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A Novel J8 Domain Gene, *IbJ8*, in *Ipomoea batatas* (L). Lam.

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Sweet potato cDNAs that encode IbJ8, the smallest known J-domain protein, were isolated and characterized. This genome has at least two copies of *IbJ8*, which is expressed preferentially in the leaves, flowers, petioles, and stems. Spatial and temporal patterns were studied at different developmental stages, and expression was greater in younger leaves than in older ones. Moreover, expression in roots that arose from single-leaf cuttings was lower at 15, 20, and 30 d than at 40 d, and then the signal was undetectable at 60 d after planting. These results suggest that *IbJ8* expression may be related to the organ age or developmental stage.

Keywords: J8 domain gene, sweet potato (*Ipomoea batatas* (L.) Lam.)

DnaJ was first isolated as a 41-kDa heat-shock protein from *Escherichia coli* (Zylicz et al., 1985). This protein is involved in DNA replication by stimulating the capacity of DnaK to form a replication-competent complex at the phage origin of replication. Members of the DnaJ family of molecular chaperones have been identified in a variety of organisms, including the cytosol of prokaryotes and eukaryotes, and in cellular compartments such as mitochondria, endoplasmic reticulum (Silver and Way, 1993; Cyr et al., 1994), and chloroplast stroma (Schlicher and Soll, 1997). Most soluble homologues of *E. coli* DnaJ possess several different functional domains (Cyr et al., 1992). These include a J-domain, a region rich in glycine and phenylalanine residues, a cysteine-rich zinc-finger domain, and a less conserved C-terminal region (Szabo et al., 1994; Banecki et al., 1996). In addition, a large number of proteins containing a single J-domain are combined with other domains distinct from those in *E. coli* DnaJ (Kelley, 1998). The J-domain, which is the definitive region for all DnaJ proteins, interacts with Hsp70 proteins and stimulates the Hsp70 ATPase activity necessary for stable binding of Hsp70 to its protein substrate (Bukau and Horwich, 1998; Cho and Hong, 2004; Yoon et al., 2005). Membrane-bound forms of DnaJ proteins invariably contain this functional domain as well as numerous transmembrane regions (Kelley, 1998). The J-domain may be involved in recruiting Hsp70 to interact with a specific set of substrates defined by other domains in the DnaJ protein (Kelley, 1998).

In plants, *Arabidopsis thaliana* J-proteins comprise a family that is larger and more diverse than that reported from any other organism (Caplan et al., 1993). Many AtJ proteins are Type III homologues. The only known role for protein J-domains involves interactions with the Hsp70 chaperone (Caplan et al., 1993; Laufen et al., 1999). To increase our knowledge regarding the J8 domain gene in the sweet potato, we have isolated a clone encoding that gene, and have examined the spatial and temporal expression of *IbJ8*.

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MATERIALS AND METHODS

Plant Materials

Plants of the sweet potato (*Ipomoea batatas* L. cv. Kokei 14) were grown in the field and harvested when 50-days-old to analyze the *IbJ8* gene in different organs.

Isolation of the *IbJ8* Gene from Sweet Potato

The cetyltrimethylammonium bromide (CTAB) method (Kim and Hamada, 2005) was revised in order to extract RNA from sweet potato. Specific primers (5′-AGAAGATGGCAGGAAATCAGAAG-3′ and 5′-CAATTATTTCCTCTGCAAACGTC-3′) were designed according to the J8 domain-like gene in the GenBank database (Accession number BU692118; You et al., 2003). First-strand cDNA was synthesized from tuberous roots according to the manufacturer's instructions for the SMART RACE cDNA Amplification Kit (Clontech, USA). This was amplified with templates of cDNA using a forward specific primer and an adaptor primer. The remaining 5′-sequences of the cDNAs were obtained by 5′-RACE, using the total RNA from the tuberous roots.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and Southern Hybridization

Total root RNA samples were treated extensively with RNase-free DNase I to remove any contaminating genomic DNA. First-strand cDNA was then synthesized using MMLV Reverse Transcriptase (Stratagene, USA) from 1 μg of total RNA in a 20 μL reaction volume. Then, 2 μL of the reaction mixture was subjected to PCR in a 50 μL reaction volume. *IbJ8* (5′-AGAAGATGGGAG-GAAATCAGAAG-3′ and 5′-CAATTATTTCCTCTGCAAAC-GTC-3′), and *Tublin* (5′-CAACTACCAGCCACCAACTGT-3′ and 5′-CAAGATCCTCACGAGCTTCAC-3′) were amplified with the indicated primers for 25 cycles of the following condition: 94°C for 0.5 min, 62°C for 0.5 min, and 72°C for 1 min. This was followed by a final cycle at 72°C for 5 min to allow completion of the polymerizations. A revised CTAB method (Kim and Hamada, 2005) was used to extract genomic DNA (about 10 μg), which was first restricted with *Eco*RV, *Bam*HI, *Hin*dIII, and *Kpn*I, then separated on 0.8% agarose gel, and transferred to Hybond-N$^+$ Nylon membranes (Amersham Pharmacia, UK). Filters were hybridized with *IbJ8*-specific probes labeled with DIG direct labeling reagent (Amersham Pharmacia). Hybridization, washing, and detection were performed according to the instruction manual for DIG labeling and the detection system with CDP-Star (Amersham Pharmacia).

Sequence Analysis

Plasmids were purified from selected colonies, and the full lengths of sequences for both strands of inserted DNA were determined with an ABI Prism TM 3100 genetic analyzer (PerkinElmer Applied Biosystems, USA). All sequencing data were examined using the CLUSTAL-W program (http://www.ebi.ac.uk/clustalw/), PSORT (http://wolfpsort.seq.cbrc.jp/), and ChloroP (http://www.cbs.dtu.dk/services/ChloroP/). The homology of amino acid sequences was analyzed via CLUSTAL-W against sequences in the GenBank and EMBL DNA databases.

RESULTS AND DISCUSSION

Isolation of the J8 Domain Gene from Sweet Potato

Total RNA was extracted from sweet potato tuberous roots to isolate the J8 domain gene. This RNA was subjected to 3′-RACE using the J8 domain forward specific

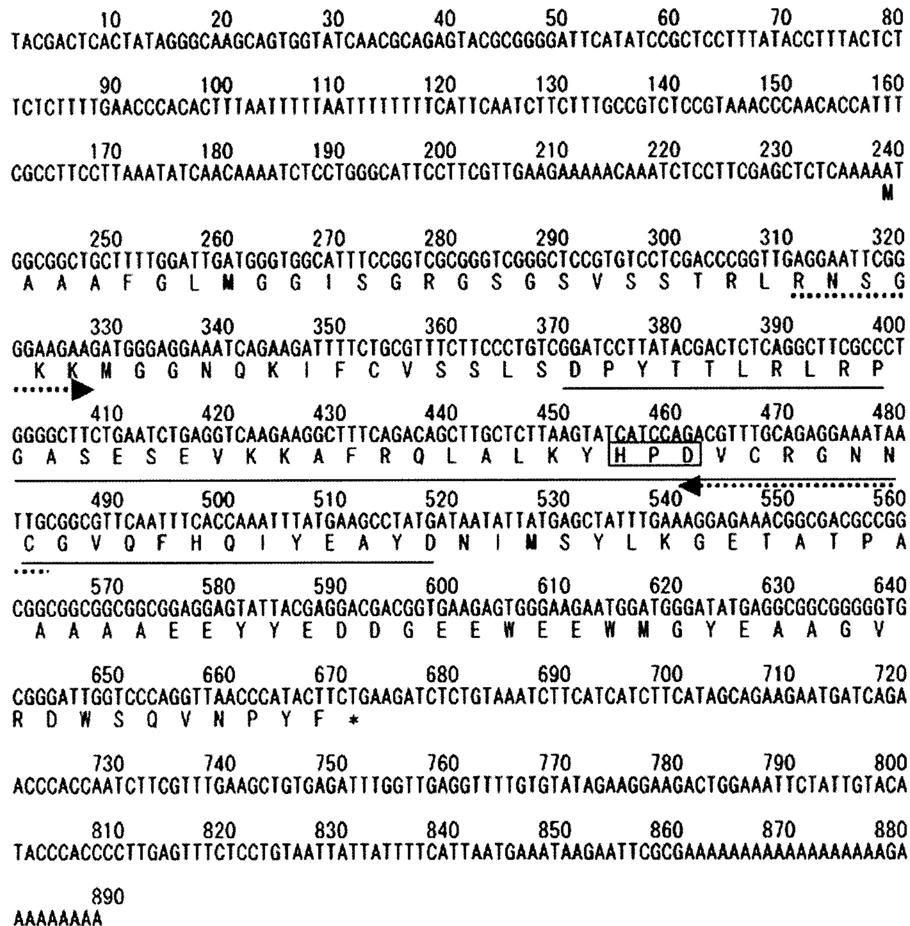

Figure 1. *IbJ8* nucleotide and deduced amino acid sequences. J-domain is underlined, and HPD motif is boxed. RT-PCR primer site is indicated by dotted line.

A

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IbJ8  MAAAFGLMG-GISGR-GS---GSVSSTRLRNSGKK---MGGNQKIFCVSS--LSDPYTTL 50
AtJ8  MTIALTIGGNGFSGLPGSSFSSSSSSFRLKNSRRKNTKMLNRSKVVCSSSSSVMDPYKTL 60
      *: *: : * *:**  **   .* ** **:** :*   * ...*:.* **  : ***.**

IbJ8  RLRPGASESEVKKAFRQLALKYHPDVCRGNNCGVQFHQIYEAYDNIMSYLKGETATPAAA 110
AtJ8  KIRPDSSEYEVKKAFRQLAKKYHPDVCRGSNCGVQFQTINEAYDIVLKQIKNQMEGTEEF 120
      ::**.:** ********** *********.******: * **** ::. :*.:    .

IbJ8  AAEEYYED--------DGEEWEEWMGYEAAGVRDWS-QVNPYF 144
AtJ8  EPFDVYDEGLNGMNDPDCDTWEEWMGWEGAGTRDYSSHVNPYA 163
       . : *::        * : *****:*.**.**:* :****
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B

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1 -DPYTTLRLRPGASESEVKKAFRQLALKYHPDVCRG-NNCGVQFHQIYEAYD------
2 -DYYEILGVSKTAEEREIRKAYKRLAMKYHPDRNQGDKEAEAKFKEIKEAYEVLTDSQ
3 MDPYKTLKIRPDSSEYEVKKAFRQLAKKYHPDVCRG-SNCGVQFQTINEAYDIVLKQL
   *  *  * :   :   *::**: : * . ***        .    *: : :* :
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Figure 2. Comparison of IbJ8 amino acid sequences. (**A**) Alignment of *IbJ8* with *AtJ8* (GenBank accession number AAC72399). (**B**) Alignment of regions with sequence homology between *AtJ8* and *IbJ8* domain proteins: (1) *IbJ8* (BU692118), (2) *E. coli* DnaJ (M12565), and (3) *A. thaliana AtJ8* (AAC72399). HPD motif is boxed.

primer, following the asymmetric amplification of first-strand cDNA (synthesized from the poly-A tail). A 562-bp band was subcloned and found to contain, almost exclusively, a J8 domain cDNA fragment. This cDNA fragment was identified as being similar to known J8 domain genes in other plant species. The remaining 5´-sequences of the cDNAs were obtained by 5´-RACE from the total RNA of the tuberous roots.

Sequence Characterization of *IbJ8*

The 888-bp cDNA was completely sequenced (Fig. 1), the clone of which comprised 238 b of 5´-UTR and 214 b of 3´-UTR. The cDNA reading frame encoded a polypeptide of 144 amino acids. Amino acid residues 45 through 94 constituted the highly conserved J-domain, and included the His-Pro-Asp (HPD) tripeptide (Fig. 1, 2B). This has been shown to be necessary for regulating the ATPase activity of Hsp70 proteins (Brodsky, 1996). The J-domain of *IbJ8*, which is highly conserved in the *Arabidopsis* protein, contained 50 amino acid residues after the J-domain; this region shared less similarity with *AtJ8* (Fig. 2A), suggesting it might have other important functions. However, whether this region is large enough to provide the necessary specificity for the interaction of substrate proteins with Hsp70 remains to be determined.

A comparison with *E. coli* DnaJ (Zylicz et al., 1985; Ohki et al., 1986) showed that *IbJ8* had identical residues at 30% of the positions over the J-domain. Using conservative amino acid replacements, this similarity was calculated to be 53% (Fig. 2B).

For the *Arabidopsis J8* gene, which is localized in the plastid, the J-domain is preceded by a transit peptide (Kroczynska et al., 2000). Therefore, we analyzed the sequence of our *IbJ8* gene for clues regarding its cellular function. Two computer algorithms were employed to search for sequence motifs involved in protein interactions. The prediction that the N-terminal region was a chloroplast transit peptide was examined using PSORT and ChloroP. These analyses predicted, with >89% probability, that IbJ8 was localized in the chloroplasts. A transit peptide of 63 amino acid residues also was predicted on the basis of a cleavage site between Pro-63 and Gly-64. However, this would appear unlikely because it was within the conserved J-domain region. Thus, the small size of the IbJ8 protein and the lack of any functionally identifiable regions flanking the J-domain make predictions of its function difficult.

Southern Blot and RT-PCR Analyses of *IbJ8*

Southern blot analyses under high stringent conditions against sweet potato genomic DNA revealed more than two bands when digested with *Bam*HI, *Eco*RV, *Hin*dIII, and *Kpn*I (Fig. 3). The number of hybridizing bands indicates the presence of at least two copies of *IbJ8* in the sweet potato genome, but also suggests that more copies of the J8 domain may exist in this hexaploid plant. For now, it is unclear whether our finding is due to the hexaploid and heterozygous nature of the sweet potato.

You et al. (2003) previously reported high expression of *IbJ8* in the tuberous roots of the sweet potato. Our RT-PCR analyses were performed using cDNA from green and non-green tissues to examine whether the gene was expressed throughout the plant, rather than being restricted to the green portions. *IbJ8* was weakly expressed in the leaf, but transcript levels were greater in the stem and highest in the flower and petiole.

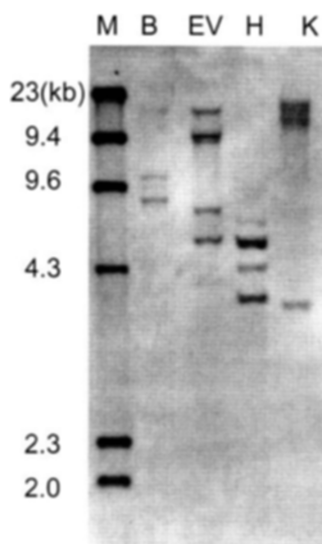

Figure 3. Southern hybridization analysis of *IbJ8*. Lane M, molecular size marker (λ/*Hin*dIII). Genomic DNA (10 μg) was digested with *Bam*HI (B), *Eco*RV (EV), *Hin*dIII (H), and *Kpn*I (K). Membrane was hybridized with DIG-labeled *IbJ8*-specific probe containing *Eco*RI-digested fragments spanning about 62 amino acids of C-terminus plus 3′ untranslated region.

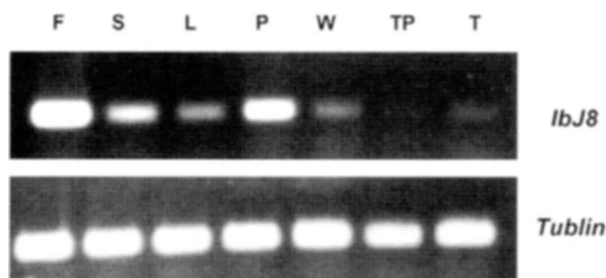

Figure 4. Expression analysis of *IbJ8*. RT-PCRs were performed with equal amounts of cDNA from flowers (F), stems (S), leaves (L), petioles (P), white fibrous roots (W), thick pigmented roots (TP), and tuberous roots (T). *Tublin* was used as control.

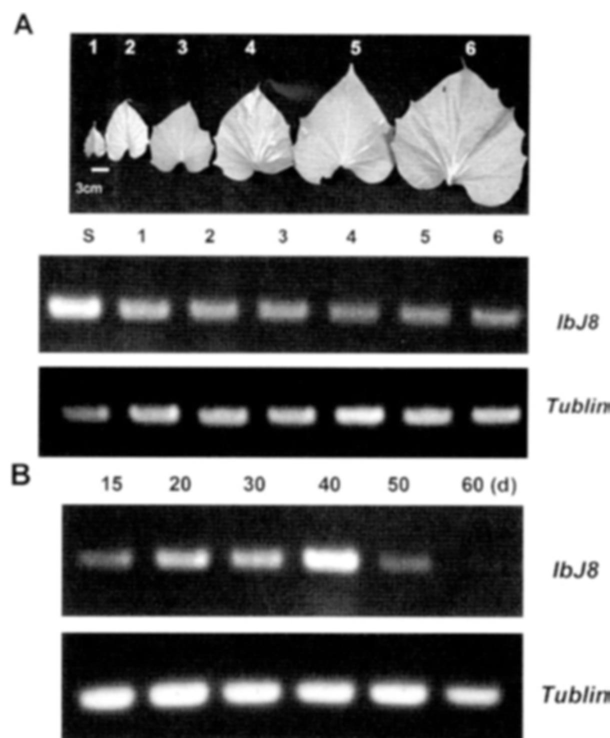

Figure 5. Expression patterns of *IbJ8*: (**A**) for each size of sweet potato leaf, from approx. Sixty-day-old plants grown in field; and (**B**) in total root tissues. Total RNA was isolated from each developmental stage. S, shoot apical meristems. *Tublin* was used as control.

However, in contrast to You et al. (2003), *IbJ8* mRNA here was also weakly detected in our root tissues, i.e., in the white fibrous roots, thick-pigmented roots, and tubers (Fig. 4). Therefore, these current results contradict those reported earlier. Because this gene was detected in all plant tissues examined, we can hypothesize that its functioning might not be limited to the chloroplasts. As such, it is likely to be required for all plastid types. Similarly, the *Bsd2* gene is expressed in both bundle sheaths and mesophyll cells, although Rubisco accumulates only in the former cell type (Waegemann et al., 1990; Brutnell et al., 1999). This perhaps suggests a wider function for this protein.

Spatial and Temporal Expression Patterns of *IbJ8*

We performed RNA gel blot analyses to examine the accumulation profile of *IbJ8*. Here, transcripts were localized in the shoot tissues, including the leaves. If this gene does have a direct role in leaf development, one might expect it to manifest a different expression profile based on leaf size. Therefore, to examine this possibility, we compared accumulation patterns for *IbJ8* transcripts, and found that a developmental gradient existed in which younger leaves near the shoot tips differentiated later than older leaves located near the shoot base. Transcript levels also were tightly coordinated with this gradient, with levels declining near the basal leaf and peaking toward the tip (Fig. 5A). Interestingly, this profile of *IbJ8* transcript accumulation was more similar to that of the Bsd2 protein, which also gradually increases from the base to the tip of the leaf (Brutnell et al., 1999).

By using a single-rooted-leaf method to profile *IbJ8*, we were able to more precisely elucidate the relationship between root development and gene expression (Kim et al., 2002a). Our sweet potato roots stopped elongating and began to thicken about 40 d after planting. Total root dry weight increased rapidly, to about five times the initial value (Kim et al., 2002b). This indicated a close relationship between starch accumulation and cell proliferation for tuber bulking during root development ≥40 d after planting. *IbJ8* expression was weak from Day 15 to 40 (Fig. 5B). Expression levels of this particular mRNA may be related to age and developmental stage. In root tissues

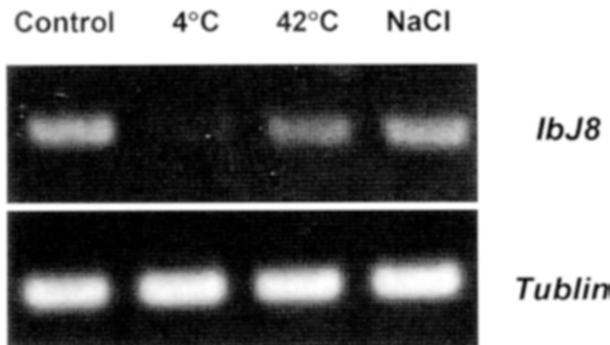

Figure 6. Expression patterns and RT-PCR analyses of *IbJ8* expression in 4-week-old plants subjected to salt (3 h at 300 mM NaCl), or high- (6 h at 42°C) or low- (6 h at 4°C) temperature stresses. Only leaf tissues were collected. Tublin was used as control.

at 15, 20, and 30 d post-planting, the amount of PCR product generally seemed lower than at Day 40. However, the signal was not detectable 60 d after planting, again suggesting that expression may be related to age or developmental stage.

DnaJ, a heat-shock protein usually expressed at low levels during normal growth, is greatly induced following heat stress (Schroder et al., 1993). Many eukaryotic DnaJ homologues are constitutively expressed, with less than a 10-fold increase in response to thermal stress (Georgopoulos and Welch, 1993). Although our RT-PCR analyses involved plants exposed to moderate heat shock (6 h at 42°C) or NaCl stress (3 h at 300 mM), no significant difference was found between them and the control plants (Fig. 6). However, the *IbJ8* gene seemed to be negatively regulated by a low temperature (6 h at 4°C; Fig. 6), suggesting that it may have an important function in the stress response. In other research, expression of a *Phaseolus vulgaris* gene that encodes a J-domain has been shown to be regulated by heavy-metal stress, virus infection, and wounding treatments (Chai and Burkard, 1996). Unfortunately, responses to other stresses have not been reported.

In conclusion, we have isolated the *IbJ8* gene and demonstrated its expression pattern during leaf and root development. Further studies on the regulation of *IbJ8* gene expression in transgenic plants are required.

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