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Full Length Research Paper

Isolation of *AhDHNs* from *Arachis hypogaea* L. and evaluation of *AhDHNs* expression under exogenous abscisic acid (ABA) and water stress

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The peanut *(Arachis hypogaea* L.*)* is an important oil and cash crop all over the world. It is mostly planted in arid and semi-arid regions. To determine the mechanism by which dehydrins (DHNs) are regulated by abscisic acid (ABA) in peanuts, three *Arachis hypogaea* L. dehydrins (*AhDHNs*) were isolated from peanut plants and sequenced. By blasting the protein sequences of these *AhDHNs*, *AhDHN1* was found belonging to the YnSKn subfamily. *AhDHN2* and *AhDHN3* were found belonging to the SKn and YnKn types, respectively. 100 µM ABA enhanced *AhDHNs* expression in peanut leaves. When peanut plants were treated with ABA and then with the ABA synthesis inhibitor sodium tungstate 12 h later, *AhDHN* expression was suppressed. However, *AhDHN2* was inhibited by sodium tungstate at 2 h, though other *AhDHNs* were not. *AhDHNs* expressions increased greatly in peanut leaves treated with 30% polyethylene glycol (PEG). Sodium tungstate along with PEG inhibited the expression of *AhDHNs*. This study found that exogenous and endogenous ABA can both affect the expression of *AhDHN* independently. The differential expression of *AhDHNs* to exogenous ABA may be because of differences in the structure of different *AhDHNs*.

Key words: *Arachis hypogaea* L. dehydrins (*AhDHNs*), peanut, abscisic acid (ABA), expression, sodium tungstate, water stress.

INTRODUCTION

Plants are frequently threatened by harsh environmental conditions. Of these, drought is the most devastating, inhibiting factor to plant germination, growth, development, and crop productivity (Huang et al., 2011). Plants can adapt climate changes by altering their architecture and physiology. Abscisic acid (ABA) is an important phytohormone. It can regulate the protective physiology in plants experiencing water deficient.

Specifically, the accumulation of ABA promotes stomatal closure to minimize water loss and induces the biosynthesis of protective substances (Nishiyama et al., 2011). ABA can also induce functional genes that help the plant adapt to drought conditions. The kinase cascade of MMK4 (homologous to mitogen-activated protein (MAP) kinase in alfalfa) seems to be conserved in modular form throughout evolution, mediating distinct ABA signal transduction pathways (Jonak et al., 1996). Three members of the nonspecific lipid transfer protein (nsLTP) gene family were isolated from *Lycopersicon pennellii* and found to be strongly induced by exogenous ABA. The nsLTPs exhibited a broad range of substrate specificity. They were found capable of transferring several classes of phospholipids and glycolipids (Marcela and Mary, 1998). *Nicotiana tabacum* Syntaxin 1 (NtSyr1 or SYP121) is a SNARE protein required for ABA control of ion channels (Leyman et al., 1999; Leyman et al., 2000). It appears to be involved in exocytosis (Di

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Abbreviations: ABA, Abscisic aid; **DHNs,** dehydrins; **PCR,** polymerase chain reaction; **RT-PCR,** reverse transcription polymerase chain reaction; **RT-qPCR** , real time quantitative PCR; **HPLC,** high performance liquid chromatography.

Sansebastiano et al., 2006). In addition to these proteins, there is a large protein family, the dehydrins (DHNs), which is believed to be induced by ABA.

DHNs were first found in rice, barely, and corn. Each DHN is extremely hydrophilic, glycine-rich, cysteine- and tryptophan-free, and contains repeated units in a conserved linear order (Close et al., 1989). They mainly exist in higher plants, algae, yeast, worms, and germs. DHNs belong to group 2 of the late embryogenesis abundant proteins (LEAs). They are some of the most extensively studied dehydration-protective molecules (Sunderlíková et al., 2009). All DHNs have one distinctive feature: the K-segment (EKKGIMDKIKEKLPG). Every DHN contains at least one well-conserved, lysine-rich stretches of 15 amino acids in its C terminal. Some DHNs may contain S-segments, Y-segments, or both (T/VDEYGNP) (Scott and Close, 1997; Choi et al., 1999). The DHNs can be assigned to five subclasses by the permutations in the number and arrangement of conserved domains: YnSKn, SKn, Kn, YnKn and KnS (Rorat, 2006). The K-segment can form a class A2 amphipathic α-helix, and the K-segment is required for binding to anionic phospholipid vesicles (Koag et al., 2009). The Y-segment is located at the N terminus. In DHNs, this terminus is homologous to the nucleic acid binding sites the molecular chaperones found in some bacteria and plants (Rorat, 2006). DHNs accumulate in plants during drought, dehydration, and similar stresses or when those plants are exposed to exogenous ABA. *DHN* genes were found to be abundantly expressed in drought-tolerant barley genotypes relative to drought-sensitive barley genotypes under both drought and control conditions, as assessed using a 22 K Affymetrix Barley 1 microarray (Guo et al., 2009). In barley, DHN promoter activity can be up-regulated by 0.1 μM ABA (Robertson, 2003). Northern blot analysis showed that DHN transcript levels increase in response to 25 μM ABA in Populus (Bae et al., 2009).

The peanut (*Arachis hypogaea* L.) is one of the most important oil and cash crops worldwide. Peanuts can grow in water-deficient areas because of their ability to adapt to dehydration. It has been shown that DHNs can be strongly induced by exogenous ABA. Veseva et al. (2010) found that, as ABA content increased, neutral *WZY2* DHN proteins accumulate in three varieties of winter wheat (cv. Pobeda, Katya, and Sadovo of *Triticum aestivum* L.) (Vaseva et al., 2010). The transcripts of two closely related low-temperature-induced *dhn/lea/rab*-like genes (*lti45* and *cor47*) from *Arabidopsis thaliana* L. have been shown to accumulate in response to ABA (Welin et al., 1995). However, the critical regions of DHNs, the ones involved in ABA transduction are not clear. The differential expression of DHNs under exogenous ABA treatment or under drought stress combined with endogenous ABA synthesis in *A. hypogaea* L. was studied.

This may provide a basis for further study of the relationship between the critical regions of DHNs and ABA signal transduction.

MATERIALS AND METHODS

Plant materials and growth conditions

Peanut seeds (*A. hypogaea* L. cv. YueYou 7) were sown in sterilized culture medium (vermiculite: perlite: soil, 1: 1: 1) and grown in an illuminating incubator with 16 h of light (200 μmol m^{-2} s^{-1}) at 26°C and 8 h of darkness at 22°C at approximately 60% humidity. Plants were irrigated every 3 days with half-strength Murashige and Skoog nutrient solution (Murashige, and Skoog, 1962).

Isolation of *Arachis hypogaea* L. dehydrins (*AhDHNs*) from peanut expressed sequence tags (EST) library

To obtain the full-length open reading frame (ORF) of *AhDHNs*, we searched EST fragments with high homology to DHNs of different plants using http://www.ncbi.nlm.nih.gov and http://www.gdaas.cn. We classified the ESTs into different branches and spliced them into several full-length ORFs. Specific primers were designed for the isolation of ESTs, and the primer sequences were designed according to UTRs of these fragments. R1-F: 5′- GAG GAT GAC GGA CAA GGT GGG AGG A-3′ and R1-R: 5′- GTA TTA CAT TAT TTG GAC ATG AAC A-3′ were used for the isolation of 305 base pair (bp) fragment of R1. R2-F: 5′-TGG CAC CAC AAC TGA ATC CGG CA-3′ and R2-R: 5′-TAC CCC ATC CCT GTC GTT CCA C-3′ were used for the isolation of a 306 bp fragment of R2. C1-F: 5′-AAT CAT GGC AGA GGA GCA CCA CAA G-3′ and C1-R: 5′-GCA GAC AGC AGG ATG AGG GTG AAC T-3′ were used for the isolation of 746 bp fragment of C1. As an internal loading control, primers 18S-F: 5′- CAA TGA TCC TTC CGC AGG TTC AC -3′ and 18S-R: 5′- ATT CCT AGT AAGCGC GAG TCA TCA G-3′, which were specific to a peanut 18S rRNA gene were used to amplify a 226 bp fragment. Polymerase chain reaction (PCR) fragments were gel-purified with an Agarose Gel DNA Purification Kit (TaKaRa). They were then linked to the pMD 19-T Vector (TaKaRa) and transformed into *Escherichia coli* strain DH5α. The recombinant clones were isolated randomly. Then plasmid DNA was extracted and the insert fragment was examined using PCR. Positive clones were directly sequenced by Shanghai Invitrogen Biotech Co. Ltd.

Treatment of peanut plants

Whole, 4-week old peanut plants were carefully removed from the soil and soaked in tap water in a 1 L beaker for 2 h to recover. Then the soaked peanut plants were divided into groups and treated for either 2 h or 12 h in each of the following solutions: (1) 100 μM ABA; (2)100 μM ABA and 5mM sodium tungstate. The treatments were performed at 25°C and approximately 60% humidity in an illuminating incubator. Leaves of water-treated peanut plants were as blank controls. Whole, 4-week-old peanut plants were treated for 12 h in each of following solutions: 5 mM sodium tungstate; 30% polyethylene glycol (PEG); and 5 mM sodium tungstate with 30% PEG. Treatments were performed at 25°C and 60% humidity an illuminating incubator. Leaves of water-treated peanut plants were used as blank controls.

Reverse transcription and real time quantitative PCR (RT-qPCR)

Total RNA was isolated using a TRIzol reagent (Invitrogen, Carlsbad, CA, U.S.) on samples collected from the treated peanut leaves. Two micrograms (2 μg) of total RNA from DNaseI-treated plants was reverse transcribed with Superscript III Reverse Transcriptase (Invitrogen, Carlsbad, CA, U.S.) in a reaction volume

of 20 µl to generate first-strand cDNA. RT-qPCR was performed as later described. For analysis of gene expression of different groups, reactions were conducted in an ABI Prism® 7300 Sequence Detection System (Applied Biosystems, San Francisco, CA, U.S.). Specific primers were designed for real-time PCR. AhDHN1-F: 5′-GGC AAG ATG TAC GGC AGT GGT-3′ and AhDHN1-R: 5′-CGT TCC ACC CAT TCC TCC ACT-3′ were for *AhDHN1* quantification. AhDHN2-F: 5′- GTT ACT TCC TTC CAT TAC GC-3′ and AhDHN2-R: 5′- GCT TAT TCT CAC CCT CCT TT-3′ were for *AhDHN2* quantification. AhDHN3-F: 5′- ACT TCC TTC CTC GGA GAC CC-3′ and AhDHN3-R: 5′- CCG CCA TTG TTT CCT CAC TT-3′ were for *AhDHN3* quantification. For the gene expression experiments, total reaction volume was 20 µl, containing 20 ng of template cDNA, 10 µl of 2× SYBR Premix Ex TaqTM mix (Applied Biosystems), 2 µl of primer mix (1 µM) in a final volume of 20 µl. All samples were amplified in triplicate assays using the following thermal cycle: 50°C for 2 min and 95°C for 10 min, for 1 cycle, followed by 35 cycles of 95°C for 15 s and 60°C for 30 s. The dissociation stage was performed at 95°C for 2 min, followed by 60°C for 15 s and then at 95°C for another 15 s. Relative expression levels were calculated using the relative 2-ΔCT method described by Livak and Schmittgen (2001).

ABA content determination

500 mg samples of peanut leaves were collected from the different treatment groups as previously described. The plant materials were frozen using liquid nitrogen and then stored at -80°C. Endogenous ABA was extracted using methanol, chloroform, and ethyl acetate. Extraction took place in 80% (v/v) aqueous methanol. Pre-purification was performed using Sep-Pak C18 cartridges (Waters, U.S.). High performance liquid chromatography (HPLC) fractionation took place in a Kro-masil C18 column (150 × 4.6 mm, 5 µm, Chenhang Company, Shenzhen, P.R.China). The standard ABA (Sigma) was diluted to 10 mM, 1 mM, 100 nM, and 10 nM as needed. The ABA level was determined in triplicate for each group.

RESULTS

Isolation of *AhDHNs* by ESTs splicing

The conservative regions of *COR47* and *RAB18* were found by blasting DHNs in different plants, such as *Arabidopsis* and *Glycine max*. The 16 ESTs found to be the most homologous to the conservative regions were obtained from the peanut EST library. An analysis of the homology of these ESTs is shown in Figure 1.

EY396017, EZ723109, GO337779, EZ725858, GO337673, and EZ729811 were different fragments of the same gene. They were spliced to an intact ORF, which we named R1 for its homology with *RAB18*. The ORF that could be spliced with EE127176, E747923, ES719633, and EZ747922 was named R2. Similarly, for its homology with *COR47*, the ORF that could be spliced with DQ889511, EZ727608, EZ728161, HM543578, EZ733401, and sun2541 was named C1.

Using the specific primers that we designed using the spliced sequences, three fragments of R1, C1, and R2 were isolated (Figure 2). Then the nucleotide sequences of these fragments were compared using the sequences we had spliced. They were found to have 100% identity. R1, C1, and R2 had the same K-segment in their C termini. This was the obvious characteristic of the DHN family. The nucleotide and predicted protein sequence of the R1 ORF, denoted *AhDHN1* for *A. hypogaea* late embryogenesis abundant protein group 2 protein DHN, is available in the National Center for Biotechnology Information databases under accession number HM543578. C1 and R2 were named *AhDHN2* and *AhDHN3*. As shown in Figure 3, AhDHNs proteins had similar Y-segments (a1, a2), S-segments (b1, b2), and K-segments (c1, c2, c3) in their ORFs. However, *AhDHN1* and *AhDHN3* (homologous to *RAB18*) showed some differences from *AhDHN2* (homologous to *COR47*) in the structures of the Y-segment (a1, a2) and K-segment (c1), and in the location of the S-segment (b1, b2).

Expression of *AhDHNs* in peanut tissues and influence of exogenous ABA

The expression of *AhDHNs* in peanut roots and leaves by sq-PCR is shown in Figure 4. Among all, *AhDHNs*, *AhDHN1* and *AhDHN3* expressed more in leaves than in roots. *AhDHN2* was not expressed differently in leaves or roots. The expression of 18S was used as an internal control.

As shown in Figure 5, the *AhDHNs* were all strongly induced by 100 µM ABA at 2 h and 12 h. At 2 h, *AhDHN2* was found to be more induced 11.8 times more than in the water-treated group. *AhDHN1* and *AhDHN3* were 2.4 times and 1.7 times more concentrated, respectively, than in the water-treated group. At 12 h, *AhDHN2* was induced 15.0 times more than in the water treated group. *AhDHN1* and *AhDHN3* changed slightly relative to the 2 h, 100 µM ABA treatment group. *AhDHN1* and *AhDHN3* were not found to be inhibited 2 h after 5 mM of sodium tungstate was added. Only when the plants were treated with inhibitor for the full 12 h did concentrations of *AhDHN1* and *AhDHN3* decrease. *AhDHN2* was inhibited by sodium tungstate both at 2 and 12 h. *AhNCED1*, which was used as positive control, reflected the condition of ABA synthesis. It showed that the expression of *AhDHN2* was more sensitive to solid tungstate than the other *AhDHNs*.

AhDHN expression in peanuts treated with PEG

The levels of *AhDHN* expression in peanut leaves given various treatments for 12 h are shown in Figure 6. *AhDHN1* and *AhDHN3* were expressed at low levels when the plants were treated with water or sodium tungstate relative to the negative control. *AhDHN2* showed increased expression when the plants were treated with water for 12 h. Like *AhDHN1* and *AhDHN3*, the expression of *AhDHN2* decreased when the plants were treated with tungstate. When the leaves were exposed to PEG, the expression of *AhDHN1*, *AhDHN2*,

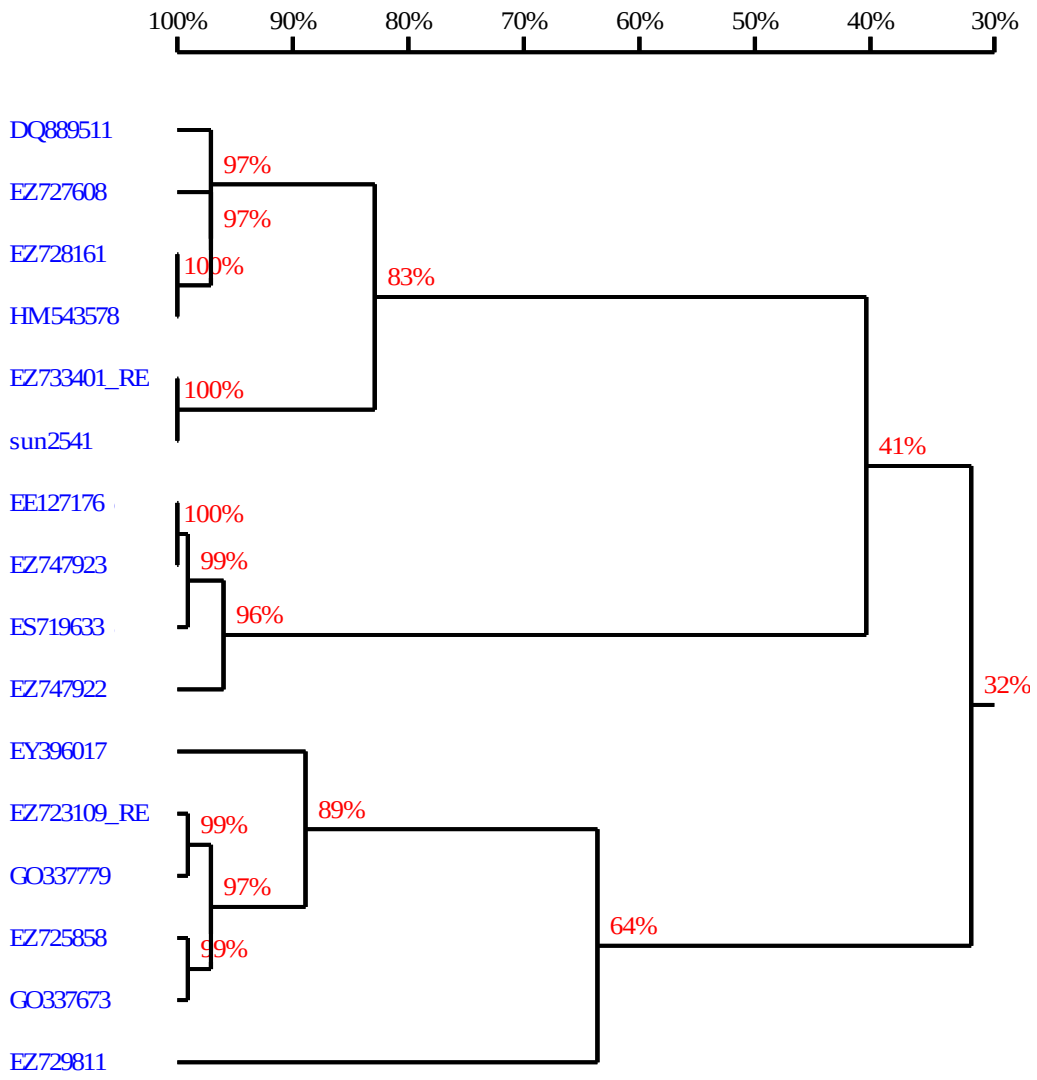

Figure 1. Homology of ESTs of *DHNs in A. hypogaea* L.

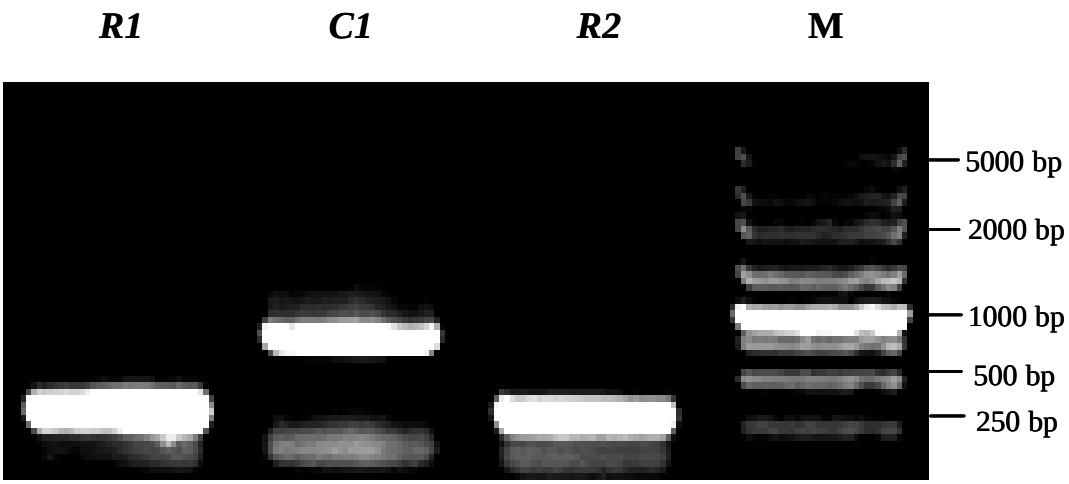

Figure 2. Amplification of homologous fragments of COR47 and RAB18 in *A. hypogaea* L. (R1, PCR product of R1; C1, PCR product of C1; R2, PCR product of R2; M, Takara 250 bp marker).

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RAB18.pro     .MASYQNRPGGQ............ATDEYGNP.....IQQQYDEYGNPMG      32
AhDHN1.pro    MAEAQLRDQHGNP.........IQLTDEQGN......PVKLTDEHGQPIH      35
AhDHN3.pro    MSQEYRDQARG.............RTDEYGN......TMRQTDEYGNPVQ      31
COR47.pro     MAEEYKNNVPEHETPTVATEESPATTTEVTDRGLFDFLGKKEE.EVKPQE      49
AhDHN2.pro    MAEEHHKQY..........EGSESRDVEVQDRGVLDFLGKKKEGENKPQE      40
                                          a1          a2

RAB18.pro     GGGYGTGGGGGATGGQG.........................YGTGGQGY      57
AhDHN1.pro    LTGVAAPASATTGFGSIP.....................TTTAGFGGTPP      64
AhDHN3.pro    QGGGTTGYGTTTESGKM.........................YGSGG...      53
COR47.pro     TTTLESEFDHKAQISEPELAAEHEEVKENKITLLEELQEKTEEDEENKPS      99
AhDHN2.pro    E.VIATEFDDKVKVSD.........................EADHEKKPS      64

RAB18.pro     GSGGQGYGTGGQGYGTGTGTEGFGTGGGARHHGQ...EQLHKESGGGLGG     104
AhDHN1.pro    TTTGFESTATTGHATGGGGIAGLGTLGGGPYGGG...ATREKGTVGDMVG     111
AhDHN3.pro    ..GAHGHGTGLGGDTTGMATGGYGTAGMGTGMG..........TGMGTGG      91
COR47.pro     VIEKLHRSNSSSSSSSDEEGEEKKEKKKKIVEGEEDKKGLVEKIKEKLPG     149
AhDHN2.pro    LLEKLHRSDSSSSSSSEEEVEEGGKKIRKKKE....KKGLKEKVEEKIG.     109
                        b1                  c1

RAB18.pro     ......MLHRSGSGSSSSSD.....DGQGGRRKK.GITQKIKEKLPGH..     140
AhDHN1.pro    R..DKKDDDKSSSSSSSSED.....DGQGGRRKKKGLKEKIKEKIPGVGG     154
AhDHN3.pro    ......GYGTTGTGEYGSTG.....TGAAGGYG..TTGGSYGTTGGGE..     126
COR47.pro     HHDKTAEDDVPVSTTIPVPVSESVVEHDHPEEEKKGLVEKIKEKLPGHHD     199
AhDHN2.pro    ..HKKEEEHHEESGAVPVEK....VEVHHTEE.KKGFLDKIKEKLPGGAN     152
                        b2                  c2

RAB18.pro     ...HDQSGQAQAMGGMGSGYDAGGYGGEH.........HEKKGMMDKIKEK     179
AhDHN1.pro    GMAKDHSPHQGTTTSTTTTTTATSAAGQHPG.....DHEKKSFMDKIKEK     199
AhDHN3.pro    ...YASSGGMGGTTGMGYGSTETGQGGHHGQHDQSHGGEKKGIMDKIKEK     173
COR47.pro     EKAEDSPAVTSTPLVVTEHPVEPTTELPVEHP.....EEKKGILEKIKEK     244
AhDHN2.pro    KKAEEHTAITTPPPPAAECVETAHQHHEHEAQGE..TKEKKGILEKIKEK     200
                                                    c3

RAB18.pro     LPGGGR...............                               185
AhDHN1.pro    MPGHHHNH.............                               207
AhDHN3.pro    LPGGHGGGHHDS.........                               185
COR47.pro     LPGYHAKTTEEEVKKEKESDD                               265
AhDHN2.pro    LPGYHPKS.EDDKEKEKQSAS                               220
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Figure 3. Sequence of *AhDHNs*, *COR47*, and *RAB18*.

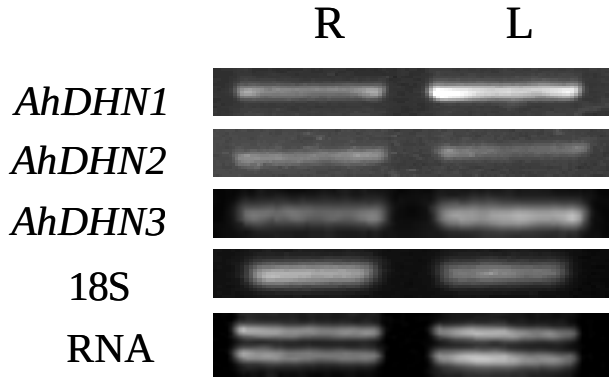


Figure 4. Expression of *AhDHN* genes in roots and leaves of *A. hypogaea* L. (R, Peanut roots; L, peanut leaves).

and *AhDHN3* all increased greatly (Figures 6A, 6B, 6C). When the plants were treated with tungstate and PEG, the *AhDHNs* expression was suppressed to a relatively low level. *AhDHNs* could be induced by endogenous ABA, which was in turn influenced by PEG. Meanwhile, *AhDHNs* were partially suppressed by inhibitors of ABA synthesis. Untreated plant leaves were used as blank controls. The expression of *AhNCED1* was used as the positive control. ABA content increased in the leaves of peanut plants soaked in PEG solution for 12 h (Figure 7). ABA content was suppressed to the untreated level when the plants were treated with tungstate, and it remained relatively low in plants treated with tungstate and PEG together.

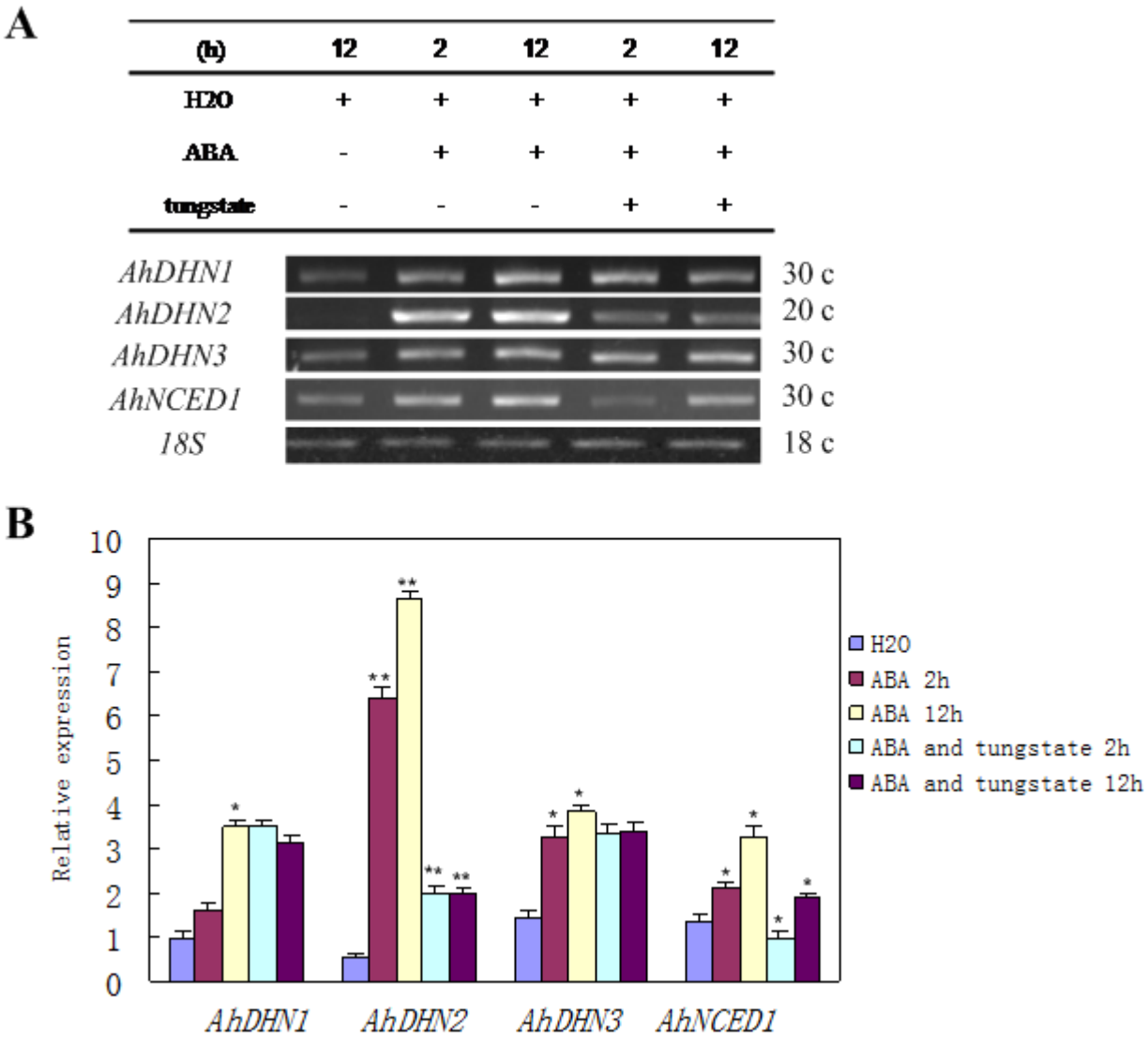


Figure 5. Expression of *AhDHNs* in leaves of peanut plants treated with ABA and ABA inhibitors. A, The electrophoretogram of *AhDHNs* under different treatment conditions. (+ represents cases in which this substance was applied; - represents cases without this substance; c represents RT-PCR cycles; 18S was used as the internal control); B, the relative expression of *AhDHNs*. The transcription levels of *AhDHNs* were expressed relative to the level of *18S*. The data are expressed as the means of three replicates. * and ** indicate significant differences between the control group and treated group, $P < 0.05$.

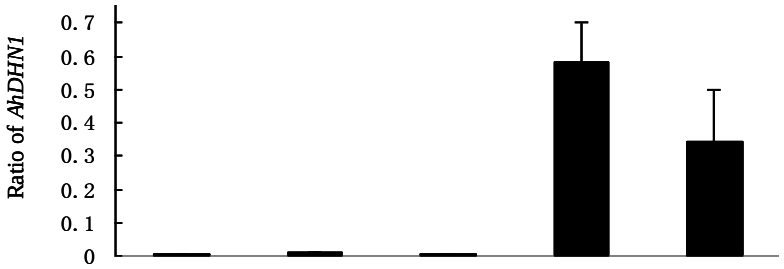
DISCUSSION

DHNs are a group of anti-stress proteins that have been studied for more than 20 years. They have strong hydrophilic tendencies and are stable in heat. Their structures are not well organized. They existed not only in the nucleus but also in the cytosol and some vesicles (mitochondria, chloroplasts) (Soulages et al., 2003). Most of the studies on DHNs evaluate protein structure and function. The critical domains of DHNs are the Y-segments, S-segments, and K-segments. Many studies have shown that DHNs play important roles in stress responses, such as those to cold, drought, disease, and heat. ABA is also involved in protecting plants from these stresses. It has been shown that exogenous ABA can upregulate DHN expression. However, the mechanism by which it does so is not clear.

In this study, three *AhDHNs* from *A. hypogaea* L. were isolated and identified using EST fragment splicing (Figure 2). *AhDHN1* was found to be YnSKn type, *AhDHN3* YnKn type, and *AhDHN2* SKn type. The structures of *AhDHN1* and *AhDHN3* were closely related them imply that this may have to do with the C1 or C2 regions or the locations of the S-segments.

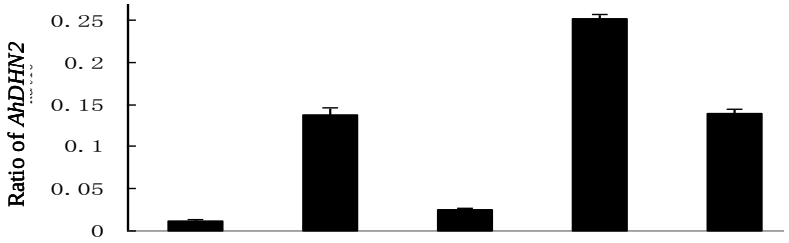
Tungstate here was shown to block the formation of ABA from ABA aldehyde by impairing ABA-aldehyde to

A



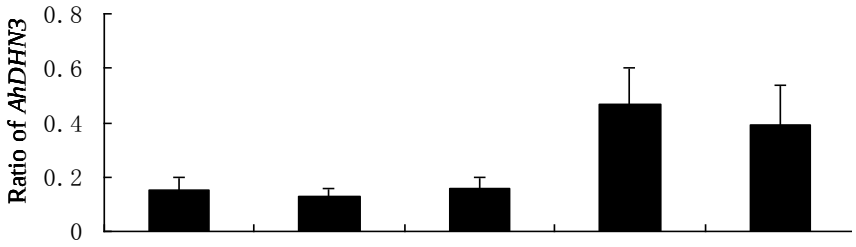
(h)	0	12	12	12	12
H_2O	-	+	+	+	+
Tungstate	-	-	+	-	+
PEG	-	-	-	+	+

B



(h)	0	12	12	12	12
H_2O	-	+	+	+	+
Tungstate	-	-	+	-	+
PEG	-	-	-	+	+

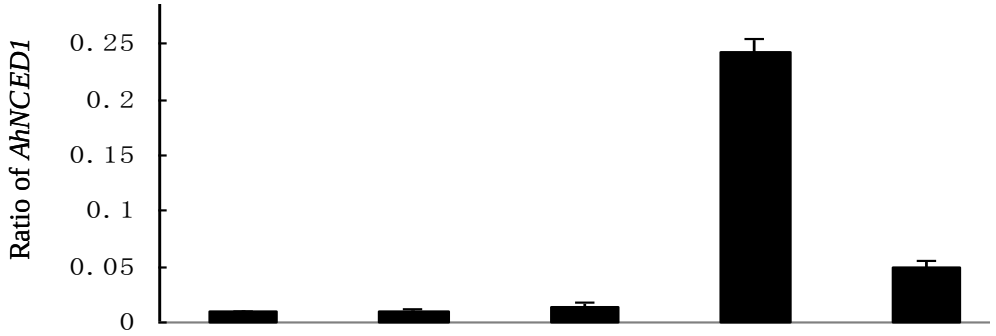
C



(h)	0	12	12	12	12
H_2O	-	+	+	+	+
Tungstate	-	-	+	-	+
PEG	-	-	-	+	+

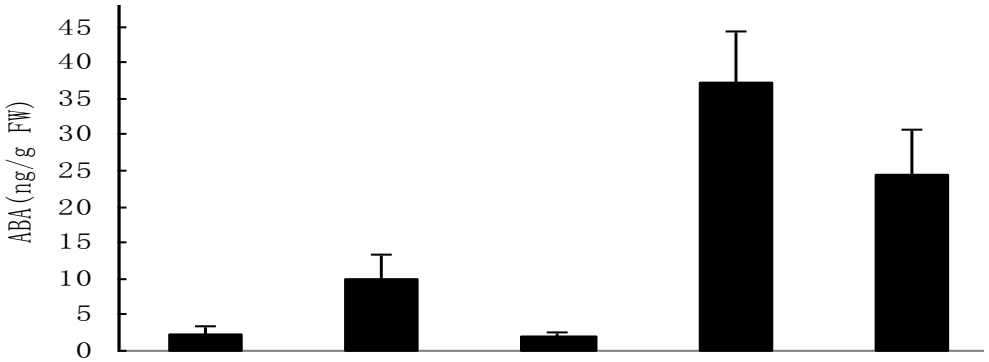
Figure 6. Expression of *AhDHN*s in peanut leaves as assessed using real-time PCR. A, Relative expression of *AhDHN1*; B, relative expression of *AhDHN2*; C, relative expression of *AhDHN3*; D, relative expression of *AhNCED1*.

D



（h）	0	12	12	12	12
H_2O	-	+	+	+	+
Tungstate	-	-	+	-	+
PEG	-	-	-	+	+

Figure 6. Contd.



（h）	0	12	12	12	12
H_2O	-	+	+	+	+
Tungstate	-	-	+	-	+
PEG	-	-	-	+	+

Figure 7. Expression of *AhDHN*s in the leaves of peanut plants treated with tungstate or given PEG solution.

to *RAB18* and that of *AhDHN2* to *COR47*. *RAB18* and *COR47* are subfamilies of DHNs in *Arabidopsis*, and they are induced by exogenous ABA. The structures of these subfamilies can influence the ability of the plant to respond to ABA. *AhDHNs* are induced by exogenous ABA. *AhDHN2*, which is homologous to *COR47*, responds more sensitively to exogenous ABA than the other *AhDHNs* do. The structural differences between

oxidase (Hansen and Grossmann, 2000). Pre-treatment with tungstate significantly suppressed the accumulation of ABA and also reduced the increased generation of reactive oxygen species (ROS) and the up-regulation of these antioxidant enzymes in water-stressed leaves (Jiang and Zhang, 2002). In our experiments, we treated peanut roots with tungstate and PEG. The expression of *AhDHNs* was reduced by tungstate (Figure 5). The experiment proved that *AhDHNs* could be induced by endogenous ABA.

ABA regulates nearly 10% of the protein-coding genes in *Arabidopsis* plants. Many studies on ABA signal transduction have focused on ABA receptors and the related upstream transcription factors. Gene expression is mainly regulated by two different families of bZIP transcription factors (TFs), ABI5 in the seeds and AREB/ABFs in the vegetative stage (Fujita et al., 2011). All the downstream genes are regulated by these TFs. Our research aim is to find out why these homologous genes are so differently expressed. In future studies, downstream functional genes will be used to determine how ABA affects these downstream genes. The critical regions of these functional genes may be identified by knocking down their expressions in bacteria or in higher plants. Using these clues, another means of studying ABA transduction may be developed.

ACKNOWLEDGEMENTS

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