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Hahb-4, a homeobox-leucine zipper gene potentially involved in abscisic acid-dependent responses to water stress in sunflower*

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ABSTRACT

Homeodomain-leucine zipper proteins constitute a family of transcription factors found only in plants. We have characterized a full-length cDNA encoding the sunflower homeobox-leucine zipper protein *Hahb-4* (*Helianthus annuus h*omeo*b*ox-4). The complete cDNA is 674 base pairs long and contains an open reading frame of 177 amino acids that belongs to the Hd-Zip I subfamily. Northern blot and RNAse A protection analysis show that the expression of *Hahb-4* is rapidly, strongly and reversibly induced by water deficit in whole seedlings, roots, stems and leaves. A similar fast induction of *Hahb-4* expression is observed when seedlings are subjected to a treatment with the hormone abscisic acid (ABA). Nuclei prepared from seedlings treated with ABA or subjected to water stress show a significant increase of protein(s) that specifically bind the sequence 5′-CAAT(A/T)ATTG-3′, recognized *in vitro* by Hahb-4, suggesting that an active protein is synthesized in response to these treatments. The promoter region of the *Hahb-4* gene contains sequences that fit the consensus for a G-box present in some ABA responsive elements (ABREs). We propose that Hahb-4 may function in signalling cascade(s) that control(s) a subset of the ABA-mediated responses of sunflower to water stress.

Key-words: *Helianthus annuus*; drought stress; homeodomain; plant transcription factor; water deficit.

INTRODUCTION

The homeodomain is a 60 amino acid motif present in a number of eukaryotic transcription factors involved in developmental processes (Gehring 1987). Genes containing homeoboxes have been isolated from many eukaryotic organisms including fungi, mammals and plants (Gehring, Affolter & Bürglin 1994). Plant homeoboxes can be divided into several families according to sequence conservation and structure in and outside the homeodomain (Chan *et al*. 1998). Members of one of these families have a distinct feature: they code for proteins termed Hd-Zip, because they contain an homeodomain associated with a leucine zipper, a coiled-coil structure involved in dimerization (Ruberti *et al*. 1991; Mattsson *et al*. 1992; Schena & Davis 1992; Gonzalez *et al*. 1997). Hd-Zip proteins bind DNA efficiently only as dimers (Sessa, Morelli & Ruberti 1993; Palena, Gonzalez & Chan 1999). It has been suggested that these proteins may be involved in regulating developmental processes associated with the response of plants to environmental conditions (Carabelli *et al*. 1993; Schena, Lloyd & Davis 1993; Chan *et al*. 1998).

One of the most common environmental stresses to which plants are exposed is dehydration. Although many seeds tolerate extreme dehydration, tolerance is rare in vegetative parts of the plant. Plants respond to water stress with the expression of a specific set of genes, which allows them to adapt to the altered environmental conditions (Almoguera, Coca & Jordano 1993; Coca *et al*. 1996; Bray 1997; Shinozaki & Yamaguchi-Shinozaki 1997). The hormone abscisic acid (ABA) plays an important role in a subset of these responses (Skriver & Mundy 1990; Shinozaki & Yamaguchi-Shinozaki 1997; Leung & Giraudat 1998).

We have previously reported the isolation and characterization of several homeobox genes from sunflower (Gonzalez & Chan 1993; Chan & Gonzalez 1994; Valle *et al*. 1997; Gonzalez *et al*. 1997). Herein we report studies on a member of the sunflower Hd-Zip family, *Hahb-4*. We have characterized a full-length cDNA and genomic sequences. Expression studies indicate that *Hahb-4* is up-regulated by drought and ABA in sunflower roots, stems, and leaves. We propose that this gene is involved in ABA-dependent responses of sunflower to water stress.

MATERIALS AND METHODS

cDNA isolation

For the isolation of partial cDNA clones containing homeobox sequences, a polymerase chain reaction (PCR)-based strategy was carried out as previously described (Gonzalez & Chan 1993) on total DNA from a sunflower stem cDNA library constructed in lambda gt10 (A kind gift

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of Dr A. Steinmetz and colleagues, Institut de Biologie Moleculaire des Plantes-CNRS, Strasbourg, France). Sequences representing the 3′-end of the *Hahb-4* transcript were obtained by PCR using lambda gt10 sequencing primers and the specific primer H41 (5′-GGCGGATCCAACA GAAACAACCACCAGG-3′), that matches nucleotides 81–100 of the cDNA sequence (Fig. 1). The 5′-end of the transcript was obtained applying rapid amplification of cDNA ends (RACE) to polyA$^+$ RNA obtained from water-stressed sunflower stems using the specific oligonucleotide 5′-GGCGGATCCCCTGGTGGTTGTTTCTGTT-3′ and primers Qt and Qo according to Frohman (1994).

Isolation of Hahb-4 genomic sequences

The 5′ non-transcribed region of *Hahb-4* was characterized using inverse PCR according to Ochman, Ayala & Hartl (1993). Sunflower genomic DNA, prepared according to Kaiser, Murray & Whittaker (1996), was partially digested under controlled conditions with *Sau*3A or *Hin*dIII. Circularization of DNA after digestion and purification was carried out overnight in the presence of 5 U of T4 DNA ligase (Promega Corp., Madison, WI, USA) according to the manufacturer's instructions. Primer pairs used for amplification were 5′-GGCG GATCCCCTGGTGGTTGTTTCTGTT-3′ and 5′-GCCGAATTCAGATTGAGCAAGAGTATAAC-3′, or 5′-ACCTTTATAAAGACCACTC-3′ and 5′-ACGC AATG GTGAGTTGTAC-3′.

DNA sequence analysis

The PCR products were cloned into either pUC119 or pGEM-Teasy (Promega Corp.). The nucleotide sequence of inserts was obtained by the chain termination method using the fmol sequencing system (Promega Corp.).

Plant material, growth conditions and water stress treatments

Helianthus annuus L. (sunflower cv. contiflor 15, from Zeneca, Balcarce, Argentina, or cv. Sunweed, from Rhône-Poulenc, Lyon, France) seeds were surface sterilized and grown on filter paper inside Petri dishes for a period of 4 d. Seedlings were then transferred to plastic supports containing Hoagland's medium and grown until they had six leaves (approximately 3 weeks).

Water stress was imposed either by transferring 4-day-old-seedlings to Petri dishes with dry filter paper or by removing plants from the hydroponic culture. The treatment times were as indicated in the figures.

RNAse protection analysis

For RNAse protection analysis, total RNA (15 μg) prepared as described (Almoguera & Jordano 1992) was hybridized to a specific *Hahb-4* riboprobe synthesized by *in vitro* transcription using T3 RNA polymerase and [^{32}P]CTP following the instructions of the manufacturer (Boehringer Mannheim, Mannheim, Germany). The template consisted of an insert corresponding to the coding region between +81 and +429 (Fig. 1) cloned into the *Spe*I/*Bam*HI sites of pBluescript SK$^-$. The *Bam*HI site, not present in the cDNA, derives from amplification with oligonucleotide H41 as described above. Restriction digestion of this template DNA with *Eco*RI allows transcription of a 411-nucleotide RNA probe that contains 63 nucleotides from the vector (from the T3 promoter to the *Spe*I site in the polylinker, and from the *Bam*HI to the *Eco*RI sites). Conditions for riboprobe preparation, hybridization, digestion with RNAse A, and subsequent electrophoretic analysis of protected RNA fragments were as previously described (Coca *et al*. 1996).

Northern analysis

Total RNA (20 μg) was denatured with formamide and formaldehyde, separated in a 1·5% (w/v) agarose/6% formaldehyde gel, and blotted onto nylon membranes (Hybond N; Amersham-Pharmacia, Buckinghamshire, UK) essentially as described by Sambrook, Fritsch & Maniatis (1989).

Hybridization was performed overnight at 65 °C in 6 × SSC (1 × SSC is 0·15 M NaCl, 0·015 M Na_3-citrate, pH 7·0), 0·1% (w/v) polyvinylpirrolidone, 0·1% (w/v) bovine serum albumin, 0·1% (w/v) Ficoll, 0·5% (w/v) sodium dodecyl sulphate (SDS). An *Spe*I/*Eco*RI fragment (from +424 to +674), corresponding to the 3′-non coding region of the *Hahb-4* cDNA plus the last 177 nucleotides of the coding region, which does not include the Hd-Zip domain, was labelled with [^{32}P]dATP (1×10^8 dpm μg^{-1}) by random priming (Sambrook *et al*. 1989) and used as probe. The filters were autoradiographed using Bio-Max films and transcreen (Eastman Kodak, Rochester, NY, USA) overnight. To check the amount of total RNA loaded in each lane, filters were then re-probed with a 25S rRNA from *Vicia faba* under similar conditions as those described above, except that hybridization was performed at 62 °C

Preparation of nuclei

Sunflower nuclei and nuclear extracts were prepared from control, water-stressed, or ABA-treated seedlings (4-days-old) according to the technique described in Maliga *et al*. (1995). Protein patterns were analysed by SDS-polyacrylamide gel electrophoresis (PAGE) and total protein concentration was measured as described (Sedmak & Grossberg 1977).

DNA-binding assays

For electrophoretic mobility shift assays (EMSA), aliquots of purified nuclear proteins (30 μg) were incubated with double-stranded DNA (0·3–0·6 ng, 30 000 c.p.m., labelled with [α-^{32}P]dATP by filling-in the 3′-ends using the Klenow fragment of DNA polymerase) generated by either hybridization of the complementary oligonucleotides 5′-AAT TCAGATCTCAATAATTGAGAG-3′ and 5′-GATCCTC TCAATTATTGAGATCTG-3′ (binding site for Hahb-4 is

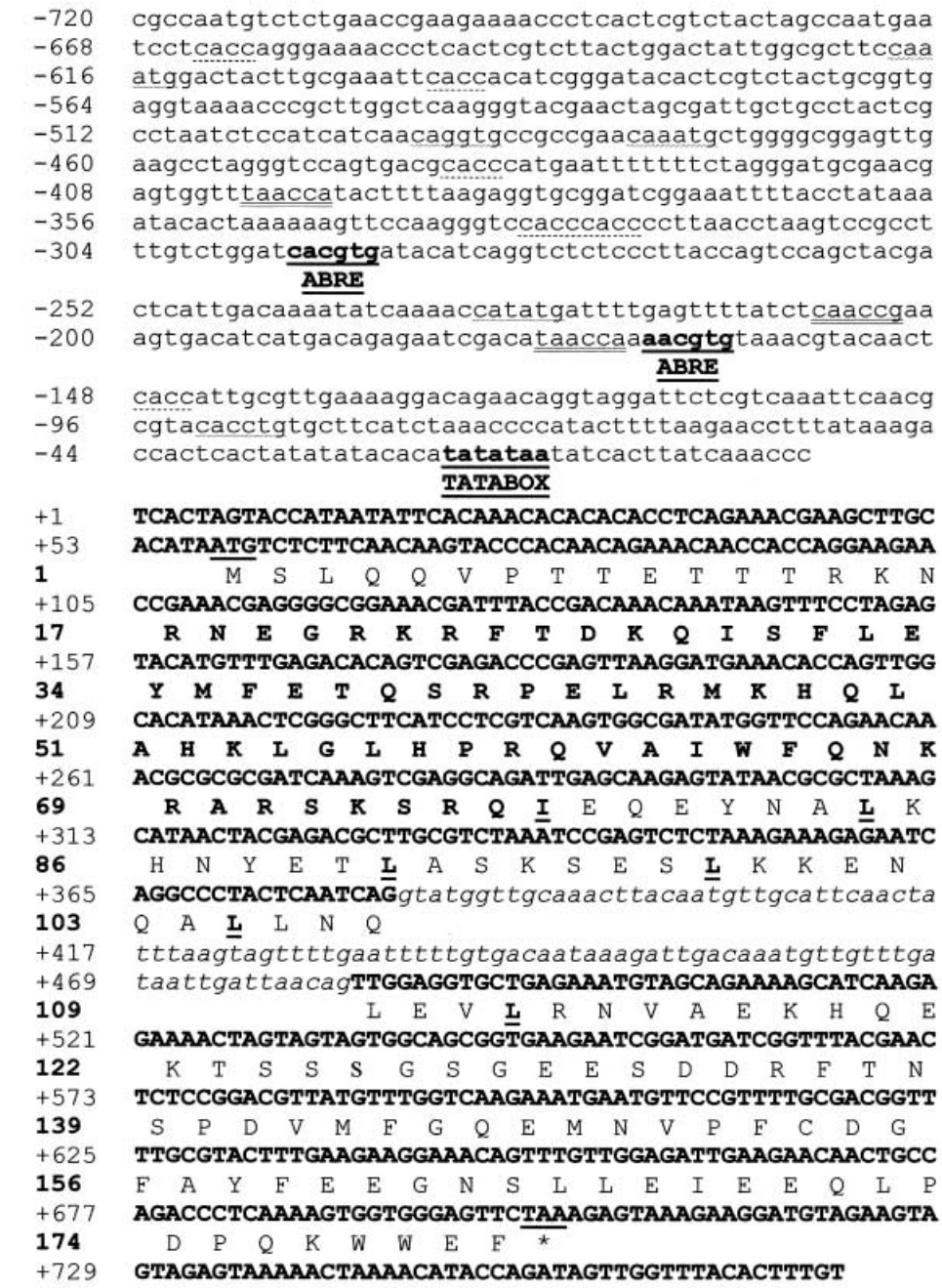

Figure 1. Genomic sequence encoding sunflower Hahb-4. The deduced protein sequence of the open reading frame is indicated below the nucleotide sequence. The homeodomain is shown in bold; leucines from the leucine zipper are shown in bold and underlined. Numbers to the left correspond to either nucleotide or amino acid sequences. The transcription initiation site, as determined by primer extension analysis, is indicated as +1. The TATA-box and two putative ABREs are shown in bold and underlined. Myb and Myc recognition sequences are doubly and wavy-underlined, respectively; putative coupling elements are underlined with a dotted line. The lower part of the Figure shows an alignment of the Hd-Zip domain of Hahb-4 with those of Athb-1, -6, 7 and 12. Shaded boxes indicate identical amino acids.

underlined). Binding reactions (20 μL) containing 20 mM HEPES–NaOH (pH 7·6), 40 mM NaCl, 0·2 mM ethylenediaminetetraacetic acid (EDTA), 1·0 mM dithiothreitol (DTT), 0·5% Triton X-100, 20% glycerol, and 1·5 μg poly(dI-dC), were incubated for 20 min at 25 °C, supplemented with 2·5% (w/v) Ficoll and immediately loaded onto a running gel (5% acrylamide, 0·08% bis-acrylamide in 0·5 × TBE plus 2·5% glycerol; 1 × TBE is 90 mM Tris-borate, pH 8·3, 2 mM EDTA). The gel was run in 0·5 × TBE at 30 mA for 1·5 h and dried prior to autoradiography.

RESULTS

Isolation of an Hahb-4 full-length cDNA

In order to characterize homeobox-containing sequences from sunflower, we have previously isolated several partial clones from a stem cDNA library (Gonzalez & Chan 1993). One of the clones represents a member of the Hd-Zip family that was named *Hahb-4*. Sequences corresponding to the 5′ and 3′ ends were obtained by PCR as described in Materials and methods. The complete cDNA sequence obtained in this way is 674 bp long and contains an open reading frame of 177 amino acids (Fig. 1). Primer extension analysis confirmed that the fragment obtained by RACE extends up to the transcription initiation site of the *Hahb-4* gene (data not shown).

Comparison of the encoded protein with other Hd-Zip protein sequences indicates that it is most related to Hd-Zip I proteins, sharing about 50% identical amino acids within the homeodomain with other members of this subfamily, with the exception of Athb-7 (Söderman *et al*. 1994) and Athb-12 (Lee & Chun 1998), that have 60 and 53% identity, respectively, within this region (Fig. 1). It is also noteworthy that the Hahb-4 homeodomain is located almost at the amino terminal end of the protein (amino acids 17–76). As a consequence, Hahb-4 lacks the acidic domain present in other members of the Hd-Zip protein family adjacent to the N-terminal end of the homeodomain. This characteristic is also shared with Athb-7 and Athb-12.

In order to investigate the genomic structure of the *Hahb-4* gene, we have amplified genomic DNA with several oligonucleotides comprising the entire cDNA. A single intron of 101 bp was detected between nucleotides 381 and 382 (aminoacids 108 and 109) of the cDNA (Fig. 1). The intron is located just between sequences encoding the fifth and sixth heptads of the leucine zipper domain.

To isolate the promoter region of *Hahb-4*, we used inverse PCR on genomic DNA. We were able to isolate a fragment of 720 bp located upstream from the transcription initiation site determined by primer extension analysis. We have identified the TATA box 24 bp upstream from the transcription initiation site, two putative ABREs (abscisic acid responsive elements; Bray 1997; Shinozaki & Yamaguchi-Shinozaki 1997; Leung & Giraudat 1998) in the –290 and –165 regions, and several putative coupling elements (Shen & Ho 1995; Fig. 1). Consensus sequences for other transcription factors involved in responses to water stress, namely Myb and Myc proteins (Shinozaki & Yamaguchi-Shinozaki 1997), were also identified (Fig. 1).

Hahb-4 is strongly and rapidly induced by water deficit

Northern blot analysis indicated that the *Hahb-4* gene is expressed at very low levels in sunflower plants grown under controlled environmental conditions. Only faint signals were obtained with total RNA extracted from several tissues and developmental stages. As it has been suggested that Hd-Zip proteins may function in regulating phenotypic responses to environmental signals (Schena & Davis 1992), we have analysed the expression of *Hahb-4* under different environmental conditions (i.e. water, osmotic, salt, cold, heat, and oxidative stress) by RNAse protection, which is more sensitive than the Northern technique (Fig. 2). Figure 2b shows that *Hahb-4* was not detected in 4-day-old seedlings grown under normal conditions. In water-stressed seedlings, however, a strong signal was observed. Mannitol also induced *Hahb-4* expression, although at lower levels, probably reflecting the decrease in water activity caused by this compound (Fig. 2b). A similar result was obtained with NaCl (not shown). As many responses to water stress are mediated by ABA (Shinozaki & Yamaguchi-Shinozaki 1997), we have also analysed the effect of this hormone on the expression. As shown in Fig. 2b, induction was observed after 24 h of treatment of irrigated seedlings with 100 μM ABA. A smaller, but significant increase in transcript levels was also observed with 10 μM ABA (not shown). No detectable effect was observed when the seedlings were subjected to either cold (4 °C) or heat (42 °C) stress conditions (Fig. 2b). These results indicate that the effect of water stress is specific. Figure 2c shows that the response to water stress conditions is also observed in roots, stems, and leaves of older (21-day-old) plants. The level of induction in aerial parts of the plant is similar to that observed in seedlings. Roots, on the contrary, show considerably lower transcript levels under water stress conditions. Since ABA also participates in seed development during late embryogenesis, which involves a desiccation process (Skriver & Mundy 1990; Leung & Giraudat 1998), we analysed *Hahb-4* transcript levels in sunflower embryos (20 d after pollination) and in dry seeds. No signal was obtained in RNAse protection experiments (Fig. 2c), indicating that the response of *Hahb-4* to water stress and ABA is characteristic of vegetative phases of development.

The high level of induction observed under water stress conditions allowed us to analyse the time-dependent increase in transcript levels by Northern experiments. Figure 3 shows that seedlings exposed to drought significantly increased their *Hahb-4* transcript levels after only 30 min; the response reached a maximum after 1 h of withholding water. After this time, no further increments in *Hahb-4* transcript levels were observed (not shown). Rehydration of the seedlings lowered *Hahb-4* transcript levels more slowly, with only about a 50% decrease after 2 h (Fig. 3, lanes 4 and 5). However, the effect of water stress was eventually fully

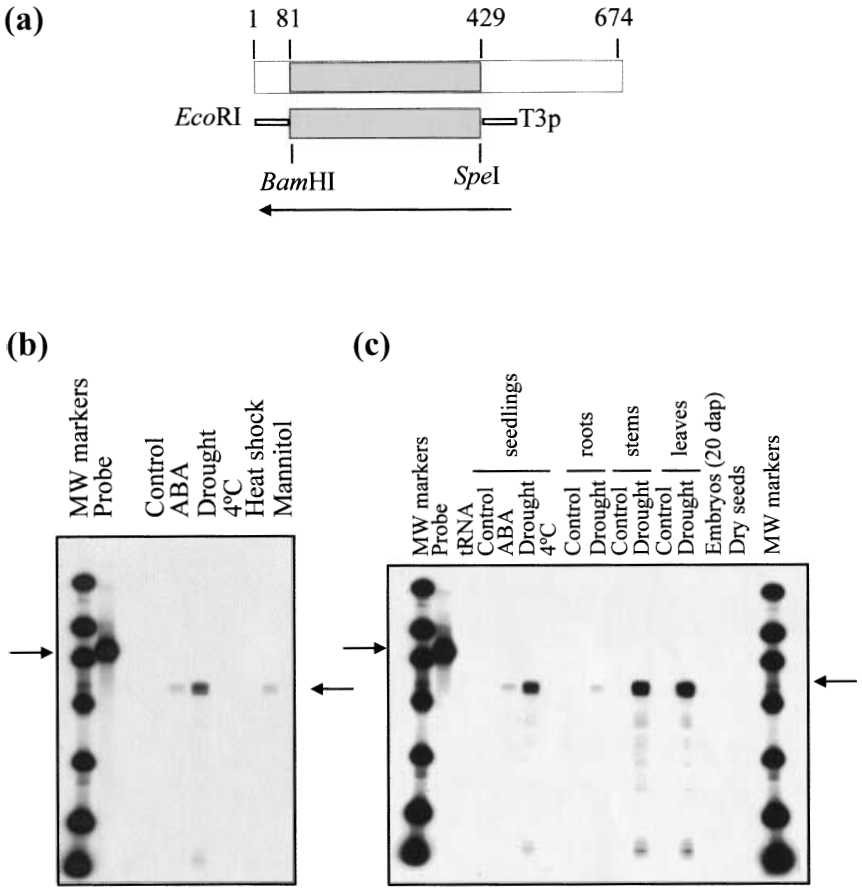

Figure 2. *Hahb-4* expression is strongly induced by water stress in sunflower plants. Total RNA samples (15 μg each) from plants subjected to different treatments were hybridized to a *Hahb-4* riboprobe. RNA hybrids were digested with RNAse A and the products were analysed by denaturing PAGE. (a). Scheme of the *Hahb-4* cDNA showing the length and polarity of the riboprobe (arrow), and the region protected by the *Hahb-4* mRNA (+81 to +429; shaded box). Additional riboprobe sequences arise from pBluescript SK$^-$ (T3p, T3 RNA polymerase promoter). (b) Four-day-old seedlings were treated as follows: 100 μM ABA during 24 h; water stress during 2 h; 4 °C during 24 h; 42 °C during 2 h; 0·5 M mannitol during 4 h. (c) Different organs from plants subjected to water stress during 2 h, embryos, and dry seeds were analysed. Arrows on the left and right of (b) and (c) indicate the migration of the full-length riboprobe and the fully protected RNA fragment, respectively. The size of DNA molecular weight standards are 680, 489, 404, 328, 242, 190 and 157 bp.

reversed (not shown). The effect of ABA treatment was also time dependent. As shown in Fig. 4, the response to ABA was detectable within 1 h and reached a maximum after 3–6 h. After that, transcript levels decreased slowly, but were still significantly high after 24 h of treatment.

The response of *Hahb-4* to water stress in different organs of hydroponically grown 3-week-old plants was also rapid. In roots, transcript levels reached a maximum after 60 min of stress treatment (Fig. 5). In leaves and stems, a maximum response was observed after more prolonged treatments. This may be related to the fact that the initial perception of water stress is accomplished by the root, which synthesizes ABA that is then translocated to aerial parts of the plant (Bray 1997; Sauter & Hartung 2000). This, together with the results previously shown in Figs 2–4, most likely indicates that *Hahb-4* expression is related to endogenous ABA levels in water-stressed plants.

The presence of functional DNA binding proteins in sunflower nuclei was analysed by electrophoretic mobility shift assays using a synthetic double-stranded oligonucleotide comprising the sequence 5′-CAAT(A/T)ATTG-3′, bound *in vitro* by Hahb-4 expressed in bacteria (Palena *et al*. 1999). Nuclear extracts prepared from 4-day-old seedlings showed distinct shifted bands, indicating the existence of at least two different protein complexes that bind to this sequence (Fig. 6). The amount of both complexes was significantly increased in extracts obtained from ABA-treated plants. On the contrary, only the slower-migrating complex increased when plants were subjected to water stress. Formation of both complexes was almost completely abolished by an excess of the same unlabelled DNA, but not by an equivalent amount of a similar DNA containing the sequence 5′-CACT(A/T)AGTG-3′ (changed positions underlined) not bound by Hahb-4 (Fig. 6). This result strongly suggests that at least one functional protein with the same DNA-binding specificity as Hahb-4 is synthesized and translocated to the nucleus in the presence of ABA or under water stress conditions.

DISCUSSION

This report describes the characterization of *Hahb-4*, a sunflower Hd-Zip gene induced by water stress conditions. Within the homeodomain, Hahb-4 shows partial homology with Athb-7 and Athb-12, two closely related *Arabidopsis thaliana* Hd-Zip proteins. Hahb-4 also shares with these two proteins the fact that the Hd-Zip domain is located near the N-terminal end, and that the typical stretch of acidic residues found in other Hd-Zip I proteins is not present in them. The *Hahb-4* gene contains an intron that separates the coding regions for the fifth and sixth heptads of the leucine zipper motif. All genes encoding Hd-Zip I proteins described up to now possess an intron in this

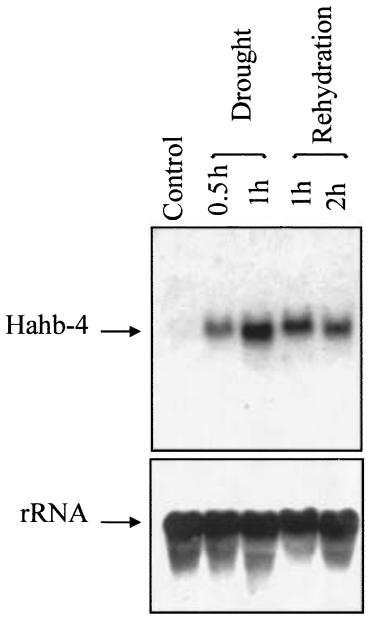

Figure 3. Induction of *Hahb-4* by water stress is reversed by rehydration. Total RNA (20 μg) was extracted from 4-day-old seedlings treated as indicated, electrophoresed, blotted onto nylon membranes, and probed with ^{32}P-labelled *Hahb-4* cDNA. Seedlings were subjected to water stress during 0·5 or 1 h, or rehydrated at different times after a 1 h drought treatment. The same filter was hybridized with an rRNA probe as a control for RNA loading and transfer (lower panel).

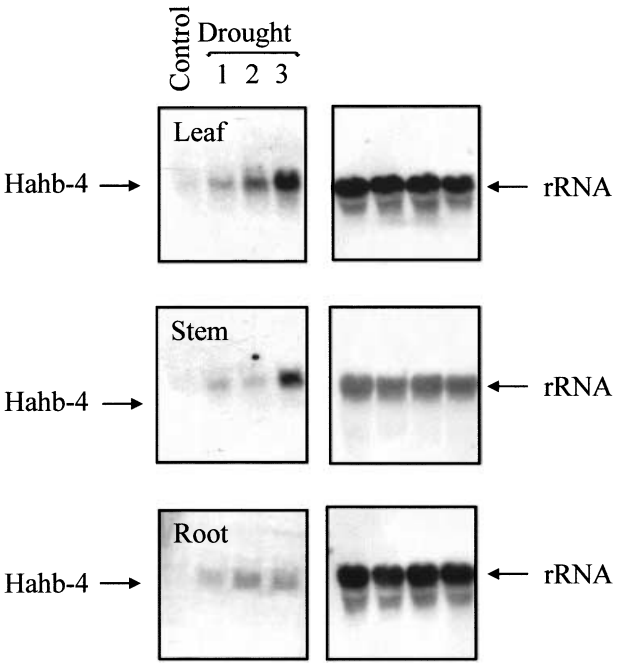

Figure 5. Time dependence of *Hahb-4* induction by water stress in roots, stems and leaves. Total RNA (20 μg) isolated from roots, stems, or leaves was probed with ^{32}P-labelled *Hahb-4* cDNA. RNA was prepared from different organs of control, 30 min- (1), 60 min- (2), or 90 min-water-stressed plants (3). The same filters were hybridized with an rRNA probe as a control for RNA loading and transfer (right panels).

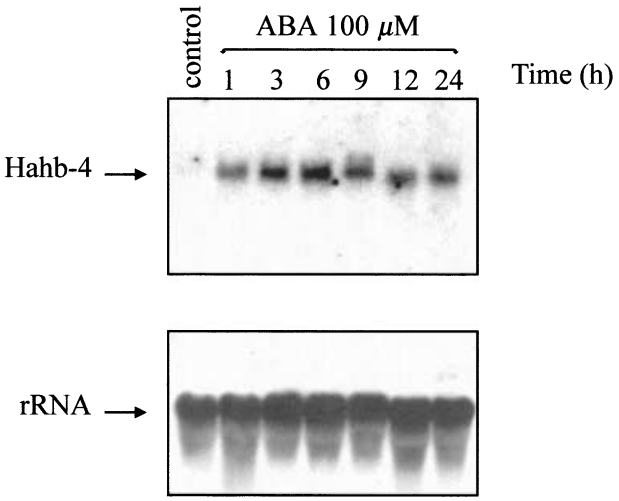

Figure 4. Time dependence of the response of *Hahb-4* to ABA. Total RNA (20 μg) was isolated from seedlings that were either untreated, or treated with 100 μM ABA for different times as indicated in each lane. In the lower panel, the same filter was hybridized with an rRNA probe as a control for RNA loading and transfer.

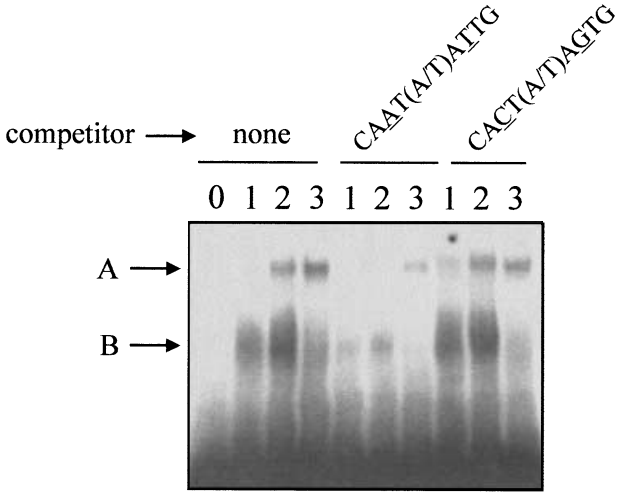

Figure 6. ABA and water stress induce the expression of nuclear proteins that specifically bind to the Hahb-4 DNA target sequence. Nuclear extracts (30 μg) prepared from plants treated under different conditions were analysed by an electrophoretic mobility shift assay for the presence of proteins that bind to labelled DNA containing the sequence CAAT(A/T)ATTG. Where indicated, a 50-fold molar excess of unlabelled DNA containing either the same, or a mutated sequence, was included in the assay. Lane 0, DNA alone; Lane 1, control plants; lane 2, ABA-treated plants; lane 3, water-stressed plants; A and B indicate two different shifted bands observed with nuclear extracts.

position, with the exception of *Athb-3*, which has a termination codon near the end of the fifth heptad (Mattsson *et al*. 1992). All other Hd-Zip I protein coding genes, however, have introns in other positions, with the exception of *Athb-7* and *-12*. It is noteworthy that *Athb-7* and *-12* are both induced by water stress and ABA (Söderman, Mattsson & Engström 1996; Lee & Chun 1998). Outside the Hd-Zip domain, however, there is no significant homology between these proteins and Hahb-4. This, and the low percentage of identity at the amino acid level within the homeodomain, makes it difficult to ascertain whether *Hahb-4* is the orthologue of any of the *Arabidopsis* genes. Phylogenetic analysis indicate that *Athb-7* and *-12* are more related to each other than to *Hahb-4*, suggesting that they have arisen by a recent duplication event. We have not obtained any evidence on the existence of an additional gene closely related to *Hahb-4* in sunflower. Another important difference between Hahb-4 and the *Arabidopsis* proteins is that while the first one is able to bind the sequence CAAT(A/T)ATTG *in vitro* (Palena *et al*. 1999), Athb-7 and -12 do not bind this or related sequences (Johannesson, Wang & Engström 2001).

The observations described above may indicate the existence of a group of related Hd-Zip I proteins involved in water stress responses. *Athb-6*, a less related *Arabidopsis* gene encoding an Hd-Zip I protein, is also induced (although less significantly) by water stress and ABA (Söderman *et al*. 1999). In addition, two Hd-Zip proteins induced by desiccation in the resurrection plant *Craterostigma plantagineum* (Frank *et al*. 1998) belong to the Hd-Zip II subclass, suggesting that different members of the Hd-Zip family have acquired different expression characteristics upon diversification. We have tested the existence of water-stress-/ABA-mediated induction of other members of the sunflower Hd-Zip family using probes comprising the Hd-Zip domain coding regions of Hahb-1 and Hahb-10. We have not obtained any evidence on a general induction of Hd-Zip protein expression by these treatments.

Intense expression of *Hahb-4* was detected only when plants were subjected to either water stress or ABA treatment. Neither cold or heat shocks, nor oxidative stress (data not shown), induced the expression. Salt and osmotic treatments produced a small increase in transcript levels. The effect of water stress was completely reversed when the plants were rehydrated. All these characteristics suggest that the expression of *Hahb-4* responds directly to the water status of plant cells, and that the induction is not an effect of general damage or stress responses. Accordingly, the hormone ABA induced *Hahb-4* expression in watered plants to levels similar to those observed under water stress conditions. ABA plays an important role in various plant developmental and physiological processes, including responses to drought and salt stress. In most cases, these processes are associated with an increase in ABA levels, which in turn produces changes in gene expression (see Skriver & Mundy 1990; Leung & Giraudat 1998 for a review). From sequence comparisons and functional analysis of the promoters of genes whose expression is induced by ABA, abscisic acid responsive elements (ABREs) have been identified (Shinozaki & Yamaguchi-Shinozaki 1997). The ABREs found in genes encoding late embriogenesis abundant (LEA) and LEA-related proteins have a core sequence (T/G/C)ACGTG to which members of a group of the b-Zip class of DNA-binding proteins bind specifically (Guiltinan, Marcotte & Quatrano 1990). Sequences bearing resemblance with the ABRE consensus have been identified in the *Hahb-4* promoter region, suggesting that regulation by ABA may act, at least in part, through interaction of nuclear proteins with this region.

Our results indicate that ABA is involved in the signal transduction pathway that induces *Hahb-4* expression in response to water stress. Since Hahb-4 is a transcription factor, we propose that it may participate in the regulation of the expression of a subset of genes involved in the response of plants to desiccation. The formation of specific complexes with nuclear proteins that bind to the Hahb-4 DNA target sequence increases upon treatment of plants with either ABA or drought conditions. Although other proteins may be involved, it is likely that Hahb-4 contributes to this behaviour. The formation of different complexes under ABA treatment or water stress may indicate that Hahb-4 interacts with different protein partners or that additional proteins that bind the same sequence are also induced by these treatments. Further studies will be necessary to elucidate this point.

Induction kinetics are sufficiently fast to support the view that Hahb-4 may function in the regulation of genes that respond more slowly to water stress and ABA treatments as, for example, those coding for dehydrins (Close 1997; Giordani *et al*. 1999). Genes that show faster responses, such as those encoding small heat shock proteins (Almoguera *et al*. 1993; Coca *et al*. 1996), must be regulated by other mechanisms.

Several *Arabidopsis* genes induced by water stress that carry the sequence bound by Hahb-4 in their promoter regions have been identified. Further studies will determine if the expression of any of them is modified by the presence of Hahb-4. These experiments will help to elucidate the complex transduction signal pathways involved in the response of plants to water stress.

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