.

2. Materials and methods

2.1. Plant material and NaCl treatment

The seeds of *A. nanus* were soaked in 60 °C water for 5 min, and sown in sterilized nutrient soil. The seedlings were grown at 26/30 °C under the light/dark cycle of 12/12 h for four weeks, and then subjected to salt stress by adding 500 mmol L^{-1} NaCl, in order to increase the expression of the *AnBADH* gene. Five hours later, the leaves were harvested, snap frozen in liquid nitrogen and stored at −80 °C.

2.2. Cloning of full-length cDNA

Total RNA was extracted from the stored leaf sample by using RNAiso plus kit (TaKaRa, Japan), and treated with RNase-free DNase to remove probable genomic DNA.

The first strand of the cDNA was reverse transcribed by using the total RNA sample as the template, and PrimeScript™ II 1st Strand cDNA Synthesis kit (TaKaRa, Japan). A pair of primers (5′-CTGTGAATGGCGACACGGAAG-3′/5′-GATTGTTCCACGCTCGCTCTTAG-3′) was designed according to the partial cDNA sequence (GenBank accession number: DQ288723) of the *BADH* gene in *Ammopiptanthus mongolicus*, the other species of the *Ammopiptanthus* genus, and used to amplify the core sequence of the *AnBADH* gene with the sample of the first strand of the cDNA, and high fidelity DNA polymerase (TaKaRa, Japan).

The first strand of the cDNA for 3′-RACE (rapid amplification of cDNA ends) was reverse transcribed by using the total RNA as the template, and the 3′-RACE adaptor with 3′-Full RACE Core Set Ver.2.0 (TaKaRa, Japan). The product was used for the first amplification of the nested PCR with the specific outer primer (5′-ACTGGAAGCTCTGCAACTGGGACCAAGA-3′) designed according to the core sequence, and the 3′-RACE outer primer (5′-TACCGTCGTTCCACTAGTGATTT-3′). The product was used for the second amplification of the nested PCR with the specific inner primer (5′-TCAAGCCTGTTTCACTAGAGCTCGGTGG-3′) designed according to the core sequence, and the 3′-RACE inner primer (5′-CGCGGATCCTCCACTAGTGATTTCACTATAGG-3′).

The 5′-RACE was conducted using First Choice® RLM-RACE kit (Ambion, USA). The total RNA sample was dephosphorylated with calf intestinal alkaline phosphatase, decapped with tobacco acid pyrophosphatase, ligated with the 5′ RACE adaptor, and used for the reverse transcription of the first strand of the cDNA with the random

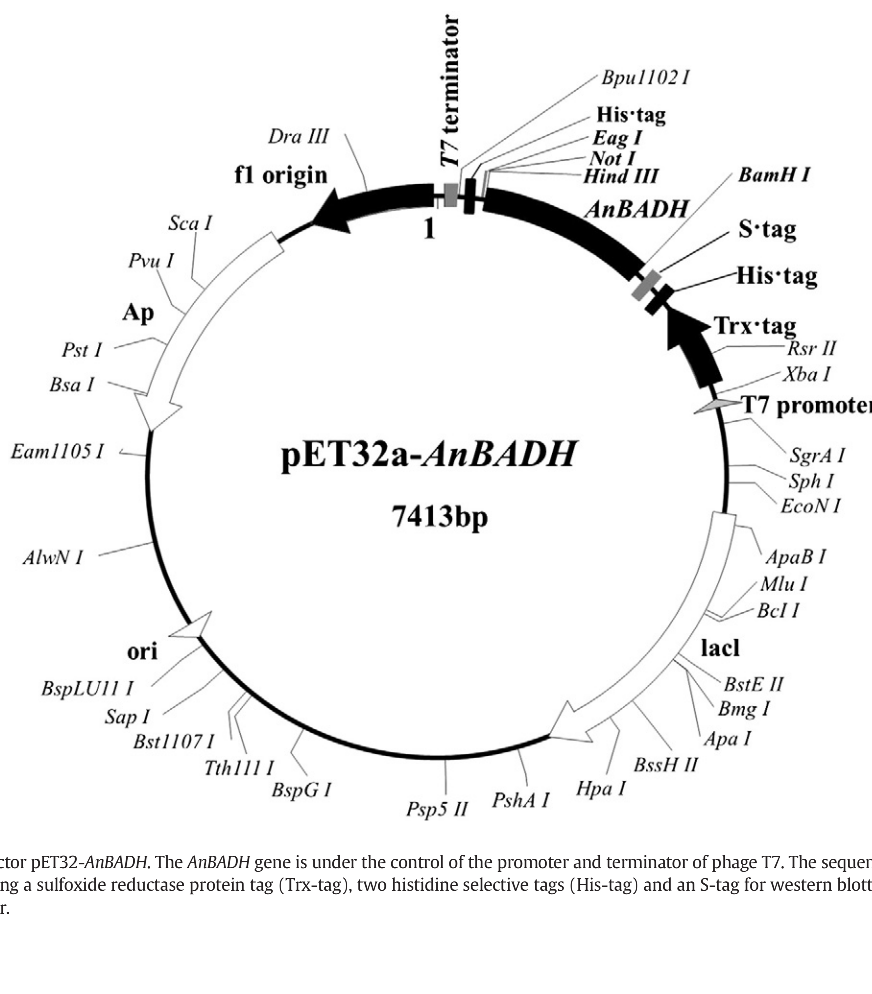

Fig. 1. Prokaryotic expression vector pET32-*AnBADH*. The *AnBADH* gene is under the control of the promoter and terminator of phage T7. The sequence immediately after the promoter encodes a leader peptide including a sulfoxide reductase protein tag (Trx-tag), two histidine selective tags (His-tag) and an S-tag for western blotting. The ampicillin resistance gene *Ap* is used as the selective marker.