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Cloning and characterization of dehydrin gene from *Ammopiptanthus mongolicus*

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Abstract Based on the sequence of an expressed sequence tag, the full-length cDNA of 1,008 nucleotides was cloned from *Ammopiptanthus mongolicus* by rapid amplification of cDNA ends. It was designated as *AmDHN*, encoding a protein of 183 amino acids. The calculated molecular weight of the *AmDHN* protein is 18.4 k Da, and theoretical isoelectric point is 5.78. The *AmDHN* localized in nucleus. Under normal growth conditions, differential expression of *AmDHN* exhibited that the expression was the highest in seeds and the lowest in flowers. *AmDHN* could be induced by NaCl, PEG6000, ABA and drought treatments. Salt and drought resistances of transgenic plants with overexpression of *AmDHN* are improved. Taken together, these results demonstrated that *AmDHN* could regulate the expression of abiotic-responsive genes and plays important roles in modulating the tolerance of plants to abiotic stresses.

Keywords Dehydrin · *AmDHN* · *Ammopiptanthus mongolicus* · Drought · Salt

Abbreviate

ABA	Abscisic acid
CDS	Coding domain sequence
GFP	Green fluorescent protein
RACE	Rapid amplification of cDNA ends
RT-PCR	Reverse transcriptase polymerase chain reaction
SSH	Suppression subtraction hybridization
MW	Molecular weight

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Introduction

Abiotic stress limits crop productivity [1, 2], and plays a major role in determining the distribution of plant species across different types of environments. Drought and salt stress are important factors that limit crop production. And many abiotic genes had been identified [3–5].

Dehydrin is one of the most abundant plant proteins produced during late embryogenesis or in response to drought, low-temperature, salinity, and ABA [6]. Dehydrins have been found in many plants including *Arabidopsis*, wheat, barley, and rice [7]. They are thought to act as chaperons, and thus to stabilize vesicles, proteins, and membrane structures in stressed plants [6, 8, 9], although their exact function remains uncertain.

Ammopiptanthus mongolicus (Maxim.) Cheng f., an ancient relic of the tertiary period, is a rare and endangered species of Fabaceae in Mid-Asia desert and has been listed as one of primary protection plants by the Chinese government. As a kind of typical drought-resisting resources plant, it mainly grow in the desert and semi-desert area in the northwest of China, which displays prominently characteristics of draught resisting, cold resisting and salt and alkali resisting. Because of its high value of study and ecological usefulness in defending desert, this plant has been called 'live fossil' [10]. *A. mongolicus* distinctively distributed in the northwestern desert area of China, where is marked by seasonally extreme drought and temperatures (over 40 °C in the summer and under −30 °C in the winter).

Previous studies have demonstrated that some dehydrin proteins play important roles in drought and salt resistances. As mentioned above, dehydrin proteins play important roles in response to stresses. If we clone dehydrin protein gene from *A. mongolicus* and over-express it in other plants, we might improve the ability of other plants to tolerate various stresses to support the development of agriculture.

Table 1 Primers used in this study

Primer name	Primer type	Sequence (5′–3′)
96F	Forward	GCATCTCATGGTGCGCCTGGTGGGT
96R	Reverse	ATACCCACCAGGCGCACCATGAGAT
DHN1	Forward	CAACAAACTTCACTTTCTTTGTGCATTC
UPM		CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT and AAGCAGTGGTATCAACGCAGAGT
DHN-f	Forward	TATGGAAACCCTGTGAGGCAAAC
DHN-r	Reverse	CTTATTCATTATCCCTTTCTTCTCG
Actin-1	Forward	GCATTGTAGGTCGTCCTCGTCAC
Actin-2	Reverse	GGAAGGGCATAACCCTCGTAGAT
D-Gf	Forward	CTCGAGATGGCACACATTCAGAGTGGG
D-G-r	Reverse	ACTAGTACGACGTCGCGGTGTCCAGGAAGCTTC
pBI-DHN-f	Forward	GGGTCTAGAATGGCACACATTCAGAGTGGG
pBI-DHN-r	Reverse	CCCGGATCCGACGTCGCGGTGTCCAGGAAGCTTC
BADH-RT-f	Forward	GGACTTGATAACTACTTGA
BADH-RT-r	Reverse	GAACTAGACAGACTGATAA
CAT-RT-f	Forward	CTCAATATGCTCCTCTTACG
CAT-RT-r	Reverse	AATGGATGATAGTGATACTTGTT
DREB-RT-f	Forward	GGATGGTAATGGATTCTT
DREB-RT-r	Reverse	GTGTTCTGTAGTTCTCTATA
UBQ-RT-f	Forward	GAATACCTCCTTGTCCTGGATCT
UBQ-RT-r	Reverse	GTACTTTGGCGGATTACAACATC

A SSH cDNA library induced by salt stress had been constructed and some abiotic genes had been screened by reverse Northern blot methods. An expressed sequence tag (EST) of *AmDHN* gene was one of these differential expression sequences. In the present study, the full-length dehydrin cDNA, designated *AmDHN*, from *A. mongolicus* was obtained with rapid amplification of cDNA ends (RACE). Its expression patterns were analyzed with RT-PCR. When overexpressing this gene in *Arabidopsis thaliana* and tobacco, stress-resistances were improved. The characterization of *AmDHN* might provide insight into the physiological processes of stress response in higher plants.

Materials and methods

Plant materials and treatments

Seeds of *A. mongolicus* (Maxim.) Cheng f. and *A. thaliana* (ecotype Col-0) were obtained from Chinese Academy of Agricultural Sciences. Seeds of *A. mongolicus* were gown on 1/2 MS agar media. Seeds were placed on soil and germinated in the dark at 25 °C for 1 day, then were grown under a regime of 16 h light and 8 h dark at 25 °C. For growth on the soil, *Arabidopsis* seeds were sown on soil and incubated at 22 °C under LD (16 h light and 8 h dark) conditions. For growth on the agar medium, *Arabidopsis* seeds were sown on a 1/2 MS plate and incubated at 22 °C under continuous light.

Amplification of Full-length *AmDHN* cDNA

An EST of 432-bp was isolated from a SSH cDNA library of *A. mongolicus* induced by PEG6000. RACE was performed to amplify its unknown 3′ and 5′ ends. Total RNA was isolated from *A. mongolicus* seedlings of 40-day-old by a Trizol extract method. Any contaminated genomic DNA was removed by incubating the total RNA with RNase-free DNase (Promega) at 37 °C for 30 min. Total RNA was used to synthesize 5′-RACE-Ready-cDNA and 3′-RACE-Ready-cDNA according to the manufacturer's recommendation of SMARTTM RACE cDNA Amplification Kit (Clontech, USA). Based on the EST sequence already obtained, gene-specific primers: 96F and 96R (seen in Table 1) were used to amplify the 3′-cDNA end and 5′-cDNA end, respectively, with Advantage^{TM} 2 PCR Enzyme Kit (Clontech). Thermocycling was performed at 35 cycles with 94 °C for 30 s, 68 °C for 30 s and 72 °C for 3 min, and an additional polymerization step at 72 °C for 5 min. PCR product was separated by electrophoresis on a 1 % agarose gel stained with ethidium bromide, purified using the DNA gel extraction kit (TaKaRa, Janpan). The products were cloned into the pMD-18T (TaKaRa, Japan)

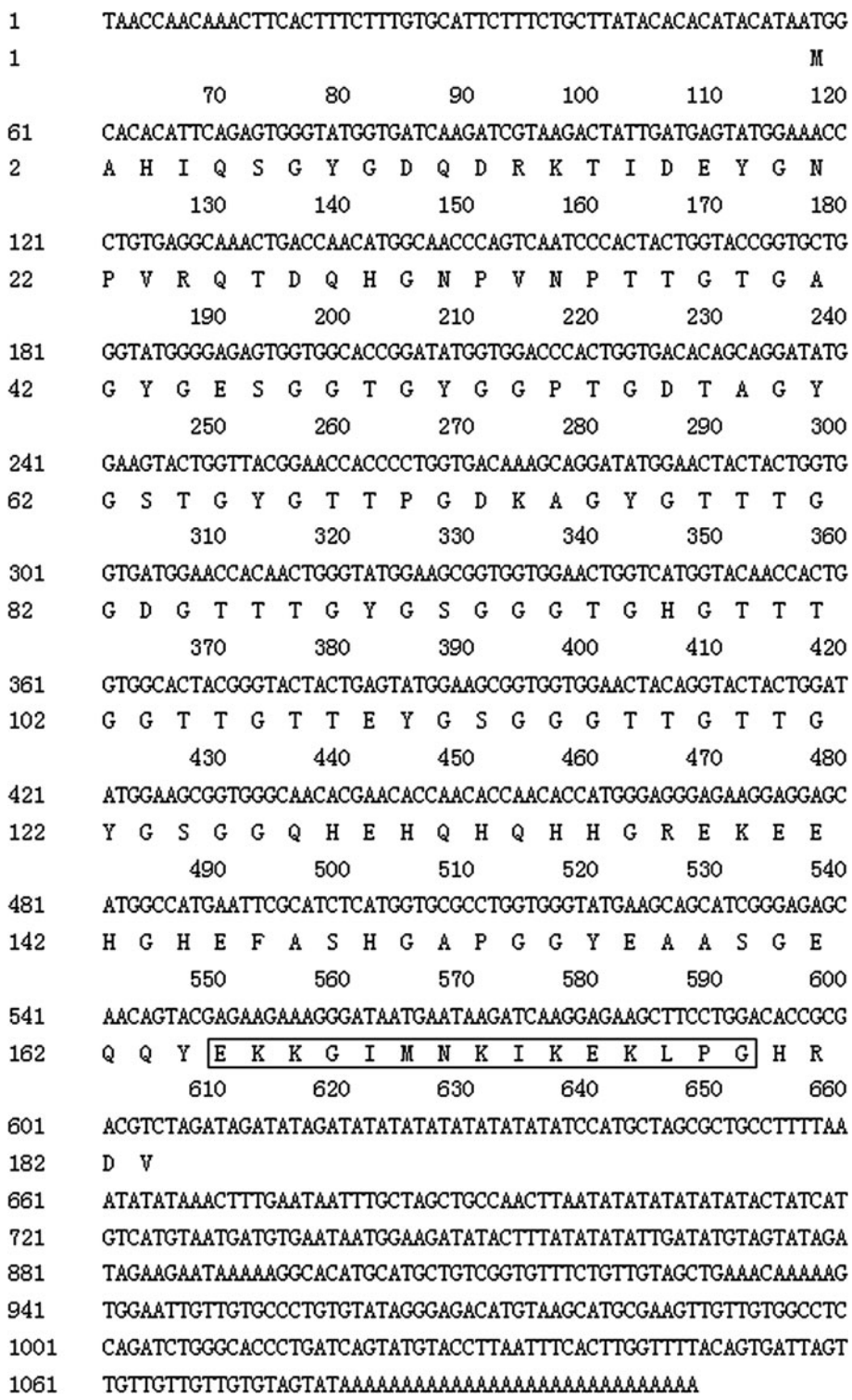

Fig. 1 The nucleotide sequence of *AmDHN* cDNA and the deduced amino acid sequence. One K fragment is boxed

vector and then transformed into *Escherichia coli* DH5α. Recombinant plasmids were sequenced by Beijing Genomics Institute (Beijing, China).

To obtain the full-length of the gene, the 3′-RACE cDNA was used as temple. The cDNA template was then amplified using gene-specific primer DHN1 and UPM (Universal Primer A Mix) (seen in Table 1).

Bioinformatics analysis

The overlapping and assembly of cDNA fragments were done by the tool of "align two sequences" (bl2seq) in GenBank. Sequences homology analysis was against nucleotide and protein database of GenBank using BLAST tools. Conserve sequences was analyzed with ExPaSy ScanProsite

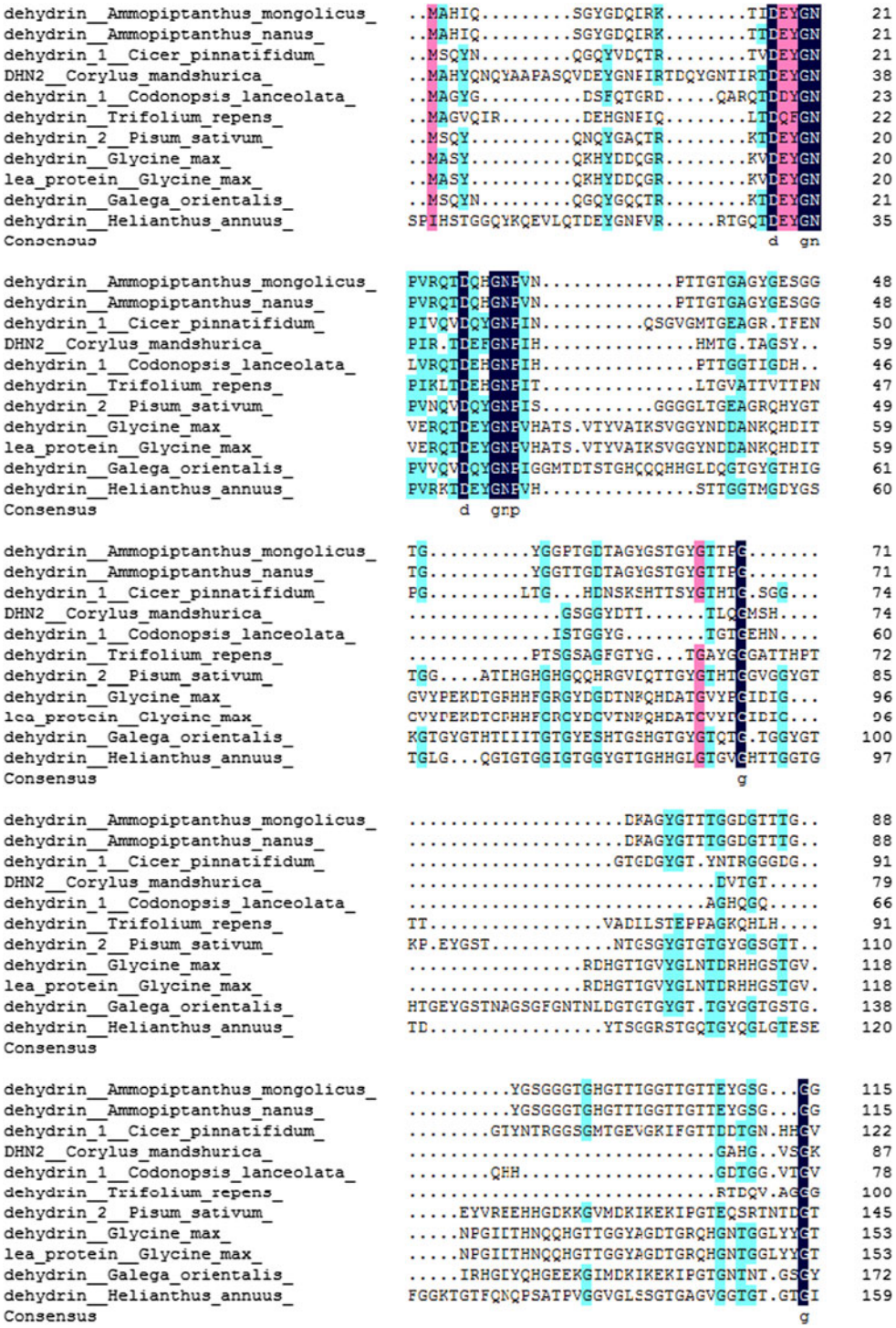

Fig. 2 Comparison of amino acid sequences of *AmDHN* with other reported dehyrin proteins in NCBI database. Ten dehyrin proteins from other plants were compared with *Ammopiptanthus mongolicus* dehydrin, including *Ammopiptanthus nanus*, *Cicer pinnatifidum*, *Corylus mandshurica*, *Codonopsis lanceolata, Trifolium repens, Pisum sativum, Glycine max, Galega orientalis, Helianthus annuus*

(http://www.expasy.org/tools/scanprosite/). Potential signal peptide cleavage site was identified using Signal P 4.0 (http://www.cbs.dtu.dk/services/SignalP/). And pI/MW were predicted on the ExPaSy website (http://us.expasy.org/tools/pitool.htm). Amino acid sequences of *AmDHN* and its related proteins were obtained from the NCBI database. The neighbor-joining trees were generated using the MEGA version 5.0 software, with bootstrap values obtained from 1,000 replications.

Expression analysis

In order to investigate the expression pattern of *AmDHN* in different tissues including dry seeds, roots, stems, leaves and

Fig. 2 continued

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dehydrin__Ammopiptanthus_mongolicus_  TTGTTGYGSGGQH..EHQHQHHG.................  136
dehydrin__Ammopiptanthus_nanus_       TIGTTGYGSGGQ....NQHQHHG.................  134
dehydrin_1__Cicer_pinnatifidum_       DQTTTDYGTHN...TGSYGTHTG.................  142
DHN2__Corylus_mandshurica_            LHRSGSSSSSSSEDDGHGGRRKKK................  111
dehydrin_1__Codonopsis_lanceolata_    LHRSGSSSSSSSEDDGMGGRRKK.................  101
dehydrin__Trifolium_repens_           HRRSSSSSSSSSEDDGQGGRRKKK................  124
dehydrin_2__Pisum_sativum_            GYGSTGYGASGG.GIGNTGQEYVREERHVHPG......DK  178
dehydrin__Glycine_max_                DTADTGAGSGSGNTGGTGYGGTGGTDYG.............  181
lea_protein__Glycine_max_             DTADTGAGPFSGNTGGTGYGGTGGTDYG.............  181
dehydrin__Galega_orientalis_          GTTGTGYGTIGGYGTGSTGVRHGDHQHHGEDKGIMDKIKE  212
dehydrin__Helianthus_annuus_          LHRSGSSSSSSSEDDGQGGRRKKK................  183
Consensus

dehydrin__Ammopiptanthus_mongolicus_  ........REKEEHG.........HEFASHGAPGGYEAAS  159
dehydrin__Ammopiptanthus_nanus_       ........REKEEHG.........HEFASHGAPGGYEAAS  157
dehydrin_1__Cicer_pinnatifidum_       ...GYGNTNIGSGYG........NTKVGQEFGREEPRHHG  171
DHN2__Corylus_mandshurica_            ...GLTGKIKEKIPGV.....GHKDHRSNTSATTPYGYDE  143
dehydrin_1__Codonopsis_lanceolata_    ...GIKEKIKEKLPGG.....RKEEQRTSTTTPGTGAVYG  133
dehydrin__Trifolium_repens_           ...GVKDKVKEKLPGVGG...GKDHNSQTTTVPAATATHH  158
dehydrin_2__Pisum_sativum_            KHGSAGQEYVREEHHVHP.GDKKHGSAGQEYVKEERRGIG  217
dehydrin__Glycine_max_                .TAGGTGYGSGTGYGIN....TGGAHTEAGYGKEHRQHEQ  216
lea_protein__Glycine_max_             .TAGGTGYGSGTGYGIN....TGGAHTEAGYGKEHRQHEQ  216
dehydrin__Galega_orientalis_          KIPGTGNTNIGSGYGTTGYGGTGSTGVGQEYVREERHHHG  252
dehydrin__Helianthus_annuus_          ...GVMQKIKEKLPGGH....RQEEQYQSQTTTTTTGGGA  216
Consensus

dehydrin__Ammopiptanthus_mongolicus_  GEQQ..........YEEKGIMNKIKEKLPGHRDV...  183
dehydrin__Ammopiptanthus_nanus_       GEQQ..........YEEKGIMNKIKEKLPGHRDV...  181
dehydrin_1__Cicer_pinnatifidum_       GEQQ..........HGEKGIMDKIKEKLPGTGH....  195
DHN2__Corylus_mandshurica_            GQLHH.........QEEKGVMEKIKEKLPGHH.....  166
dehydrin_1__Codonopsis_lanceolata_    GHEVE.........PEEKGLVEKIKEKLPGAHAHH..  159
dehydrin__Trifolium_repens_           PAEPT.........PEEKGILDKIKEKLPGHHNH...  183
dehydrin_2__Pisum_sativum_            NTGQEYVREEHRVDHGEEKGIMDKIKEKLPGTGGCTGH  255
dehydrin__Glycine_max_                SHGG..........QPEEKGILDKIKEKLPGGHSDK..  242
lea_protein__Glycine_max_             SHGG..........QPEEKGILDKIKEKLPGGHSDK..  242
dehydrin__Galega_orientalis_          DQQQ..........HGEEKGIMDKIKEKLPGTGSCTGH  280
dehydrin__Helianthus_annuus_          GYGET.........PEEKGMMEKIKEKLPGHH.....  239
Consensus                                           ekkg   kikeklpg
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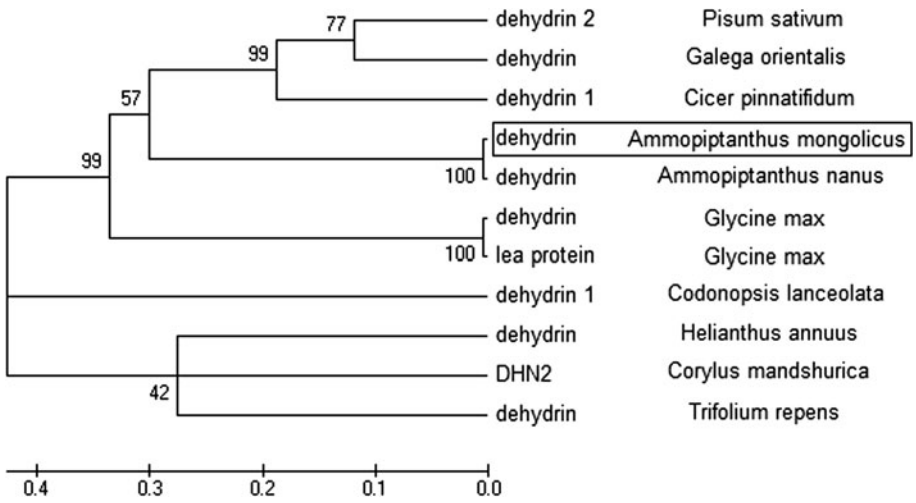

Fig. 3 Evolutionary relationships of taxa. *AmDHN* is *boxed*

flowers of *A. mongolicus*, semi-quantitative RT-PCR was carried out. Expression profiles of *AmDHN* under different kinds of abiotic conditions including NaCl, PEG6000, drought and ABA were also investigated. Seedlings of 40-day-old were grown on soil were used to extract RNA. All RNA templates were digested with DNase I (RNase-free). Aliquots of total RNA (0.5 μg) extracted were used as templates in one-step RT-PCR with the forward primer DHN-RT-f and reverse primer DHN-RT-r (seen in Table 1) specific to coding sequence of *AmDHN*. Meanwhile the RT-PCR reaction for the housekeeping gene (*Actin* gene, which is highly conserved in plants) using specific primers Actin-1 and Actin-2 (seen in Table 1) designed according to the conserved regions of plant *Actin* genes were performed to

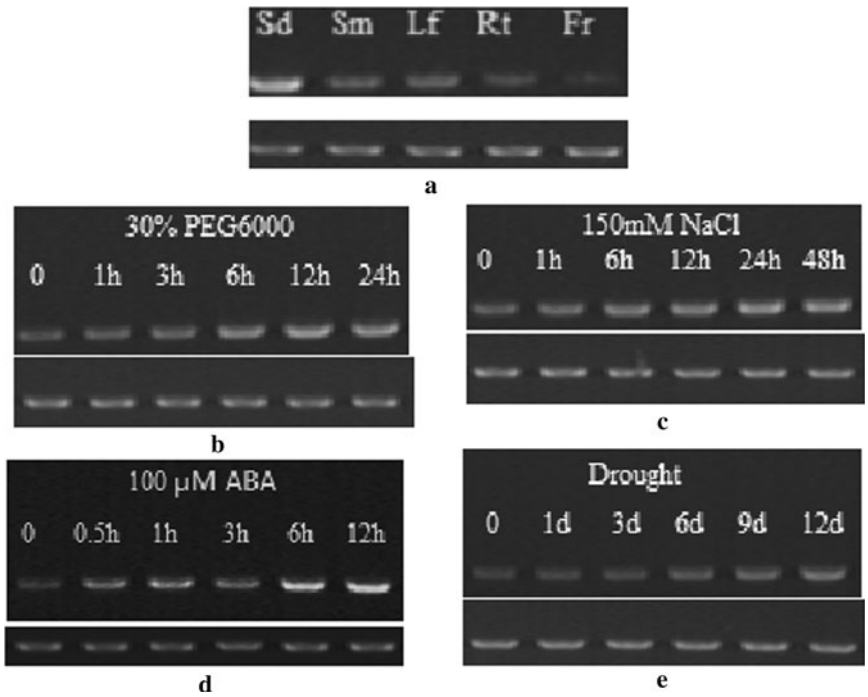

Fig. 4 Expression pattern of *AmDHN*. *AmDHN* expression level was detected by RT-PCR. The *Ammopiptanthus mongolicus Actin* gene was used as an internal control. (**a**) RT-PCR analysis of seeds (*Sd*), stems (*Sm*), leaves (*Lf*), roots (*Rt*) and flowers (*Fr*). (**b**) RT-PCR analysis of 20-day seedlings treated with 30 % PEG6000 for 0, 1, 3, 6, 12 and 24 hours (h). (**c**) RT-PCR analysis of 20-day seedlings treated with 150mM NaCl for 0, 1, 6, 12, 24 and 48 h. (**d**) RT-PCR analysis of 20-day seedlings treated with 100 μM ABA for 0, 0.5, 1, 3, 6 and 12 h. (**e**) RT-PCR analysis of leaves from mature plants without water for 0, 1, 3, 6, 9 and 12 days (d)

estimate if equal amounts of RNA among samples were used as an internal control. Amplifications were performed under the following condition: 94 °C for 3 min followed by more than 25 cycles of amplification (94 °C for 58 s, 60 °C for 30 s and 72 °C for 40 s). RT-PCR images were captured using a UVP transilluminator.

Subcellular localization of *AmDHN*

AmDHN-GFP (green fluorescent protein) fusion was constructed and transformed into onion epidermal cells to express the fusion protein. To amplify the coding sequence of *AmDHN*, two primers were designed, one primer D-Gf with *Xho* I restriction site and another primer D-Gr with *Spe* I restriction site (seen in Table 1). The PCR product was digested with *Xho* I and *Spe* I, and ligated with the vector pA7-GFP which was digested with the same restriction enzymes. The fusion was then transformed into onion epidermal cells using a gene gun. Subcellular localization of transiently expressed GFP-*AmDHN* fusion was detected by a confocal laser scanning microscope.

Plasmid construction

For the overexpression experiments, the 35S promoter was used to drive the expression of the *AmDHN* gene. The whole CDS of *AmDHN* gene was amplified by PCR with the primer pair pBI-DHN-f and pBI-DHN-r (seen in Table 1). The PCR products were digested by *Xba* I and *Bam*H I and used to insert into the enzyme sites of pBI121 (Clontech, Palo Alto, CA) to generate 35S::*AmDHN*, which was used to generate transgenic tobaccos and transgenic *A. thaliana*.

Generation of transgenic plants

Agrobacterium GV3101, which had the expression vector pBI121 with the full-length cDNA of *AmDHN* gene, was infected to *Arabidopsis* using the floral dip method [11]. Transformed *Arabidopsis* seeds were selected on a 1/2 MS medium containing 50 mg L^{-1} kanamycin and 0.8 % agar.

Stresses response assay

The germination response to the salt was measured by placing *Arabidopsis* seeds on plates with 1/2 MS containing 100 mM NaCl. After incubation at 4 °C for 2 days, seeds were transferred to room temperature (22 °C) and the germination rate was scored every day.

Arabidopsis with 35S::*AmDHN* and wild-type plant seeds were planted on 1/2 MS plates with 100 mM NaCl and 15 % PEG6000 under long-day conditions. After incubation at 4 °C for 3 days, seeds were transferred to

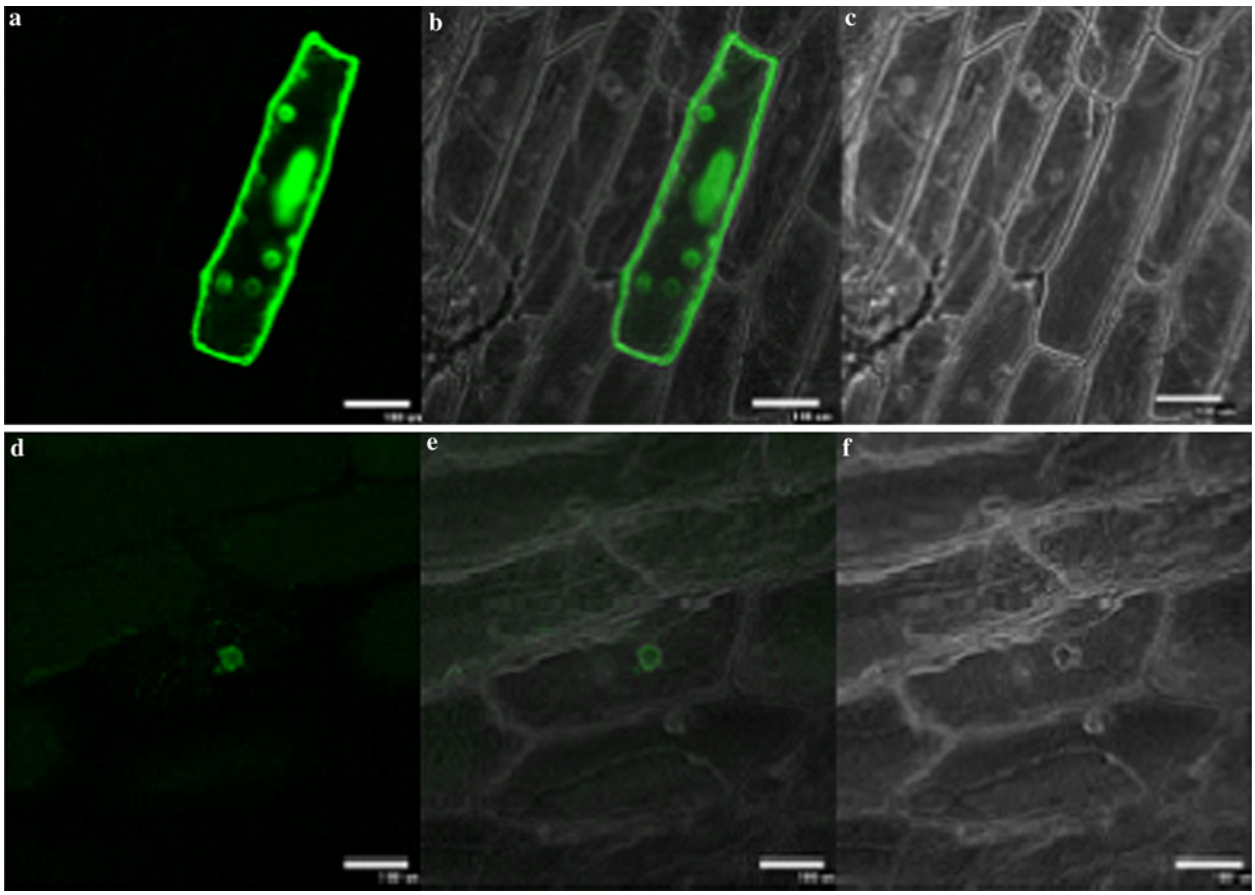

Fig. 5 Subcellular localization of *AmDHN*-GFP fusion in onion epidermal cells. The *AmDHN*-GFP fusion and the pA7-GFP control plasmid were transformed into onion epidermal cells using a gene gun. The fluorescence signals were examined by a confocal laser scanning microscope. The GFP fluorescence from cells expressing *AmDHN*-GFP fusion protein was localized to the nucleus of the cells (**d**–**f**). The GFP fluorescence was distributed throughout the entire cells expressing GFP empty vector (**a**–**c**). The photographs were taken in dark field vision (**a**, **d**), bright light vision (**c**, **f**), and superposition of the bright and dark vision (**b**, **e**). *Bar* = 100 μm

room temperature and representative pictures were taken 5 days after PEG6000 treatment.

Real-time PCR assays of abiotic genes in transgenic plants

Transgenic *Arabidopsis* with 35S::*AmDHN* were selected to analyze relative expression level of abiotic genes compared with *Arabidopsis* with 35S::*GUS*. Total RNA was isolated from 10-day-old seedlings, and total RNA preps were then treated with DNase (TaKaRa, Japan) for 30 min at 37 °C. Quantitative analysis of gene expression was performed by Realtime RT-PCR using ABI 7500 apparatus and SYBR Green I detection. For the quantification of gene expression the following primers were used (seen in Table 1). *Actin* was used as a control to normalize the amount of cDNA.

Results

Isolation of the full-length cDNA of *AmDHN* and sequence analysis

Base on the sequence of 432 bp isolated from a SSH cDNA library of *A. mongolicus*, two primers were designed to obtain 5′-cDNA end and 3′-cDNA end. A 519-bp fragment was isolated by using 3′ RACE and a 483- bp fragment was obtained by 5′ RACE. The 1,008-bp full-length cDNA of *AmDHN* was amplified from cDNA with primers DHN1 and UPM, tentatively designated *AmDHN*.

Sequence analysis indicated that the full-length cDNA contained an open reading frame of 549-bp encoding a putative protein of 183 amino acids. Ahead of the original code ATG and after the stop code TAA, there were a 56-bp 5′-UTR and a 400-bp 3′-UTR. Conserve sequence

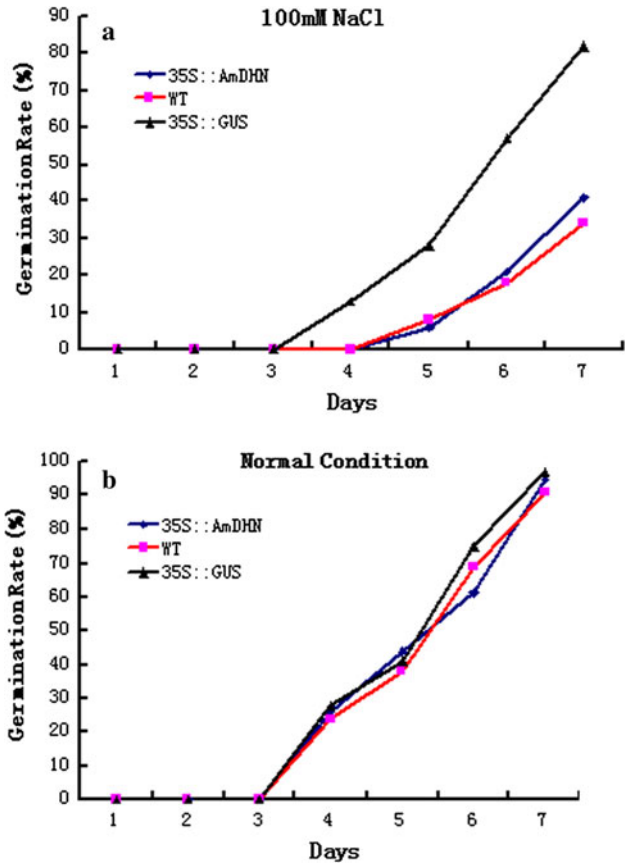

Fig. 6 Seeds germination of wild-type and *AmDHN* transgenic tobacco strain in response to NaCl. **a** Germination in the presence of 100 mM NaCl; **b** Germination in the absence of NaCl

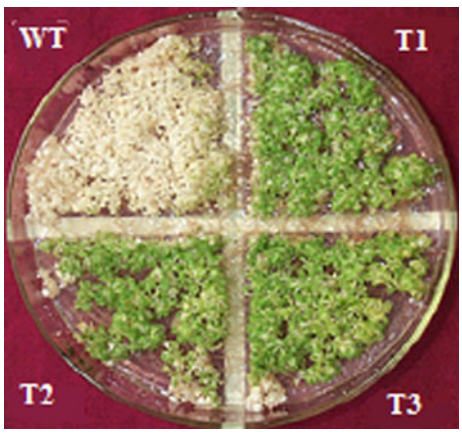

Fig. 7 Growth analysis of transgenic *Arabidopsis thaliana* in plates with 15 % PEG6000

was a K fragment at the C-terminal end of the protein, which sequence was EKKGIMNKIKEKLPG (Fig. 1). Its amino acid sequence was used to search the protein databank and showed that it could be aligned with other dehydrin proteins from different species (Fig. 2). Its theoretical pI and MV were 5.78 and 18.4 kDa. The signal peptide analysis showed that the sequence had no signal peptide (Fig. 3).

Expression analysis of *AmDHN* gene

Transcripts of *AmDHN* were found in all organs tested, including seeds roots, stems, leaves and flowers, and the expression in seeds was the highest (Fig. 4a). The expression of *AmDHN* could be improved under different abiotic conditions including salt, PEG6000, ABA and drought treatments (Fig. 4b–e). This expression pattern implies that *AmDHN* might play a role in the response of plants to abiotic stresses.

AmDHN localizes in nucleus

To examine the subcellular localization of *AmDHN*, the fusion *AmDHN*-GFP was constructed. The recombinant DNA and pA7-GFP vector were transformed into onion epidermal cells by a gene gun. When the cultured onion epidermal cells with *AmDHN*-GFP fusion proteins were examined by epifluorescence microscropy, a strong fluorescence signal was observed only in the nucleolus (Fig. 5d). In contrast, the GFP signal distributed throughout the onion epidermal cells with control pA7-GFP vector (Fig. 5a). Taken together, these results indicated that *AmDHN* is a nuclear-localized protein.

Overexpression of *AmDHN* enhances abiotic stresses tolerance of transgenic lines

The increased expression level of *AmDHN* under different abiotic stresses helped us to evaluate the gene functions, if any, of *AmDHN* overexpression on the salt and drought stresses response. The CDS of *AmDHN* under the control of the 35S promoter was transformed into wild-type plants. Based on PCR, RT-PCR and Southern blot analysis, several independent transgenic plants showing increased expression of the *AmDHN* transgene were selected for further investigation. We used transgenic *Arabidopsis* plants with 35S::*AmDHN* to test their germination efficiencies in the presence of NaCl. In the absence of NaCl, all plants, including those transformed with 35S::*AmDHN*, 35S::GUS and WT seeds germinated with similar efficiencies (Fig. 6b). When adding 100 mM NaCl, WT and 35S::GUS seeds showed reduced germination efficiencies compared with transgenic lines expressing 35S::*AmDHN* (Fig. 6a). To test whether overexpression of *AmDHN* could enhance drought resistances, *Arabidopsis* plants were treated with 15 % PEG6000 simulating the drought condition. Transgenic plants with *AmDHN* showed higher survival rate after 15 % PEG6000 treatments compared to the WT *Arabidopsis* (Fig. 7). These data indicated that

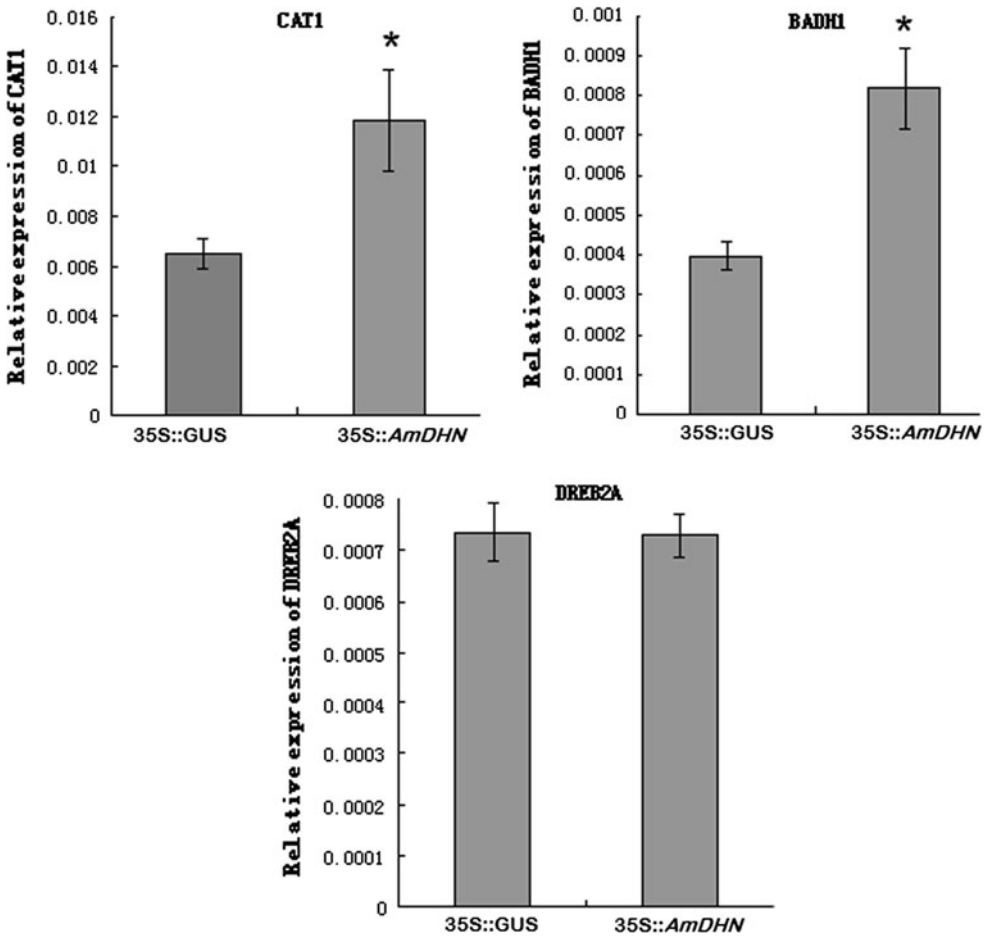

Fig. 8 Transcript levels of CAT1, BADH1 and DREB2A were detected by real-time PCR in 35S::GUS and 35S::*AmDHN Arabidopsis* plants of 10-day old. The data are mean ± SE from three independent experiments. *Asterisk* indicates $P < 0.001$ compared with 35S::GUS

Arabidopsis lines over expressing *AmDHN* are more salt and drought-resistant than WT *Arabidopsis*.

The *AmDHN*-overexpressing *A. thaliana* were then used to determine whether increased expression of *AmDHN* would affect the expression of other abiotic-resistant genes and, therefore, modulate the tolerance of plants to salt or other stresses. To examine the expression of other abiotic genes in transgenic plants, we carried out relative quantitative real-time RT-PCR analysis using gene-specific primers of CAT1, BADH1 and DREB2A (seen in Table 1), with cDNA from 35S::*AmDHN* and 35S::GUS seedlings of *Arabidopsis* thaliana as templates. The relative expression was calculated as:

$$2^{-\triangle Ct} = 2^{-[Ct,t-Ct,r]}$$

Ct,t: The threshold cycle of target gene; Ct,r: The threshold cycle of housekeep gene.

As shown in Fig. 8, overexpression of *AmDHN* leads to accumulate expression of *CAT1* and *BADH1*. But another major abiotic gene *DREB2A* was not affected with overexpression of *AmDHN* (Fig. 8). Taken together, these results show that overexpression of *AmDHN* leads the transgenic plants to become more tolerant to salt and drought stresses.

Discussion

In this study, we isolated the full-length cDNA of *AmDHN* from *Ammopiptanthus mongolicus* on the basis of 432-bp EST fragment from the SSH cDNA library. Sequence analysis of *AmDHN* indicated that it contained one K fragment, which amino acid sequence is EKKGIMN KIKEKLPG (Fig. 1). The K fragment is an obvious character of dehydrin protein. And this sequence has been found in many dehydrin proteins from other plants (Fig. 2). The expression patterns of *AmDHN* in different *A. mongolicus* organs were examined using RT-PCR analysis. In all tissues tested, the expression of *AmDHN* could be observed, whereas *AmDHN* is expressed highest in seeds and lowest in flowers.

Expression of *AmDHN* is highly induced by various stresses (Fig. 4). *AmDHN* is strongly and continuously expressed in response to ABA stress. Many dehydrin genes could be induced under ABA conditions [12–15]. A dehydrin gene *RAB21*, from *Arabidopsis*, could be induced with ABA [16]. And some researches show that, the longer plants under stresses conditions, the higher expression level

of dehydrin could be detected in wheat, barley and populus species [17–21].

High-salt and drought stresses are major adverse environmental conditions that affect plant growth and development and crop yield. So far, some dehydrin proteins in the salt stress response pathway in plants have been identified. We have demonstrated that the transcriptional expression of gene of *AmDHN* was induced by salt, drought and other stresses. And the gene encoding *AmDHN* was obtained from a SSH cDNA library induced by PEG6000. Transgenic plants overexpression of *AmDHN*, are improved in salt and drought stresses.

We used the 35S::*AmDHN* constructs described above to transform *Arabidopsis* plants and test their germination efficiencies in the presence of 100 mM NaCl. In the absence of NaCl, all plants, including those transformed with 35S::GUS and WT seeds germinated with similar efficiencies (Fig. 6b). When adding 100 mM NaCl, WT and 35S::GUS seeds show reduced germination efficiencies compared with transgenic lines expressing 35S::*AmDHN* (Fig. 6a).

To identify drought resistance in transgenic plants with *AmDHN* gene, transgenic *Arabidopsis* with 35S::*AmDHN* (T1, T2 and T3) were transferred to plates with 15 % PEG6000. Transgenic plants with *AmDHN* showed higher survival rate after 15 % PEG6000 treatment compared to the WT plants (Fig. 7). These data indicated that *Arabidopsis* lines over expressing *AmDHN* are more PEG6000-tolerant than control lines. These results imply that the *AmDHN* may be involved in the salt, drought and other stresses response in *A. mongolicus*. Previous studies have demonstrated that some dehydrin proteins play important roles in drought and salt resistances [6, 17, 22–25]. A wheat dehydrin *DHN-5* play important roles in salt and drought resistances, and overexpression of *DHN-5* enhances tolerance to salt and osmotic stress in *A. thaliana* [17]. Overexpression of the barley and wheat LEA protein genes, *HVA1* and *PMA1959* increased tolerance to drought and salt stress in transgenic rice [26, 27].

In transgenic *Arabidopsis*, expression level of *CAT1*, *BADH1* was improved, but the expression level of *DREB2A* was not obviously changed. Those results may display that overexpression of *AmDHN* in *Arabidopsis* causes high expression level of some abiotic genes, including *CAT1* and *BADH1*. *AmDHN* gene may act downstream of *DREB*, so the expression level of DREB2A is not changed when overexpression of *AmDHN* in *Arabidopsis*. Previous experiment may support this view. Expression of *OsDhn1* is highly up-regulated in CBF1 transgenic rice, indicating that *OsDhn1* is a target of CBF/DREB1 signaling [28].The identification of the genes will help to understand how *AmDHN* act in the regulation of the salt, drought or other stresses response.

In conclusion, a abiotic stresses-induced dehydrin protein *AmDHN* was isolated from *A. mongolicus.* The putative protein localized in the nucleus, and it could be induced in different stresses conditions. There is one K fragment at the C-terminal ends of the protein. *AmDHN* has function in salt and drought resistances.

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