Journal of Experimental Botany, Vol. 57, No. 6, pp. 1391–1398, 2006
doi:10.1093/jxb/erj118 Advance Access publication 10 March, 2006
This paper is available online free of all access charges (see http://jxb.oxfordjournals.org/open_access.html for further details)

Journal of Experimental Botany

RESEARCH PAPER

A root-specific bZIP transcription factor is responsive to water deficit stress in tepary bean (*Phaseolus acutifolius*) and common bean (*P. vulgaris*)

Laura Rodriguez-Uribe and Mary A. O'Connell*

Department of Agronomy and Horticulture, New Mexico State University, Las Cruces, NM 88003, USA

Received 6 July 2005; Accepted 11 January 2006

Abstract

Root cDNA libraries were differentially screened to isolate water deficit-responsive transcripts in the relatively drought-resistant plant tepary bean (*Phaseolus acutifolius*). A novel root-specific, water deficit-responsive transcript was identified and predicted to encode a bZIP transcription factor. The orthologous form of this gene was isolated from the drought-sensitive *P. vulgaris* and the patterns of expression of these genes compared. These genes have predicted amino acid sequences in the bZIP domain that are 64% similar to a soybean bZIP protein. There were three amino acid differences between the *P. acutifolius* bZIP and the *P. vulgaris* gene product. Both species transcribed this gene in a root-specific and water deficit-responsive manner. The cell-specific pattern of expression for the gene was determined using *in situ* hybridization and immunolocalization. Two tissues in the root accumulated the protein: epidermis and phloem. The nuclear localization of this protein was determined by electron microscopy. The bZIP protein accumulated in the nuclei of both the epidermal cell and the vascular cell in response to water deficit stress in both species in a similar manner.

Key words: Chromatin, drought resistance, immunolocalization, *in situ* hybridization, *Phaseolus acutifolius*, *Phaseolus vulgaris*, TEM.

Introduction

Major droughts and decreased availability of water throughout the world, is one of the most significant abiotic stresses for plants. Drought stress affects plant growth and development, as well as yield (Boyer, 1982; Tabaeizadeh, 1998; Neill and Burnett, 1999) and makes plants more susceptible to other kind of stresses by lowering the adequacy of the defensive mechanisms (English-Loeb, 1990). To overcome these stresses, plants develop adaptive strategies at morphological, physiological, cellular, and metabolic levels, which allow them to survive and succeed. These adaptive responses are modulated by sets of specific genes whose products allow plants to avoid the stress or become tolerant.

Drought-tolerant plants constitute a good source of genetic traits to improve agronomically important plants such as the legume *P. vulgaris* (common bean). Common bean is the most important food legume grown worldwide (Ramirez-Vallejo and Kelly, 1998). A major constraint to bean production in many developing countries is drought; for instance, 73% of the area planted to beans in Latin America is semi-arid (Laing *et al*., 1984). There is some variability within *P. vulgaris* for drought resistance (Terán and Singh, 2002); however, the best example of a legume adapted to grow in arid conditions is tepary bean, *P. acutifolius*. This annual is more heat and drought tolerant than common bean, produces seeds of high protein quality suitable for human consumption (Miklas *et al*., 1994), and has resistance to bacterial diseases (Garvin and Weeden, 1994). The physiological and biochemical basis for the relative drought tolerance of *P. acutifolius* has been investigated with respect to proline accumulation (Lazcano-Ferrat and Lovatt, 1999) and antioxidant biosynthesis (Türkan *et al*., 2005). However, there have been no studies on the comparative responses of *P. acutifolius* and *P. vulgaris* to drought stress at a molecular genetics level. Graft experiments among cultivars of *P. vulgaris* linked seed yields to specific root genotypes during drought stress (White and Castillo, 1989). Roots are usually the plant organs where signals for changes in water availability are

* To whom corresponding should be addressed. E-mail: moconnel@nmsu.edu

© The Author [2006]. Published by Oxford University Press [on behalf of the Society for Experimental Biology]. All rights reserved.
The online version of this article has been published under an Open Access model. Users are entitled to use, reproduce, disseminate, or display the Open Access version of this article for non-commercial purposes provided that: the original authorship is properly and fully attributed; the Journal and the Society for Experimental Biology are attributed as the original place of publication with the correct citation details given; if an article is subsequently reproduced or disseminated not in its entirety but only in part or as a derivative work this must be clearly indicated. For commercial re-use, please contact: journals.permissions@oxfordjournals.org

first perceived (Neill and Burnett, 1999). Investigations of water deficit-stress responses in roots of *P. acutifolius* and *P. vulgaris* are expected to detect stress-adaptive responses.

The list of drought-responsive genes grows bigger and bigger every year and at present there are a number of reviews describing these genes (O'Connell, 1995; Harrak *et al*., 1999; Neill and Burnett, 1999; Uno *et al*., 2000; Ndima *et al*., 2001; Bray, 2002). Drought-responsive genes have been grouped into different classes according to their possible function; for examples, genes whose products will be involved in regulation of gene expression, metabolism, ion sequestration, protection, and osmolyte synthesis have been identified (Bray, 1993). However, there are also a number of drought-responsive genes whose sequences have no similarities to other genes.

Transcription factors play crucial roles in almost all biological processes. In plants, basic region/leucine zipper motif (bZIP) transcription factors regulate processes including pathogen defence, signalling, seed maturation, and flower development (Jakoby *et al*., 2002). Gene transcription levels for proteins involved in signal transduction pathways such as transcription factors are altered by drought stress (Neill and Burnett, 1999; Uno *et al*., 2000; Bray, 2002). Studies on the role of abscisic acid in the dehydration response in vegetative tissues of *Arabidopsis* and barley have shown that this response is mediated by bZIP transcription factors (Uno *et al*., 2000; Xue and Loveridge, 2003).

This study is a report on the isolation and characterization of a water deficit-responsive bZIP transcription factor. The genes for this transcription factor were isolated from two species, *Phaseolus acutifolius* and *P. vulgaris*. The expression was characterized at the RNA and protein levels and the sites of accumulation were determined. These studies were undertaken to identify the genetic basis for the drought-resistance phenotype of *Phaseolus acutifolius*.

Materials and methods

Plant materials and growth conditions

Seeds from *Phaseolus acutifolius*, O'Odham Brown variety (Native Seeds, Schomberg, Ontario, Canada) and *P. vulgaris*, cv. Negro Jamapa (Dr Miguel Lara, UNAM) were germinated and grown in 10 inch pots, in a mix of three equal parts of perlite, Metro-Mix 360, and vermiculite, with an average day/night temperature of 29/22 °C in a greenhouse at New Mexico State University. Osmocote 14-14-14 was added as fertilizer and the plants were watered every other day up to early flowering stage. These plants were then used for water deficit-stress treatments.

The water-deficit experiments consisted of two sets of plants. One set was watered every other day while water was withheld from the other set of plants. Leaf water potential measurements were taken on both sets of plants using a Scholander pressure bomb at daily or alternate-day intervals. In multiple experiments, leaf water potentials in *P. vulgaris* reached stressed levels (−2.1 to −2.4 mPa) ~5 d after water was withheld, while *P. acutifolius* plants did not reach these leaf water potentials until 10 d after irrigation was withheld. Root tissues (from the apical meristem to the elongation zone) were harvested from both sets of plants at the indicated number of days after initiation of water stress. Samples were immediately frozen in liquid nitrogen and kept at −80 °C until total RNA was isolated. For cold-stress experiments plants were placed in the cold room at 4 °C for 24 h or 48 h, and then root samples were collected.

Nucleic acid isolation and analysis

Genomic DNA and RNA were isolated as described by Aluru *et al*. (2003). Southern blots were generated using standard methods for alkaline transfer onto nylon membranes, and then neutralized in 0.5 M Na_2HPO_4 pH 7.2; RNA blots were prepared using 10× SSC for transfer. Radiolabelled probes were synthesized by oligolabelling; the probe was boiled and added to the prehybridization solution (1% BSA, 1 mM EDTA, 0.5 M Na_2HPO_4 pH 7.2, 7% SDS) in the bag with the membrane. Blots were hybridized for 18 h and then washed in several changes of 1 mM EDTA, 40 mM Na_2HPO_4 pH 7.2, 1% SDS. The first wash also contained 3% fish gelatine and 5% SDS. The hybridizations and washes were at 65 °C. The X-ray films from the northern blots were scanned to quantify the signal of each gene. The hybridization signals were normalized using scans of the RNA detected in each lane stained with ethidium bromide.

Construction and screening of cDNA libraries

Poly(A)$^+$ mRNA was purified from total RNA by one round of oligo(dT) cellulose selection with the Ambion Micro Poly(A) Pure™ as described in the manufacturer's manual; this mRNA (6 μg) was then used to synthesize cDNA. The cDNAs from each species were fractionated in a Sepharose CL-2B column. cDNAs ranging from 0.9 to 4.0 kb were used to construct each cDNA library in UNI-Zap XR (Stratagene). The cDNA library was plated at 50 000 pfu per plate. Plates were incubated at 37 °C from 7 h to overnight until plaques were visible. Nylon membrane replica filters were hybridized with [^{32}P]dCTP-labelled cDNA from either water deficit-stressed or non-stressed plants. The plaques that hybridized strongly to cDNA from water deficit-stressed or control plants were purified.

DNA sequencing and analysis

Plasmid templates were sequenced at the New Mexico State University DNA sequencing facility using dideoxy termination sequencing methods with the products resolved on a Li-Cor DNA sequencer. DNA sequence files were edited and aligned using DNAstar or Sequencher software. Sequences were aligned to the sequences in the non-redundant GeneBank database with the BLAST X algorithm version 2.0.12 (Altschul *et al*., 1997).

Expression of the P. acutifolius *bZIP in* Escherichia coli

The Affinity™ LIC cloning (Stratagene) was used to express the bZIP transcription factor. Primers were designed with the nucleotide sequence of the bZIP open reading frame (ORF) and included the pCAL-n-FLAG vector specific sequences (12- and 13-nt) added to the 5′ end. The forward primer (bZIPEXPF: GACGACGACAAGA**ATG**GTTCCTAGTGAGATAAGA) included the start codon (ATG) of the ORF, and the reverse primer (bZIPEXPR: GGAACAAGACCCGTTC**ATT**CATGAATCATGTCTGC) included the stop codon (ATT). The PCR-amplified product, a single band of 665 bp, was purified, treated with Pfu DNA polymerase, ligated into the pCAL-n-FLAG vector and transformed into SoloPack Gold Supercompetent cells (Stratagene). After DNA sequence confirmation, this plasmid DNA was used to transform competent cells from the expression strain BL21-CodonPlus™ (Stratagene).

Cell cultures [BL21-Codon-Plus (DE3)-RIL transformants] were grown at 37 °C to 0.5–0.7 OD (595 nm). The cultures were cooled

on iced water to ~16 °C, induced with 0.3 mM IPTG and incubated at 16 °C overnight. Cells were harvested by centrifugation and proteins were extracted in $CaCl_2$ binding buffer (50 mM TRIS-HCl pH 8.0, 150 mM NaCl, 10 mM β-mercaptoethanol, 2 mM $CaCl_2$, 1 mM phenylmethylsulphonyl fluoride as described in the manufacturer's manual. Purification of the recombinant 30 kDa protein was done by passing the cell lysates through a calmodulin affinity resin column as described by the manufacturer. The CBP-tag of the fusion protein was removed with thrombin after dialysis of the protein extracts in cleavage buffer [20 mM TRIS-HCl (pH 8.4), 150 mM NaCl, 2.5 mM $CaCl_2$] overnight at 4 °C with stirring. Samples were analysed on 15% (w/v) SDS-PAGE. Cleaved protein (1.2 mg) was cut out from the gel and used for the production of polyclonal anti-bZIP antibodies. Protein concentrations were estimated with the Bio-Rad protein assay reagent. Polyclonal antibodies against the *P. acutifolius* bZIP protein were generated in rabbits by a commercial firm (Robert Sargeant, Ramona, CA, USA).

Protein analysis and western blots

Cell lysates or protein samples were mixed with an equal volume of 2× SDS gel sample buffer [100 mM TRIS-HCl (pH 6.5), 4% (v/v) SDS, 0.2% (w/v) bromophenol blue, 20% glycerol (v/v)]. The samples were then heated at 95 °C for 5 min and centrifuged. Proteins were separated by gel electrophoresis in a 15% (v/v) acrylamide/BIS gel and detected by Coomassie Brilliant Blue staining.

Western blots were prepared and detected essentially as described earlier (Aluru *et al*., 2003). Membranes were incubated with the primary anti-bZIP antibody diluted 1:2500 (v/v) in TBST [10 mM TRIS-HCl pH 8.0, 150 mM NaCl, and 0.1% (v/v) Tween 20] overnight at room temperature with gentle shaking, and washed three times with TBST for 5 min each. The antibody–antigen complexes were detected using goat anti-rabbit antibody tagged with alkaline phosphatase.

Histology

Sections (8 μm thick) were cut on a manual microtome and transferred to polylysine-coated slides. The slides were then incubated in an oven at a temperature of 55 °C for 15 min to ensure proper binding of the tissues to the slide. The paraplast was removed from the tissues with xylene; the samples were rehydrated and stained with safranin to check tissue integrity using a Leitz optical microscope.

For TEM specimens, root tissues were cut into ~1 mm pieces and processed for embedding in Spur's resin as described by Ghoshroy and Citovsky (1998). Ultrathin sections (50–70 nm) were made with the Sorval MT-2 microtome, and transferred to coated nickel 200-mesh grids.

Immunohistochemical localization

Root tissues (8 μm sections) from control and water deficit-stressed plants were treated with the anti-bZIP polyclonal antibody diluted 1:2000 (v/v) and then with the biotinylated secondary antibody as described in the VIP Substrate Kit for Peroxidase (VECTOR®). For subcellular immunolocalizations, ultrathin sections were incubated with the primary anti-bZIP antibody diluted 1:1000 and then incubated with the secondary antibody coupled to 12 nm colloidal gold particles diluted 1:50. The grids were washed and incubated in 5% (w/v) uranyl acetate and post-stained with $Pb(OH)_2$. These sections were analysed on a Hitachi H-7000 TEM, at medium (×12 000–15 000) and high magnification (×30 000). To evaluate labelling intensity, seven micrographs of each sample were visually inspected and the number of gold particles was counted in root cell and subcellular sites.

In situ *mRNA hybridization*

Root tissues for *in situ* hybridization from control and water deficit-stressed plants were harvested, washed with DEPC-treated sterile water once, and immediately placed in fixation buffer [0.1 M phosphate buffer pH 7.2, 4% (v/v) paraformaldehyde] for 18 h at 4 °C. The fixed tissues were extensively washed with 0.1 M Na_2HPO_4 pH 7.2. Dehydration and infiltration with Paraplast Plus™ was done as described by Maliga *et al.* (1995). Deparafinized root sections were pretreated with 1 μg ml^{-1} proteinase K in 100 mM TRIS-HCl, pH 8.0 and 50 mM EDTA pH 8.0 at 37 °C for 30 min, postfixed in 4% paraformaldehyde for 30 min, rinsed, and dehydrated in ethanol. Digoxigenin-labelled sense and antisense riboprobes were generated with linear plasmid according to the manual of the DIG RNA labelling kit (Roche, Basel). The riboprobes were hydrolysed into fragments of about 150 nucleotides and used at a final concentration of 100 ng ml^{-1}. Hybridization with sense and antisense riboprobes, washes, and detection were carried out as described by Wilkinson (1992).

Results

Isolation and characterization of the bZIP *gene from* Phaseolus *species*

A cDNA library of transcripts from roots of water deficit-stressed *P. acutifolius* (tepary) was differentially screened with cDNAs from roots of well-watered and water deficit-stressed tepary plants. Approximately 40 clones were identified in this screen as very abundant in water deficit-stressed transcripts and not detected in well-watered transcripts. The clone for the *bZIP* gene was one of these differentially expressed clones, the recombinant phage had an insert of 1100 bp. DNA sequence analysis of this clone indicated a full-length ORF with sequence similarity to bZIP transcription factors. The cDNA had a 5′ untranslated region (UTR) of 52 bp and an ORF of 600 bp. The basic domain was composed of 11 basic amino acids (eight R and three K), indicated by the double underline in Fig. 1A. The leucine zipper domain was composed of the following amino acids L L L M V L L, underlined and in bold. These seven amino acids, five leucines, one methionine, and one valine, were spaced with exactly seven amino acids between each another. A transactivating proline-rich domain was also found at the amino-terminus; there were 10 proline residues within the first 58 amino acids.

The *P. vulgaris* bZIP transcription factor was isolated using the clone from the tepary library as a probe to screen root cDNA library from water deficit-stressed *P. vulgaris* Negro Jamapa. A single cDNA coding for this protein, with a size of 800 bp, was isolated. Sequencing results from this cDNA showed that this was a full-length cDNA, with a 5′ UTR of 52 bp, an ORF of 600 bp, and a 3′ UTR of 157 bp, plus the poly(A)$^+$ tail. The basic domain and the leucine zippers were found in the Negro Jamapa amino acid sequence (Fig. 1A). Results from the alignment of the nucleotide sequences from the *P. acutifolius* and *P. vulgaris* bZIPs revealed a 98% similarity between both bZIPs and the amino acid sequences were 97% identical.

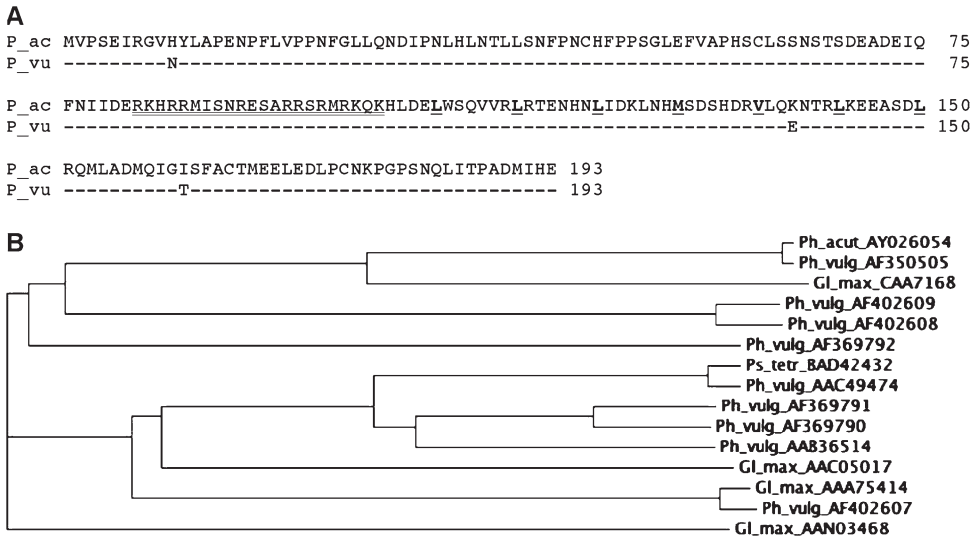

Fig. 1. Comparison of amino acid sequences of bZIP transcription factors in Phaseolideae. (A) Amino acid sequence of root-specific bZIPs from *P. acutifolius* (P_ac) and *P. vulgaris* (P_vu). The basic domain is identified as the double-underlined sequence; amino acids in the leucine zipper motif are in bold and underlined. The amino acid identities between *P. acutifolius* and *P. vulagris* are indicated by dashes. (B) Cladogram based on the bZIP domains from 15 Phaseolideae proteins aligned by Clustal W1.82; the tree was generated by the Neighbor–Joining method. Root-specific bZIP from *P. acutifolius* Ph_acut_AY026054; from *P. vulgaris*, Ph_vulg_AF350505.

Both nucleotide sequences and their corresponding amino acid sequences were submitted to the GenBank database, *P. acutifolius* bZIP transcription factor accession number AY026054 and *P. vulgaris* accession number AF350505.

Multiple sequence alignment of the bZIP domain with 15 other bZIPs in Phaseolideae generated the cladogram presented in Fig. 1B. There was 48% amino acid identity and 64% similarity between the tepary bZIP and the closest protein, soybean bZIP CAA7168. The bZIP proteins from the different taxa were distributed throughout the cladogram. Class I and II bZIP proteins, as represented by Ph_vulg_AF402607 and Ph_vulg_AF402608, respectively, were well resolved into different clades. The two bZIP proteins presented here, as water deficit-responsive and root specific, clustered with class II bZIP proteins.

The P. acutifolius *and* P. vulgaris *bZIP transcription factors belong to a small gene family*

Southern blots with DNA from tepary leaves restricted with *Bam*HI, *Eco*RI, *Xba*I, *Xho*I, or *Hin*dIII, were hybridized to the *P. acutifolius* bZIP complete cDNA (1100 bp). The X-ray films of the hybridized membranes showed many bands hybridizing to the probe. Another Southern blot was made with DNA restricted either with the same restriction enzymes and hybridized to the ORF from the tepary bZIP transcription factor amplified by PCR with *bZIP* gene-specific primers. Results from these Southern blots showed that two bands hybridized in all the different restrictions (Fig. 2). For *P. vulgaris*, there was a strong

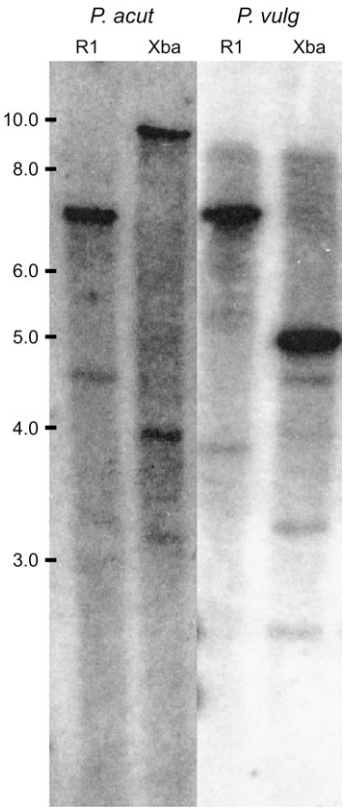

Fig. 2. Genomic Southern analysis of the *bZIP* gene family. DNA (20 mg) from *P. acutifolius* (*P. acut*) or *P. vulgaris* (*P. vulg*) leaves was digested with *Eco*R1 (R1) or *Xba*I (Xba), electrophoresed on 0.8% agarose gel, blotted to nylon membranes, and probed with the coding region of bZIP transcription factor. The positions of DNA markers in kilobases are on the left.

band and two minor bands hybridizing to the bZIP transcription factor. The results from Southern blot analysis in both plant species indicated that this bZIP transcription factor was a member of a small gene family.

Expression of transcripts for bZIP transcription factor

Northern blots with RNA from roots, stems, and leaves from control and water deficit-stressed *P. acutifolius* and *P. vulgaris* plants were hybridized with the cDNA clone for bZIP. In both species the transcripts for this gene were only detected in roots (Fig. 3). For both species, there was a relatively rapid increase in accumulation of the transcripts for bZIP in response to water deficit. Within the first few days of withholding water, 2 d for *P. acutifolius* and 1 d for *P. vulgaris*, there were noticeable increases in the transcript abundances for this gene. The elevated transcript levels persisted throughout the water deficit-stress period in both plants. *Phaseolus acutifolius* was much more drought-tolerant than *P. vulgaris*, hence the duration of the water deficit-stress period in this plant was twice as long, 10 d versus 5 d. Both plants had similar leaf water potentials at 5 d and 10 d, −2.5 MPa. The relative abundance of the bZIP transcript in the water deficit-stressed plants was quantified by scanning the autoradiographs and normalizing the signal by the abundance of the ribosomal RNA. For both species, bZIP transcript increased 6-fold in the roots in the water deficit-stressed plant relative to the well-watered plant.

The expression of these genes was also studied in response to cold stress. Northern blots with total RNA (10 μg) from roots of control and 24 h cold (4 °C)-stressed plants were hybridized with the cDNA form of the bZIP. The mRNA accumulation of the bZIP transcription factor was increased in response to cold stress ~4.6 fold (data not shown).

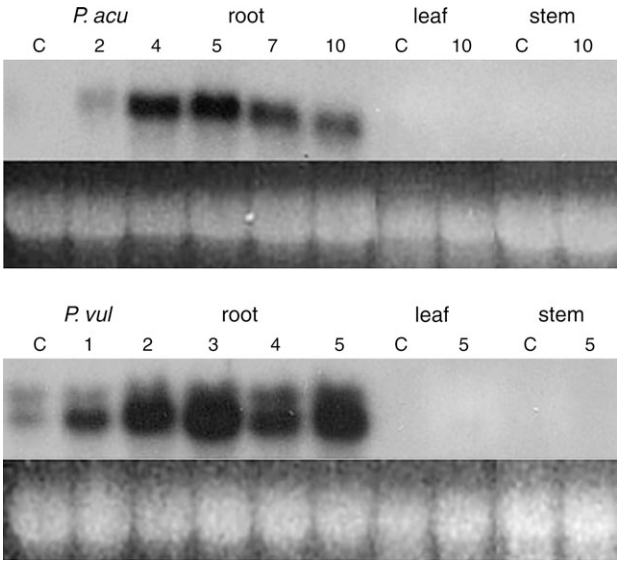

Fig. 3. Transcript accumulation of the bZIP transcription factor. RNA was isolated from roots, leaves or stems of either control or water deficit-stressed *P. acutifolius* (*P. acu*) or *P. vulgaris* (*P. vul*) plants. The samples were collected from well-watered plants (C), or after the indicated number of days of water deficit stress. Following electrophoresis, total RNA (10 mg) was blotted and hybridized to the bZIP transcription factor. The EtBr-stained image is presented in the lower panels.

In situ hybridizations were performed to determine which tissues in the root accumulated transcripts for this bZIP transcription factor. Signal was only detected when the anti-sense probe was used with root sections from water deficit-stressed plants (Fig. 4A). Transcripts accumulated in cells in the epidermal layer and the vascular tissue (arrows, Fig. 4A, G). No signal was detected when the sense form of the probe was used (Fig. 4C) or when roots from well-watered plants were hybridized with the anti-sense form of the probe (Fig. 5B). These results confirm the pattern of transcript abundance observed on the northern blots (Fig. 3).

Immunohistochemical localization of the P. actutifolius *bZIP transcription factor*

Immunoblotting was performed with extracts from roots of control and water deficit-stressed plants. The anti-bZIP antibodies reacted with a 30 kDa protein whose expression was shown to be induced by IPTG (Fig. 5). The anti-bZIP antibodies also reacted with a protein in the root tissues with a molecular weight of about 30 kDa. The abundance of this protein was increased in roots from water deficit-stressed plants. Western blots with proteins extracted from leaves, flowers, and stems of control or water deficit-stressed plants had no detectable levels of bZIP protein (Fig. 5).

The immunohistochemical localization of the bZIP transcription factor was carried out in 8-μm-thick cross-sections of Paraplast Plus-embedded roots from control and water deficit-stressed plants from *P. acutifolius*. The primary anti-bZIP antibody was diluted 1:2500. The accumulation of the bZIP transcription factor was observed in the epidermis and vascular bundles of water deficit-stressed roots. The accumulation was more intense in the vascular tissues, just between the xylem elements and in the cells of the phloem from roots of water deficit-stressed plants (Fig. 4D, F). No signal was observed in the immunohistochemical analysis when the pre-immnune serum was used or in sections treated only with the secondary antibody (data not shown).

Subcellular localization of the bZIP transcription factor

Root cross-sections (0.5–1.0 μm) from the resin-embedded (Spurr's firm) roots were made and stained with 1% toluidine blue to check for tissue integrity after fixation and embedding. The well-preserved tissues were used for ultrathin sections that were subsequently stained with the antibody against bZIP and detected by staining with secondary antibody coupled to colloidal gold 12 nm

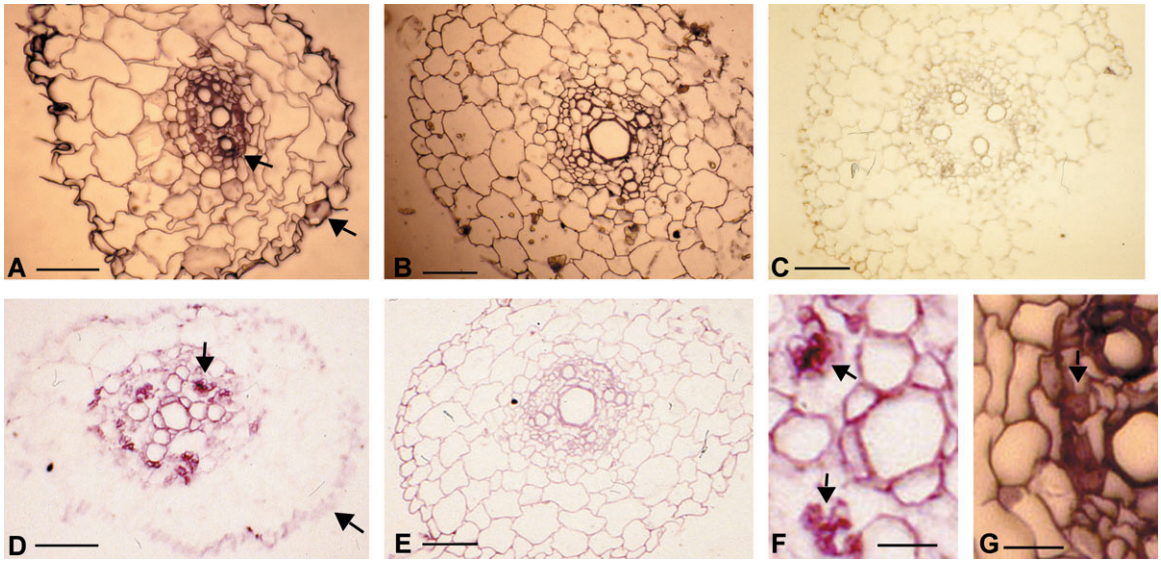

Fig. 4. Cellular localization of bZIP transcription factor in roots. *In situ* hybridization localization of the bZIP transcription factor mRNA in roots of *P. acutifolius* was performed with the bZIP antisense probe in cross-sections from a root of a water deficit-stressed (A), or control (B) plant. Panel (C) demonstrates the signal when the sense probe of the bZIP clone was used. Immunohistochemical detection of the bZIP transcription factor in cross-sections of roots from water deficit-stressed (D) or control (E) *P. acutifolius*. Sections (8 μm) were stained with the bZIP antibody and then detected by alkaline phosphatase. Photographic enlargement and comparison of bZIP protein (F) and mRNA (G) accumulation in *P. acutifolius* roots. Arrows in panels A, D, F, and G indicate regions of bZIP RNA or protein abundance. All images were acquired with a ×20 objective. Scale bars: A–E=200 μm; F, G=50 μm.

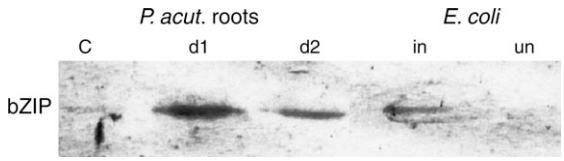

Fig. 5. Western blot detection of the *P. acutifolius* bZIP transcription factor. Protein was extracted from roots of *P. acutifolius* plants that were either well-watered (c), or had been water deficit-stressed for 5 d (d1, d2). Following electrophoresis on SDS-PAGE, the proteins were blotted to nitrocellulose and reacted with the antibody raised against the recombinant bZIP (diluted 1:2500), and then detected by alkaline phosphatase. The amount of plant protein loaded was 4.0 mg in lane c, 6.5 mg in lane d1, and 3.2 mg in lane d2. *Escherichia coli* extract lanes contained 5.0 mg protein from IPTG-induced (in) cultures or non-induced cultures (un).

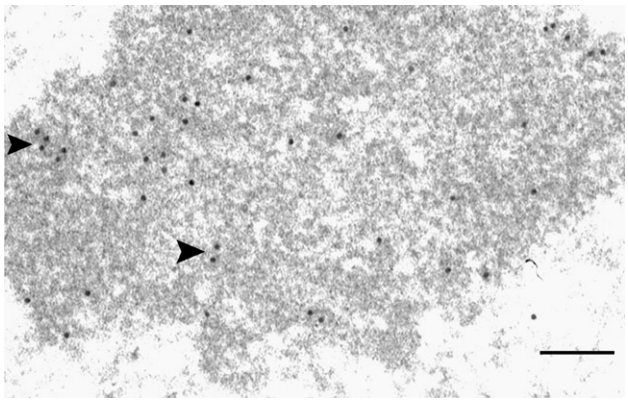

Fig. 6. Subcellular localization of the bZIP transcription factor. Transmission electron micrograph of the nucleus in a root epidermal cell from water deficit-stressed *P. acutifolius* stained with bZIP antibody, and then detected with a secondary antibody conjugated to 12 nm colloidal gold particles (arrowheads). Scale bar=1.74 μm.

particles. The same cellular pattern of staining was observed in epidermal and vascular cells in the water deficit-stressed roots, which had the biggest accumulation of bZIP. To quantify this observation, the number of gold particles in the cells in seven different cells of each tissue type was counted and the average number of gold particles determined (Fig. 6). This analysis was performed in cross-sections of roots from control and water deficit-stressed *P. acutifolius* and *P. vulgaris* (Table 1).

In *P. acutifolius* roots, water deficit-stress increased the abundance of the bZIP protein by over 2-fold in the nuclei of the epidermal and vascular cells. The signal abundance was greater in the epidermal cells; approximately twice as much bZIP protein was detected in the epidermal nuclei as in the vascular nuclei under control conditions. In *P. vulgaris* there was a similar trend, water deficit stress increased the abundance of the bZIP protein by 1.5- to 1.9-fold in the epidermal and vascular cells. The control levels of the protein were the same in the nuclei of the epidermal cells and the vascular cells. In addition to the nuclear localization of the bZIP antibody, there was a low level of staining in the cell walls of both cell types. No differences were observed in the number of gold particles in the cell walls from either species in control or water deficit-stressed samples.

Table 1. *Effect of water deficit stress on nuclear localization of the bZIP transcription factor in epidermal and vascular tissues in roots from* P. acutifolius *and* P. vulgaris

Multiple fields (n=7 for each cell type in each treatment and plant genotype) were examined using printed transmission electron micrographs of sections stained with anti-bZIP antibody and counterstained with 12 nm gold particle-tagged secondary antibody.

Plant/treatment	Nuclei of epidermal cells (no. of gold particles ±SD)	Nuclei of vascular cells (no. of gold particles ±SD)
P. acutifolius		
Control	52.9±1.9	21.4±1.0
Water deficit	121.1±5.9	46.3±1.8
P. vulgaris		
Control	62.0±23.8	57.5±29.7
Water deficit	94.4±44.7	107.8±14.2

Discussion

Differential screening of the cDNA library of water deficit-stressed *P. acutifolius* roots allowed the identification of a cDNA for a novel bZIP transcription factor. The orthologue for this gene in *P. vulgaris* was then isolated and the two genes were compared. The identification of these genes as bZIP transcription factors was initially based on predicted amino acid sequence similarity to other plant bZIPs. In this report, it is demonstrated experimentally that root specificity and nuclear localization of the protein is, as expected, based on its predicted role as a transcription factor.

The complete amino acid sequence of the two bZIP proteins showed a 46% similarity to the HBF-1bZIP transcription factor from *Glycine max* over the entire sequence and a 64% similarity in the bZIP domain. The two characteristic domains of a bZIP transcription factor (Liu *et al*., 1999) were found in the amino acid sequence of both proteins described here. A domain rich in strongly basic (R/K) amino acids containing the nuclear localization signal was followed by the zipper domain composed of a heptad of leucines or similar amino acids (Jakoby *et al*., 2002). A transactivating region rich in proline was also found at the amino-terminal region of this protein. These types of domains have also been found in other plant transcription factors like the *A. thaliana* GBF1 (Schindler *et al*., 1992), and are known to cause transactivation of transcription of the chalcone (CHS) gene promoter in parsley (*Petroselinum crispum*) up to a 6-fold (Sprenger-Haussels and Weisshaar, 2000).

bZIP transcription factor genes are usually members of gene families (Kusano *et al*., 1995; Nantel and Quatrano, 1996; Yanagisawa, 1998). The size of the root-specific *bZIP* gene family was estimated in both *P. acutifolius* and *P. vulgaris* to be at most three members, based on the complexity of the genomic Southern blot. The hybridization conditions of the Southern blot permitted detection of sequences with >84% sequence complementarity. There are clearly many more *bZIP* genes in each *Phaseolus* species (Fig. 1B). These other members of the larger *bZIP* gene family are probably involved in very different processes.

RNA abundances for the *bZIP*s in both *P. acutifolius* and *P. vulgaris* were demonstrated to be root specific, and responsive to water deficit and cold stresses. For both species the level of transcript induction by water deficit was approximately 6-fold over control levels. In plants, bZIP transcription factors regulate processes including pathogen defence, light, and stress signalling, seed maturation, and flower development (Strathmann *et al*., 2001; Jakoby *et al*., 2002). bZIP transcription factors have been found to be involved in intracellular signalling in response to water deficit (Neill and Burnett, 1999). The expression of this root-specific bZIP transcription factor in water deficit-stressed *P. acutifolius* and *P. vulgaris* plants suggested that it might also regulate the expression of other water deficit-responsive genes.

The detection of bZIP protein on western blots and histological sections also demonstrated the water deficit-responsive nature of this gene product. Only two cell types in the root accumulated mRNA or protein for this gene, epidermal cells and phloem cells, either companion cells or phloem parenchyma cells. The protein was detected primarily in the nuclei of these cells as expected for a transcription factor. Both species accumulated similar levels of the protein in the same cell types.

This gene represents a novel root-specific transcription factor, which is likely to be involved in co-ordinating gene expression in response to water deficit stress. The cell-specific pattern of expression was the same in both *Phaseolus* species. It is likely then that the targets for this transcription factor would be genes whose expression is altered by water deficit in root epidermal and vascular cells.

Vascular-specific bZIP transcription factors have been identified in rice (Yin *et al*., 1997), tobacco (Fukazawa *et al*., 2000), and tomato (Torres-Schumann *et al*., 1996). None of these genes are root specific, nor do they appear to be responsive to water deficit stress. Tobacco mutants suggest that the wild-type bZIP may regulate cell elongation through a modulation of endogenous levels of gibberellins (Fukazawa *et al*., 2000). Similar stunted shoot growth phenotypes were observed in rice mutants (Yin *et al*., 1997). One adaptive mechanism for drought-stressed plants is to increase root biomass at the expense of shoot growth (O'Connell, 1995) and, as reported by White and Castillo (1989), root genotype/physiology is an important component in *P. vulgaris* for performance under water deficit stress. A transcription factor like the bZIPs described in this report could be modelled to increase root elongation in such an adaptive response.

Acknowledgements

This work was supported in part by the NM Agricultural Experiment Station, USDA CSREES, LANL NUCOR, and CONACyT.

References

Altschul SF, Madden TT, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25,** 3389–3402.

Aluru MR, Mazourek M, Landry LG, Curry J, Jahn M, O'Connell MA. 2003. Differential expression of fatty acid synthase genes, *Acl, Fat*, and *Kas* in *Capsicum* fruit. *Journal of Experimental Botany* **54,** 1655–1664.

Boyer JS. 1982. Plant productivity and the environment. *Science* **218,** 443–448.

Bray EA. 1993. Molecular responses to water deficit. *Plant Physiology* **103,** 1035–1040.

Bray EA. 2002. Classification of genes differentially expressed during water-deficit stress in *Arabidopsis thaliana*: an analysis using microarray and differential expression data. *Annals of Botany* **89,** 803–811.

English-Loeb GM. 1990. Plant drought stress and outbreaks of spider mites: a field test. *Ecology* **71,** 1401–1411.

Fukazawa J, Sakai T, Ishida S, Yamaguchi I, Kamiya Y, Takahashi Y. 2000. REPRESSION OF SHOOT GROWTH, a bZIP transcriptional activator, regulates cell elongation by controlling the level of gibberellins. *The Plant Cell* **12,** 901–915.

Garvin DF, Weeden NF. 1994. Isozyme evidence supporting a single geographic origin for domesticated tepary bean. *Crop Science* **34,** 1390–1395.

Ghoshroy S, Citovsky V. 1998. Preservation of plant cell structure during immunolocalization of virus particles. *Journal of Virology Methods* **74,** 223–229.

Harrak H, Chamberland H, Plante M, Bellemare G, Lafontaine JG, Tabaeizadeh H. 1999. A proline-, threonine, and glycine-rich protein down-regulated by drought is localized in the cell wall of xylem elements. *Plant Physiology* **121,** 557–564.

Jakoby M, Weisshaar B, Droge-Laser W, Carbajosa JV, Tiedeman J, Kroj T. 2002. bZIP transcription factors in *Arabidopsis*. *Trends in Plant Science* **7,** 106–111.

Kusano T, Berberich T, Harada M, Suzuki N, Sugawara K. 1995. A maize DNA-binding factor with a bZIP motif is induced by low-temperature. *Molecular and General Genetics* **248,** 507–517.

Laing DR, Jones PG, Davis JHC. 1984. Common bean (*Phaseolus vulgaris*). In: Goldsworthy PR, Fisher NM, eds. *The physiology of tropical field crops*. New York, NY: John Wiley & Sons, 305–351.

Lazcano-Ferrat I, Lovatt CJ. 1999. Water content, nitrogen pools, and growth of *Phaseolus vulgaris* L. and *P. acutifolius* A. Gray during water deficit. *Crop Science* **39,** 467–475.

Liu L, White JM, MacRae HT. 1999. Transcription factors and their genes in higher plants functional domains, evolution, and regulation. *European Journal of Biochemistry* **262,** 247–257.

Maliga P, Klessig DF, Cashmore AR, Gruissem W, Varner JE. 1995. *Methods in plant molecular biology*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 95–115.

Miklas PN, Rosas JC, Beaver L, Telek X, Freytag GF. 1994. Field performance of select tepary bean germplasm in the tropics. *Crop Science* **34,** 1639–1644.

Nantel A, Quatrano RS. 1996. Characterization of three rice basic leucine zipper factors including two inhibitors of EmBP-1 DNA binding activity. *Journal of Biological Chemistry* **271,** 31296–31305.

Ndima T, Farrant J, Thomson J, Mundree S. 2001. Molecular characterization of XVT8, a stress-responsive gene from the resurrection plants *Xerophyta viscosa* Baker. *Plant Growth Regulation* **35,** 137–145.

Neill SJ, Burnett EC. 1999. Regulation of gene expression during water deficit stress. *Plant Growth Regulation* **29,** 23–33.

O'Connell MA. 1995. The role of drought-responsive genes in drought resistance. *Ag Biotechnology News and Information* **7,** 143N–147N

Ramirez-Vallejo P, Kelly JD. 1998. Traits related to drought resistance in common bean. *Euphytica* **99,** 127–136.

Schindler U, Terzaghi W, Beckmann H, Kadesh T, Cashmore AR. 1992. DNA binding site preferences and transcriptional activation properties of the Arabidopsis transcription factor GFB1. *EMBO Journal* **11,** 1275–1289.

Sprenger-Hausssels M, Weisshaar B. 2000. Transactivation properties of parsley proline-rich bZIP transcription factors. *The Plant Journal* **22,** 1–8.

Strathmann A, Kuhlmann M, Heinekamp T, Droge-Laser W. 2001. BZI-1 specifically heterodimerizes with the tobacco bZIP transcription factors BZI-2, BZI-3/TBZF and BZI-4, and is functionally involved in flower development. *The Plant Journal* **28,** 397–408.

Tabaeizadeh Z. 1998. Drought-induced responses in plant cells. *International Review of Cytology* **182,** 193–247.

Terán H, Singh SP. 2002. Comparison of sources and lines selected for drought resistance in common bean. *Crop Science* **42,** 64–70.

Torres-Schumann S, Ringli C, Heierli D, Amrhein N, Keller B. 1996. *In vitro* binding of the tomato bZIP transcriptional activator VSF-1 to a regulatory element that controls xylem-specific gene expression. *The Plant Journal* **9,** 283–296.

Türkan I, Bor M, Özdemir F, Koca H. 2005. Differential response of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol-mediated water stress. *Plant Science* **168,** 223–231.

Uno Y, Furihata T, Abe H, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K. 2000. *Arabidospsis* basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high salinity conditions. *Proceedings of the National Academy of Sciences, USA* **97,** 11632–11637.

White JW, Castillo JA. 1989. Relative effect of root and shoot genotypes on yield of common bean under drought stress. *Crop Science* **29,** 360–362.

Wilkinson DG. 1992. In situ *hybridization: a practical approach*. Oxford: Oxford University Press.

Xue G-P, Loveridge CW. 2003. *HvDRF1* is involved in abscisic acid-mediated gene regulation in barley and produces two forms of AP2 transcriptional activators, interacting preferably with a CT-rich element. *The Plant Journal* **37,** 326–339.

Yanagisawa S. 1998. Transcription factors in plants: physiological functions end regulation of expression. *Journal of Plant Research* **111,** 363–371.

Yin Y, Zhu Q, Dai S, Lamb C, Beachy RN. 1997. RF2a, a bZIP transcriptional activator of the phloem-specific rice tungro bacilliform virus promoter, functions in vascular development. *EMBO Journal* **16,** 5247–5259.