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Comparative transcript profiling in roots of *Phaseolus acutifolius* and *P. vulgaris* under water deficit stress

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Abstract

Transcript profiles in roots of *Phaseolus acutifolius* and *P. vulgaris* were compared under water deficit conditions that demonstrated the drought resistance phenotype of *P. acutifolius*. Differential gene expression was monitored with microarray analysis using slides printed with ~5200 anonymous cDNAs from libraries of *Phaseolus* spp. Those genes whose expression was significantly altered by water deficit were sequenced and functional classifications were inferred based on similarity to other annotated genes. Fewer genes ($n = 64$) were responsive in *P. vulgaris*, the more drought sensitive species, compared with *P. acutifolius* ($n = 488$). Only 25 genes were drought responsive in the roots of both species, at a drought stress level of −2.5 MPa leaf water potential. Most of the responsive genes in *P. vulgaris* were in the functional class for stress responsive genes, while the largest functional class in *P. acutifolius* was populated with unannotated or novel genes. There were a number of genes likely to be important in cell growth and metabolism that were uniquely stress responsive in *P. acutifolius.*

Keywords: Common bean; Drought; Macroarrays; Microarrays; Tepary bean

1. Introduction

Water deficit continues to be one of the most significant stresses of agriculturally important crops, affecting growth, development, and yield (reviewed in [1]). Plants adapted to grow in arid and semi-arid regions have evolved mechanisms to survive and succeed under these adverse conditions [2]. The response of plants to this environmental stress depends on several factors including the plant developmental stage and the length and severity of the stress applied [3,4]. In leaf tissues the perception of drought stress causes stomatal closure to reduce transpiration, and consequently limits carbon dioxide uptake and reduces photosynthesis rates [5]. Water deficit stress may also result in root growth or elongation at the expense of above ground growth [6]. This increases the soil volume explored by the plant for water. Plants also synthesize compounds that function as osmolytes to maintain water potential or other proteins that are modeled to protect cells from damage [7,8]. All of these responses are controlled by complex mechanisms that involve changes in gene expression. Some gene products are directly involved in protective mechanisms, such as the late embryogenesis abundant proteins (LEA), the synthesis of osmolytes; or ion transport functions; other gene products, e.g. transcription factors or kinases, which participate in signal transduction pathways that mediate cellular responses to external stimuli [4,9,10].

Common bean (*Phaseolus vulgaris* L.) is the most important food legume grown worldwide [11]. A major constraint to bean production is water deficit stress, for instance 73% of the area planted to beans in Latin America is semi-arid [12]. There is genetic diversity within *P. vulgaris* for drought resistance [13,14]; with some analysis of the likely physiological traits that could be selected for adaptation. Efforts to generate genomic resources in this essential food crop are ongoing [15,16]. Another member of the genus, *P. acutifolius* (tepary bean), is native to the Sonoran Desert in North America and remarkable in its drought resistance phenotype [6]. This plant also possesses a short duration lifespan, is more heat tolerant than common bean, produces seeds of high protein quality suitable for human

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consumption, and has resistance to common bacterial blight [17,18]. *P. acutifolius* has been cultivated by Native Americans, but is not used commonly for legume food production [19].

P. acutifolius is more heat and drought resistant than *P. vulgaris*, but the morphological and genetic basis of this resistance is under discussion. One proposed mechanism is the depth of the root system. Root length is reported to be greater in *P. acutifolius* than in *P. vulgaris* under water deficit, which allows this species to explore deeper areas of the soil to extract water. However, this result varies depending on the growing conditions. When grown in pots in the shade, both species develop long roots [20]. Root growth in *P. acutifolius* may be stimulated by stress while in *P. vulgaris* root growth is inhibited by stress [20]; however, Lazcano-Ferrat and Lovatt [6] report the opposite result. *P. acutifolius* roots may have greater hydraulic conductivity than *P. vulgaris* roots, allowing *P. acutifolius* to maintain or increase its leaf water potential [21].

We recently reported a root specific drought responsive bZip transcription factor in *P. acutifolius* and *P. vulgaris* [9]. Comparing water deficit-responsive genes in the roots of these two *Phaseolus* species will allow us to identify novel mechanisms for adaptation to water deficit stress. We hypothesize that the drought adaptive response of *P. acutifolius* involved alterations in gene expression in the roots, and that these alterations would not be observed in the more drought sensitive *P. vulgaris*. To test this idea, we generated microarrays printed with cDNA forms of transcripts from water-deficit stressed *P. acutifolius* and *P. vulgaris*. Genes associated with the drought resistant phenotype are identified by comparing the root transcriptomes in *P. vulgaris* and *P. acutifolius* under control and water deficit conditions.

2. Materials and methods

2.1. Plant material and water deficit treatment

Seeds from *Phaseolus acutifolius* (Gray) var O'Odham Brown and *P. vulgaris* (L.) cv. Negro Jamapa were germinated and grown in 25.4 cm pots, in a mix of three equal parts of perlite, Metro-Mix 360, and vermiculite with average day/night temperature of 29 8C/22 8C in a greenhouse at NMSU. Osmocote 14-14-14 was added as fertilizer and the plants were watered every other day up to the early flowering stage. Plants were divided into two sets; controls were watered every other day while water was withheld from the stressed set of plants for anywhere from 1 to 10 days (water deficit stress). Leaf water potential (C_w) was measured on seven different mature leaves representing three to five plants at each time point, using a Scholander pressure bomb (Plant Water Status Console model 3005). Root tissues were harvested from plants at the indicated times, immediately frozen in liquid nitrogen and kept at −80 8C for isolation of total RNA.

2.2. Macroarrays and northern blot analyses

RNA isolation, membrane blot hybridization, radiolabelled probe synthesis, cDNA synthesis and library construction have been described previously [9]. The inserts of recombinant cDNA phage clones from the libraries were amplified by PCR using flanking vector sequences for primers. The amplicons were electrophoresed in 1.0% agarose 96 well gels; transferred to duplicate nylon membranes and probed using standard methods. Control samples were cDNA from roots of well-watered plants (−0.70 MPa leaf C_w) and drought samples, roots of *P. acutifolius* at −2.53 MPa leaf C_w and *P. vulgaris* at −2.57 MPa leaf C_w. The nylon membranes were exposed to X-ray films, the films were scanned and band intensities quantified. Analysis of the data was performed by calculating ratios between signals of drought-treated samples over control samples after background subtraction and normalization for DNA abundance on the blot.

2.3. Microarray analysis

Microarrays consisted of 5268 prints, 2245 cDNAs from *P. acutifolius* root cDNA library of drought stressed plants, 2023 cDNAs from *P. vulgaris* root cDNA library of drought stressed plants, and 1000 cDNAs from *P. acutifolius* leaf cDNA library of drought stressed plants. DNA from lambda phage and buffer blanks were spotted within the microarray as negative controls. cDNAs from one 384-well plate were printed three times within the microarray to assess the reproducibility of the microarray hybridizations. PCR amplified products were arrayed from 384-well microtiter plates onto poly-L-ysine-coated micro slide glass (Ultra GAPS Coated Slides from Corning) using the OMNI/GRID microarray from GENE Machines. Each tip dispensed 1 nL of each PCR product per slide on 50 slides. After printing, the arrays were cross-linked and baked at 80 8C for 2 h. The arrays were kept in dust free amber plastic boxes under vacuum in the dark until hybridization was performed.

Total RNA from roots (20 mg) was used to synthesize cDNA with the SuperScript Indirect cDNA Labeling System as described by the manufacturer (Invitrogen). The methods for Cy3 and Cy5 probe preparation, slide hybridization and washing were essentially as described [22]. Independent RNA samples were collected and used to replicate the microarray hybridizations. The hybridizations were performed with control samples (roots at −0.70 MPa leaf C_w in both species) labeled with Cy3 and drought-treated samples labeled with Cy5. The drought-treated samples were obtained from *P. vulgaris* at −2.56 and −2.51 MPa leaf C_w and at −2.20 and −2.53 MPa leaf C_w in *P. acutifolius*. Arrays were scanned using the GeneTAC UC4 laser scanner (Genomic Solutions) at a resolution of 10 mm per pixel. Spots showing uneven hybridization or any other abnormality were flagged as bad spots and excluded from the analysis. Background subtraction was performed using the specified negative controls for each experiment (blank and air spots). The background median value was calculated and then subtracted from the raw values for each gene. Next a *per-spot* normalization was performed, which included the ratio calculation. Calculated ratios were converted to natural logs in order to perform statistical analysis where a normal distribution of the variables may be required. A Lowess

curve was fitted to the log-intensity versus log-ratio plot in a process called *Intensity dependent* normalization by GeneSpring GX 7.3 (Agilent Technologies). At each point, 20% of the data was used to calculate the Lowess fit. This curve was used to adjust the control value for each measurement. If the control channel was lower than 10 then 10 was used instead. Generally, the highest noise to signal ratios were observed for low intensities, consistent with observations by Zien et al. [23]. The lower limit where the standard deviation (S.D.) becomes constant can be determined using the Cross-Gene Error model [24,25]. This model, was then applied to determine the control channel value were S.D. becomes flat [25]. Data with control intensity values less than that point were not included in this analysis.

Many of the genes/clones on the microarray were printed in multiple locations on the slide. These replicated spots on the array were averaged. Each experiment was replicated, that is two independent drought stress cycles with subsequent RNA collection from roots for each species were collected and used to prepare the cDNA samples for hybridization. No dye swap treatments were performed, control samples were always labeled with Cy3 and drought samples were always labeled with Cy5. *t*-Tests ($p \leq 0.05$), comparing control and drought stress hybridization signals were used to determine clones with reproducible drought responsive patterns of expression. Clones showing regulation by water deficit stress were sequenced and submitted to dbEST at GenBank. MIAME compliant microarray data is available at NCBI-GEO (GPL5761).

2.4. DNA sequence analysis

PCR cycle sequencing was performed on the 5′ end of amplicons of phage cDNA clones and the DNA sequence was determined using Applied Biosystems, Inc., ABI 3100 DNA sequencer. DNA sequences were trimmed of vector sequences, and then analyzed with BLASTX [26]. Genes found to be differentially regulated by water deficit were classified into the general functional categories described in the MIPS database [27].

3. Results

3.1. Progress of water deficit stress in P. acutifolius and P. vulgaris

Leaf water potential (C_w) was monitored in both species throughout a drought stress cycle (Fig. 1). This analysis demonstrated that both species had similar rates of decrease in leaf C_w, although *P. vulgaris* leaves lost water potential slightly faster than *P. acutifolius.* The drought resistance of *P. acutifolius* is revealed in the continued performance of this plant when its leaf C_w drops to −2.5 MPa. *P. vulgaris* dies shortly after its leaf C_w drops to this level (day 6 in Fig. 1). In contrast, *P. acutifolius* can persist for many days at this reduced leaf C_w (days 7–10 in Fig. 1) and will recover completely with re-watering.

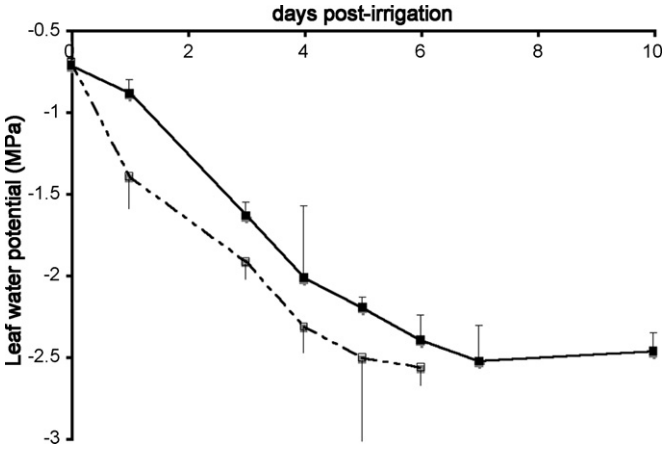

Fig. 1. Leaf water potential during a drought cycle. Solid line indicates leaf water potential in *P. acutifolius*, dashed line in *P. vulgaris* at the indicated days after irrigation stopped.

3.2. Identification of drought responsive genes using microarrays

In order to characterize drought responsive genes in *Phaseolus* spp. we used custom cDNA microarrays printed with randomly selected clones from cDNA libraries of drought-stressed *P. acutifolius* and *P. vulgaris* roots. To identify genes differentially regulated by drought we used a threshold of two-fold difference in transcript abundance (drought/control).

Panels A and B in Fig. 2 are representative scatter plots of the data from two comparisons. The normalized transcript abundances for each of the 5200 genes are plotted at their control and drought responsive levels. For both species the scatter plots have a linear relationship; further most of the genes are non-responsive to the drought stress, these genes are plotted between the two limit lines indicating two-fold induction and repression.

Many more genes were drought-responsive in *P. acutifolius* than in *P. vulgaris* (Fig. 2). The transcript levels of 328 genes were induced in roots of *P. acutifolius* at −2.52 MPa leaf C_w compared with well-watered samples (controls) while 160 were repressed. When *P. acutifolius* plants were under a moderate level of water deficit, −2.20 MPa leaf C_w, fewer genes were responsive, 81 up-regulated and 59 down-regulated; only 38 genes were scored as drought responsive under both stress conditions (Fig. 2C). In *P. vulgaris*, only the severe water deficit condition was examined, −2.5 MPa leaf C_w. Fewer clones were drought responsive in roots of *P. vulgaris*, in total, 64 clones; of these, 49 clones were induced and 15 were repressed (Fig. 2D and E). Relatively few of these genes were commonly induced in both species ($n = 23$) or repressed ($n = 2$). Altogether, DNA sequence information was obtained for 623 clones; GenBank dbEST accession numbers for these clones were obtained and this information along with the functional classification and average ratio for drought responsiveness are listed in the Supplementary Table. Empty cells in tables for drought responsiveness as measured by microarray analyses

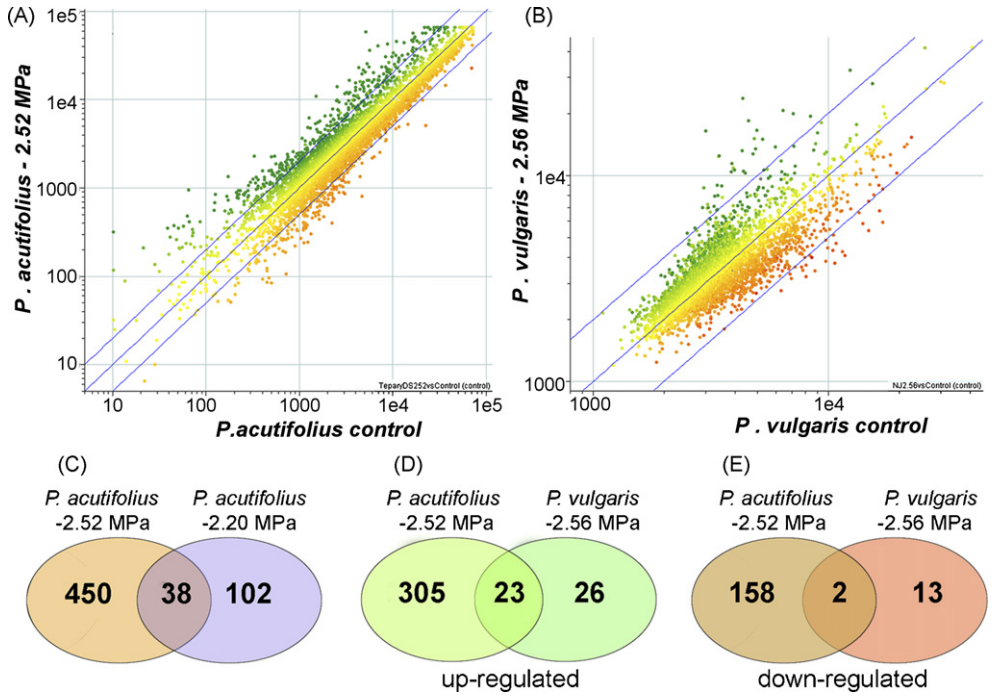

Fig. 2. Scatter plot comparisons of microarray gene expression in drought-stressed *P. acutifolius* or *P. vulgaris* and well watered control plants. The normalized expression value (signal) for each gene under well watered (control) vs. drought-stress plotted for *P. acutifolius* at −2.53 MPa leaf water potential (C_w) samples (A) and for *P. vulgaris* samples at −2.56 MPa C_w (B); Venn diagrams showing the number of genes that are drought responsive in *P. acutifolius* at two different leaf water potentials (C); the number of genes in *P. vulgaris* and *P. acutifolius* that are up-regulated (D) or down-regulated (E).

(Tables 1–3, Supplementary Table) indicate that gene was not drought responsive, i.e., the numerical value for the ratio is between 0.5 and 2.0.

3.3. Functional classification of drought responsive genes in P. acutifolius and P. vulgaris

Genes identified as drought responsive by microarray analysis in each *Phaseolus* species were classified into the general functional categories described in MIPS. These analyses were performed with the 488 genes identified in *P. acutifolius* and also with the 64 genes from *P. vulgaris* in roots of plants at −2.52 or −2.56 MPa leaf C_w. The functional annotation of these clones was based on their match to other sequences in GenBank using BLAST tools, with a threshold set at an expectation value (*E*-value) $< 10^{-10}$.

The responsive genes in *P. acutifolius* were placed in 10 different functional classifications: biogenesis of cellular components, cell division, cellular communication, cellular transport, metabolism, protein processing, protein synthesis, stress response, transcription, and unclassified proteins. The responsive genes in *P. vulgaris* were placed in nine different functional classifications; none of the responsive genes in *P. vulgaris* fell within the cell division classification. The relative sizes of these classes are depicted in two pie charts in Fig. 3. The Supplementary Table lists all of the drought responsive genes for either species in these functional classes, while Table 1 lists examples of genes in selected functional classes; these examples represent genes that are drought responsive in *P. acutifolius*.

For *P. acutifolius* the largest functional class was that of unknown genes, 27% of the sequences; within that group, 18% (89 genes) corresponded to novel sequences and 9% had similarity to hypothetical or expressed protein of unknown function. Those clones that correspond to novel sequences did have sequence similarity to unannotated plants ESTs from *P. vulgaris*, *Medicago truncatula* or *Solanum* spp. In *P. vulgaris*, only 14% of the responsive genes were in the unknown functional class, of these half correspond to novel genes and all of these novel genes are induced in both *P. acutifolius* and *P. vulgaris*.

The next largest functional class of drought responsive genes (20%) in *P. acutifolius* were in the "defense, cell rescue and stress response" category, in this group 74 were induced while 24 were repressed. This functional class was the largest class of responsive genes (49%) in *P. vulgaris*; many of the induced genes were senescence associated proteins, desiccation protective proteins and LEA proteins. Most of the drought responsive genes that were expressed similarly in both *P. acutifolius* and *P. vulgaris* were in the defense, stress response functional class. The repressed genes ($n = 6$) in *P. vulgaris* included lipoxygenases and glutamine synthetase. In *P. acutifolius* there were several induced genes ($n = 12$) for pathogenesis-related proteins (PR), while other proteins involved in pathogen recognition or infection such as receptor kinases, serine threonine phosphatases, leucine-rich proteins or disease resistance proteins were repressed. None of the induced PR genes were drought responsive in *P. vulgaris*.

The proportion and type of genes in the metabolism functional class were similar in both species, 16% of responsive

Table 1
Selected drought responsive genes in *P. acutifolius*

MIPs categories	D/C	GenBank	*E*-value	Annotation
Cellular transport ($n = 21$)	10.03	EC911524	1.00E−113	Coatomer protein gamma 2-subunit.
	3.63	EC911690	5.00E−93	Plasma membrane H^+ ATPase
	2.97	EC911334	9.00E−61	Na/H antiporter Nhx1
	2.31	EC911432	1.00E−45	Transmembrane protein FT27/PFT27
	0.33	EC911268	9.00E−24	Putative phosphate transporter 1
	0.37	EC911378	3.00E−28	Putative transport protein Sec24
	0.43	EC911230	6.00E−14	Putative phosphate transporter 1
Metabolism ($n = 80$)	11.8	EC911428	7.00E−80	ATPase-like domain-containing protein
	4.57	EC911310	1.00E−111	Aldehyde reductase.
	4.55	EC911889	4.00E−95	UTP-glucose-1-P-uridylyltransferase
	4.62	EC911502	1.00E−84	ATP citrate lyase a-subunit
	3.94	EC911539	1.00E−124	Sucrose synthase 2
	3.88	EC911458	1.00E−89	Glyceraldehyde-3-P-dehydrogenase
	2.96	EC911867	2.00E−28	*S*-adenosylmethionine decarboxylase
	2.6	EC911417	3.00E−77	UDP-D-glucuronate carboxy-lyase
	2.2	EC911306	3.00E−93	Aldehyde dehydrogenase fam 7 member
	2.1	EC911890	5.00E−74	D1-pyrroline-5-carboxylate synthetase
	2.07	EC911585	1.00E−124	Alpha-amylase
	2.01	EC911481	2.00E−44	Putative trehalose-6-phosphate synthase
	0.46	EC911765	1.00E−48	Phospholipid/glycerol acyltransferase
	0.38	EC911599	6.00E−82	Lysine-ketoglutarate reductase
	0.36	EC911736	1.00E−50	Enolase
	0.18	EC911794	1.00E−74	Peptide methionine sulfoxide reductase
Transcription ($n = 38$)	14.72	EC911536	3.00E−57	Zinc finger POZ domain protein
	4.85	EC911525	7.00E−98	Zinc finger (DHHC type) family protein
	3.95	EC911443	5.00E−22	Putative MAP kinase-activating protein
	4.1	EC911414	2.00E−36	Probable WRKY transcription factor 23
	3.82	EC911686	2.00E−73	BZip transcription factor protein.
Biogenesis of cellular components ($n = 34$)	7.14	EC911526	1.00E−110	Endo-1,3-beta-glucanase
	4.8	EC911249	4.00E−06	24 kDa vacuolar protein VP24
	2.26	EC911247	7.00E−662	Major surface like glycoprotein
	2.37	EC911320	1.00E−31	Putative glycan synthase
	2.5	EC911382	4.00E−69	Pectinesterase family protein
	2.57	EC911590	1.00E−150	Malate synthase, glyoxysomal
	0.47	EC911792	4.00E−34	Extensin
	0.48	EC911631	2.00E−86	Ferredoxin-NADP reductase
Cell growth ($n = 10$)	7.79	EC911641	1.00E−22	Histone deacetlyase
	6.46	EC911491	1.00E−114	Putative helicase
	4.06	EC911427	3.00E−76	Inosine-uridine preferring nucleoside hydrolase
	2.74	EC911520	3.00E−52	Auxin independent growth promoting protein
	2.3	EC911809	6.00E−92	Putative karyopherin-beta 3 variant
	3.02	EC911816	9.00E−19	Putative chromosome associated protein

Genes whose transcript levels changed in response to drought only in *P. acutifolius* roots (−2.52 MPa) are listed. Selected induced and repressed genes from each MIP category uniquely identified by microarray analysis of *P. acutifolius* roots GeneBank accession numbers, gene annotation and E-values are indicated together with normalized ratios (D/C) of drought over control values obtained by microarrays.

genes in *P. acutifolius* and 15% in *P. vulgaris*. In *P. acutifolius*, 16 clones were predicted to be aldehyde reductase and all of them were highly induced with ratios between 4.7 and 2.2. All but one of the genes in the metabolism class was induced in *P. vulgaris*; aldehyde dehydrogenase was repressed. Transcripts induced by drought stress included several sucrose synthases, alcohol dehydrogenase, 6-phosphogluconate dehydrogenase and transaldolase. In *P. acutifolius*, several clones ($n = 4$) for glyceraldehyde-3-phosphate dehydrogenase were induced.

In *P. acutifolius*, the transcription class accounted for 8% of the responsive genes compared with only 2% in *P. vulgaris* for this class. Several transcription factors, bZIP, WRKY and Myb were drought-regulated in *P. acutifolius*; the highest induction was observed in transcripts containing zinc finger domains (Table 1).

The biogenesis of cellular component class comprised 7% and 5% of the responsive transcripts in *P. acutifolius* and *P. vulgaris*, respectively. In *P. vulgaris* the responsive genes were primarily the cell wall protein expansin, which was not induced in *P. acutifolius*. In contrast, *P. acutifolius* induced transcripts for polysaccharide synthesis like glucan synthase, pectinesterases, and endo-1,3-beta-glucanase (Table 1).

The cellular communication/signal transduction class comprised 7% and 2% of the responsive transcripts in *P. acutifolius* and *P. vulgaris*, respectively. In some cases, kinases ($n = 9$) were induced in *P. acutifolius* in response to drought,

Table 2
Comparison of drought responsive transcripts detected using microarrays and macroarrays

CloneId/GenBank	*P. acutifolius*		*P. vulgaris*		*E*-value	Annotation
	Micro	Macro	Micro	Macro		
LR966a/CX129891	6.47	4.74	2.56	2.04	1.00E−36	Desiccation protective protein LEA5
LR968/CX129892	5.51	5.49	3.19	5.31	7.00E−26	Desiccation protective protein LEA5
LR293/EC911717	4.18	1.65	6.93	5.47	1.00E−53	Signal transduction protein
LR400/EC911737	3.01	3.81	2.18	2.16		No hits
LR696/EC911754	2.37	2.47	3.72	8.93	1.00E−10	Hypothetical protein
LR88/EC911696	2.03	3.39	2.82	3.44	2.00E−61	Aldehyde dehydrogenase protein
LR854/CX129792	5.32	8.26			5.00E−74	Pathogenesis-related protein 1
LR833/CX129772	2.84	2.12			5.00E−49	Pathogenesis-related protein 1 (PvPR1)
LR53/EC911693	2.68	4.62			3.00E−41	Drought-inducible gene
NJ911/CX129654	2.54	4.48			7.00E−54	*G. max* seed maturation protein PM37
LR300/EC911718	2.48	2.11			9.00E−42	Syringolide-induced protein 13-1-1
LR40/EC911692	2.46	24.15			3.00E−77	Pathogenesis related protein 1
LR992/CX129914	2.39	3.50			9.00E−23	Chaperone/heat shock protein
NJ889/EC911326	2.36	10.76			6.00E−25	*P. vulgaris* pathogenesis-related protein
LR24/CX129715	2.24	11.11			2.00E−36	Pathogenesis-related protein PvPR1
LR66/EC911694	2.62	9.98			1.0E−131	Hypothetical protein
NJ943/CX129682	2.28	8.90				No hits
NJ897/CX129642	2.25	2.36				No hits
NJ888/EC911325	2.18	15.93				No hits
LR857/EC911762	0.48	0.53			6.00E−51	Protein kinase; U box
LR913/EC911765	0.46	0.44			1.00E−48	Phospholipid/glycerol acyltransferase
LR951/EC911917	0.37	0.18				No hits
LR919/EC911766	0.31	0.61			5.00E−64	Cellulose synthase protein
LR953/EC911768	0.17	0.63			7.00E−22	Hypothetical protein
LR435/EC911744			4.35	6.40	3.00E−32	Seed maturation protein

Normalized ratios of drought over control transcript abundances for microarray (Micro) or macroarray (Macro) analyses in roots of *P. acutifolius* and *P. vulgaris* are listed along with the functional annotation of the clone and the E-value supporting that annotation.

while other kinases ($n = 3$) were repressed. In *P. acutifolius*, proton pyrophosphatase proteins were induced.

The protein destination/processing class had similar proportions of genes in *P. vulgaris* (6%) and in *P. acutifolius* (4%). In *P. vulgaris* this class was comprised of mainly cysteine proteases, which were repressed; in *P. acutifolius* this class was comprised of ubiquitin or polyubiquitin genes, which were induced. The protein synthesis class had the same proportion of responsive genes in both species, 5% and is represented by elongation factors and binding proteins.

No transcripts related to cell division were identified in *P. vulgaris* while 2% of responsive transcripts were classified in this group in *P. acutifolius*, including chromosome associated proteins, cullin proteins and extensin. In *P. acutifolius*, extensin was drought repressed.

3.4. Macroarray detection of drought responsive genes

A representative image of the control and drought-stressed probes hybridized against PCR amplicons in the macroarray analysis is shown in Fig. 4. All of these lanes contain samples that were scored as drought-responsive and the increase in transcript level in response to drought ranged from 2.1 to 13.7 fold in roots of *P. acutifolius*. The predicted functions for these clones correspond to desiccation protective proteins LEA5 (LR966a, LR968), a protein with no significant hit (LR400), a

Table 3
Comparison of drought responsive transcripts quantified using microarrays and northern blots

CloneID/GenBank	*P. acutifolius*		*P. vulgaris*		*E*-value	Annotation
	Micro	N-blot	Micro	N-blot		
NJ404/EC911271	2.17	8.7	3.24	3.3	8.00E−46	Putative senescence-associated protein SAG29
NJ1268/EC911384	3.56	3.2	3.73	n.d.[a]	2.00E−49	Glutathione-*S*-transferase/glutaredoxin
LR966b/EC911771	2.56	2.1		1.5	6.00E−42	Aldehyde reductase
LR23/EC911690	3.64	1.7		4.3	5.00E−93	Plasma membrane H^+ ATPase
LR214/EC911708	0.32	0.5		n.d.	7.00E−18	Calcineurin B-like-interacting protein kinase

Normalized ratios of drought over control transcript abundances for microarray (Micro) or northern blot (N-blot) analyses in roots of *P. acutifolius* and *P. vulgaris* are listed along with the functional annotation of the clone and the E-value supporting that annotation. For the northern blot ratio calculations, background corrected transcript abundances on day 10 and day 5 in the drought cycle for *P. acutifolius* and *P. vulgaris*, respectively were divided by the transcript abundances in the control sample.

[a] n.d. indicates no hybridization signal was detected on the autoradiograph.

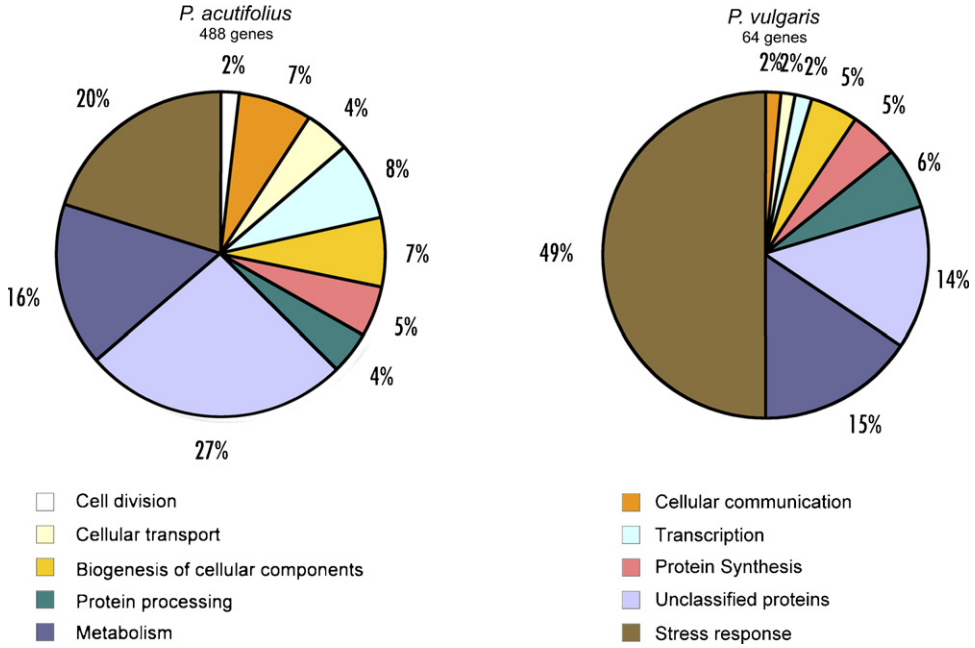

Fig. 3. Distribution of drought responsive genes in MIPS functional classes. Percentage of drought responsive genes in the various functional categories; *P. acutifolius* (left) and *P. vulgaris* (right).

nodulin (LR988), ABC transporter (LR150), aldehyde dehydrogenase protein (LR88) and two hypothetical proteins (LR696 and LR355). All these transcripts were also identified as induced in *P. vulgaris* by either micro or macroarray, suggesting that they play a role under water deficit stress but might not be related to drought resistance.

Genes that were scored as drought responsive by microarray and macroarray analyses are listed in Table 2. Both methods detected previously characterized drought responsive genes such as LEA, seed maturation proteins and aldehyde dehydrogenase. There are 18 genes that were only detected in *P. acutifolius*, not identified in *P. vulgaris*, including several

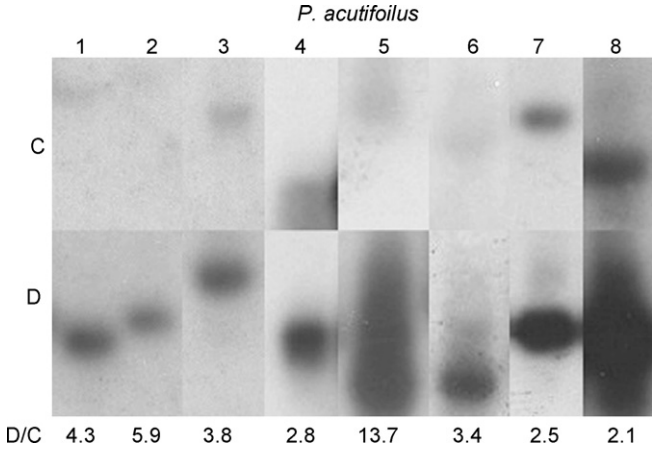

Fig. 4. Macroarray analysis. Lanes 1–8 contain PCR amplicons for the inserts from cDNA clones LR 966a, 968, 400, 988, 150, 88, 696 and 355, respectively. The radioactive signal on the X-ray film was quantified following hybridization with cDNA from roots of well watered (C) or stressed (D) *P. acutifolius* plants. The ratio of these signals, after normalization with ethidium bromide intensity for the amplicon, was recorded and is listed below each lane.

pathogenesis related proteins, syringolide-induced protein, and cellulose synthase.

3.5. Northern blot analysis of drought responsive genes

Five genes with drought responsive expression in *P. acutifolius* as determined by microarray analysis were selected for detailed characterization by northern blot analysis (Table 3). RNA was isolated from roots of control and drought stressed plants at intervals throughout the drought stress cycle, days 1, 2, 3, 4, and 5 for *P. vulgaris* and days 2, 4, 5, 7, and 10 for *P. acutifolius*. In the case of *P. acutifolius* RNA was also isolated from roots of plants after a subsequent re-watering. Transcript abundances for these genes were quantified by scanning autoradiographs and are presented in Fig. 5. One of these genes, LR214 was predicted by microarray analysis to be repressed by drought in *P. acutifolius*; the other four, LR213, LR966b, NJ1268 and NJ404, were predicted to be induced by drought stress. The northern blots confirmed the predicted drought expression patterns for these genes in *P. acutifolius*. Further, the transcript abundances for LR213, LR966b, NJ1268 and NJ404 decreased to close to control levels when the drought stressed plants were re-watered. In the case of the drought-repressed gene, LR214, re-watering did not increase the level of transcript to that of the control. No transcripts for LR214 or NJ1268 were detected by northern blot analysis in root samples of *P. vulgaris*. However, three of the genes, LR23, LR966b and NJ404 were identified as drought induced by northern blot analysis. The microarray analysis of *P. vulgaris* roots predicted that NJ404 and NJ1268 should be drought induced. Visual inspection of the autoradiograph and the resulting histogram (Fig. 5) supported the classification of LR966b in *P. vulgaris* as drought induced. The ratio for expression of this gene, 1.5 (Table 3) was below

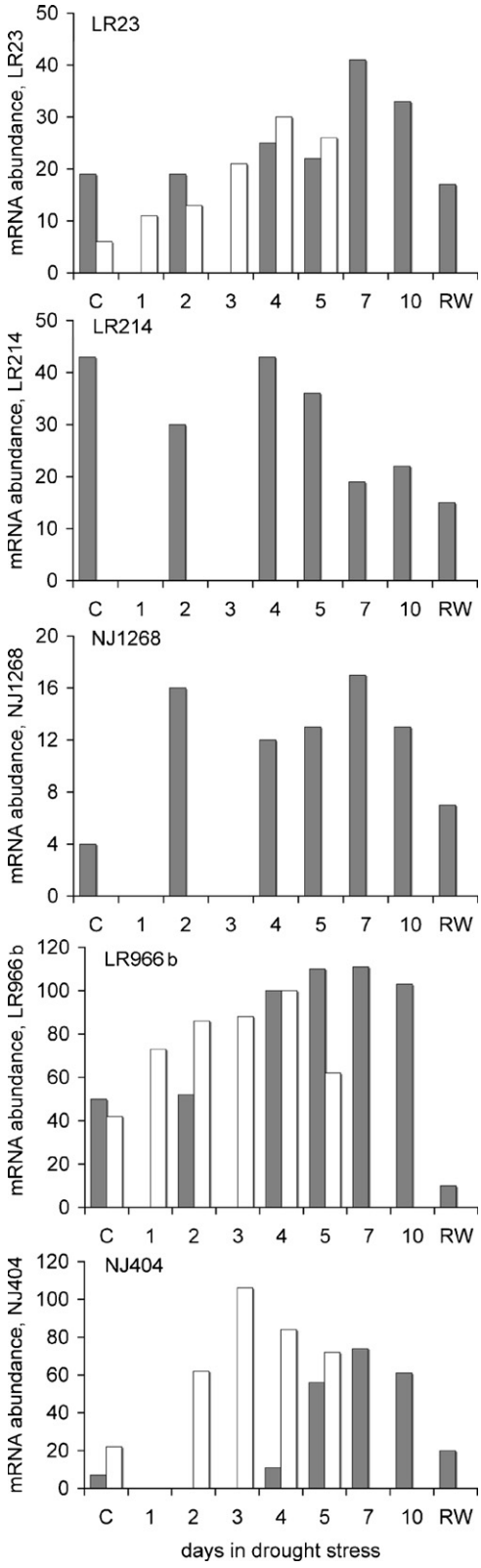

Fig. 5. Northern blot analysis. RNA isolated from roots of *P. acutifolius* (filled bars) or *P. vulgaris* (open bars); plants were well watered (C), or drought stressed for 1–10 days as indicated. After 10 days of drought stress, *P. acutifolius* plants were re-watered and then the next day RNA was isolated from roots (RW). Following gel electrophoresis, blots containing these RNA samples (10 mg/lane) were prepared and hybridized with radiolabeled probes of clones: LR23, LR214, NJ1268, LR966b, and NJ404. Autoradiographs of the hybridization signal were scanned and the signal abundances after correction for background were plotted.

the threshold for selection in a microarray experiment. Similarly, the ratio for expression of LR23 in *P. acutifolius*, 1.7, was also below the threshold. In both cases, the apparent induction of the gene was above the cut-off value of two-fold increase at an earlier time point in the drought cycle.

4. Discussion

Transcript profiles in roots of *Phaseolus acutifolius* and *P. vulgaris* were compared under water deficit conditions that demonstrated the drought resistance phenotype of *P. acutifolius*. Fewer genes ($n = 64$) were responsive in *P. vulgaris*, the more drought sensitive species, compared with *P. acutifolius* ($n = 488$). Only 25 genes were drought responsive in the roots of both species, at a drought stress level of −2.5 MPa leaf water potential.

The water potential of well-watered *P. acutifolis* is usually higher than *P. vulgaris*. Water potential of stressed *P. vulgaris* decreased rapidly from control levels after a few days of water deficit, whereas the decrease in *P. acutifolius* is much slower [17]. These results are very similar to the leaf C_w reported here (Fig. 1). Markhart [17] proposes the difference in the distribution of the roots in the soil of these two species to explain the difference in leaf C_w between the two species. Other authors report the ability of *P. acutifolius* to postpone dehydration [6,28].

One drought tolerance mechanism is to support root growth at the expense of shoot growth [29]. According to Thomas [20] and Markhart [17] *P. acutifolius* develops longer roots than *P. vulgaris* during water deficit stress. Our comparative microarray approach identified genes whose transcript abundances in roots distinguished these two species response to water deficit stress. Given that these changes were observed in plants grown in pots with a slow and progressive water deficit cycle, these changes are likely to reflect gene expression changes observed under field conditions.

We compared the gene expression patterns identified by microarray analysis with two other methods, macroarrays (reverse northerns) and northern blot analysis. The drought induction and repression patterns predicted by the microarray analysis were confirmed for most genes using these two other methods (Tables 2 and 3). In previous work we determined that a root-specific bZIP transcription factor was induced six-fold by drought stress [9]. In the microarray analyses this gene was induced 3.8 fold by drought stress (clone LR9, Supplementary Table). So while the general pattern of gene expression was correctly predicted by microarray experiments, the absolute values for the ratios were often quite different. This may be due to differences in the detector systems used for these different methods. In many cases, the ratio for gene expression appeared to have a greater magnitude in the autoradiograph detection system than in the fluorescent scanner system of the microarray detector. If the microarray system had a lower base background, then this system may record more gene expression in the control sample so that the relative increase associated with the increased signal in the drought sample will appear to be lower.

The time course aspect of the northern blots (Fig. 5) suggested that maximal accumulation of the four drought-induced transcripts occurred at day 7 for *P. acutifolius* and at day 3 or 4 for *P. vulgaris*, not at the end of the drought stress period. The RNA samples used for most of the microarray and macroarray analyses compared gene expression profiles at the end of the drought stress period. While not statistically confirmed, the apparent variability in transcript abundance among these five transcripts during the course of a drought stress cycle is likely to be a source of variability among the RNA samples used for the microarray analyses. RNA samples from roots of plants at equivalent leaf water potentials were used for the microarray experiments; there was some variability in the absolute number of days that the plants were under drought stress to reach those leaf water potentials. Replicate root samples collected from plants with similar leaf water potentials can be expected to have variable levels of drought responsive transcripts, since the abundance of these drought-responsive transcripts appeared to have unique maximal accumulation profiles.

4.1. Linking gene expression patterns to P. acutifolius phenotype in response to drought

This gene discovery process in *P. acutifolius* identified ~488 genes, which when sequenced, predicted functions that map to proposed mechanisms for drought tolerance in this plant [6,28]. For example, genes modeled to contribute to osmotic adjustment and to higher antioxidant activity were identified. On the biochemical level, the role of solutes such as proline, polyamines and changes in N metabolism has been proposed as protectant mechanisms together with higher antioxidant activity in *P. acutifolius* compared to *P. vulgaris* during drought [5,6]. In this study, transcripts for two peroxidases were induced in *P. acutifolius* at −2.20 MPa (EC911572 and EC911492). Another peroxidase was induced in *P. acutifolius* at −2.52 MPa (EC911335); none were drought responsive in *P. vulgaris*. Four lipoxygenases (EC911275, EC911285, EC911407 and EC911313) were repressed in *P. vulgaris*; none of these genes were drought responsive in *P. acutifolius*. Several enzymes related to the glutathione system showed patterns of induction or repression under both levels of water potential in *P. acutifolius* and were not detected in *P. vulgaris* (Supplementary Table).

Glyceraldehyde-3-phosphate dehydrogenase plays a role in the drought-responsive synthesis of mannitol in *Apium graveolens* [30]. *P. acutifolius* but not *P. vulgaris* roots had increased levels of transcripts for this gene in response to drought stress.

One putative aldehyde dehydrogenase (EC911696) was highly induced in both *Phaseolus* species and the same transcript was also detected as induced by macroarray analysis (Table 2). However, two other aldehyde dehydrogenases (ALDH) were only detected in *P. acutifolius*, one of them (EC911306) is a member of the subfamily 7 that is assumed to play a role in the detoxification of aldehyde [31]. Increasing ALDH represents one strategy for detoxification and stress protection in transgenic *Arabidopsis* plants and in the dessication tolerant *Craterostigma plantagineum* [32]. Another enzyme with a similar role is aldehyde reductase, which accumulates in alfalfa cells after 4–5 days of water stress and has been shown to increase the tolerance to water deficit stress in transgenic tobacco plant overexpressing this enzyme [33]. In *P. acutifolius*, 16 clones were predicted to be aldehyde reductase and all of them were highly induced with ratios between 4.7 and 2.2. Northern blot analysis confirmed the drought responsive behavior for this gene, Fig. 5 clone LR966b.

Expansins promote cell wall loosening by loosening hydrogen bonds between cellulose and all types of cross-linking glycans. They are involved in cell enlargement and other developmental processes [34,35]. In *P. acutifolius*, proton pyrophosphatase proteins were induced; this enzyme facilitates increased solute uptake to maintain cell turgor [36].

Torres et al. [4] identified several drought-responsive genes in roots of *P. vulgaris* using DDRT-PCR methods on aeroponic systems of root dehydration. Among the 42 genes identified by this group were genes in the protein processing and protein synthesis classes.

Extensins are hydroxyproline-rich glycoproteins found ubiquitously in cell walls in plants. The function of extensin is to cross-link the cell wall after elongation is completed, to make the cells less extensible [37]. A repression of extensin in drought-stressed roots suggests that cell wall cross-linking is not as extensive in drought-stressed roots as in well-watered *P. acutifolius* root cells.

Genes important for cell elongation and organ development were identified: vacuolar H^+ ATPases, plasma membrane H^+ ATPases, PEP carboxylase, sucrose synthase, sucrose transporter, K^+ transporter, ABC transporters, xyloglucanases, auxin induced proteins and expansins. Northern blot analysis confirmed the drought responsive behavior of the plasma membrane H^+ ATPase (Fig. 5, clone LR23). Some of them were found to be stage-specific and follow a pattern of induction and repression observed in other systems. A subset of these genes is down-regulated when termination of elongation or when transition from primary to secondary cell wall synthesis in pea hypocotyls and cotton fiber [38]. All these genes were drought responsive in the present study and show different patterns of expression in the *Phaseolus* spp. An expansin-related protein (EC911445) was identified as highly induced by microarray analysis of *P. acutifolius* only at −2.20 MPa while three clones with predicted function as expansins (EC911719, EC911445 and EC911266) were differentially induced in *P. vulgaris* at −2.56 MPa compared with controls.

Extensins were repressed in *P. acutifolius* at both leaf water potentials (EC911433, EC911792 and EC911798) and were not identified as drought responsive in *P. vulgaris*. Extensin stabilizes cell walls by fixing primary cell wall structure mediated by hydroxyproline residues. Endo-B1,4-glucanases are functionally related to extensins and are involved in cell wall extension and cellulose biosynthesis. Endo-B1,4-glucanase (EC911526) was drought induced (7.14 fold) and only induced in roots of *P. acutifolius*.

Many novel, unannotated genes were uniquely drought responsive in *P. acutifolius*. Determining the function of these

genes in root physiology and possibly drought resistance will be an especially valuable contribution. Breeding for drought stress resistance is difficult; the identification of genes that could be used as molecular markers for this trait will improve drought resistance breeding programs. Even though tepary and common bean are in the same genus there are significant barriers for crosses between these two species [15]. So identification of molecular tools to assist in breeding programs for drought resistance in *Phaseolus* is especially important.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.plantsci.2007.08.003.

References

[1] P.E. Verslues, S. Katiyar-Agarwal, J. Zhu, J.-K. Zhu, Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status, Plant J. 45 (2006) 523–539.
[2] R. Trejo-Calzada, M.A. O'Connell, Genetic diversity of drought-responsive genes in populations of the desert forage *Dactylis glomerata*, Plant Sci. 168 (2005) 1327–1335.
[3] E.A. Bray, Classification of genes differentially expressed during water-deficit stress in *Arabidopsis thaliana*: an analysis using microarray and differential expression data, Ann. Bot. 89 (2002) 803–811.
[4] G.A.M. Torres, S. Pflieger, F. Corre-Menguy, C. Mazubert, C. Hartmann, C. Lelandis-Brière, Identification of novel drought-related mRNAs in common bean roots by differential display RT-PCR, Plant Sci. 171 (2006) 300–307.
[5] I. Türkan, M. Bor, F. Özdemir, H. Koca, Differential responses 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 Sci. 168 (2005) 223–231.
[6] I. Lazcano-Ferrat, C.J. Lovatt, Relationship between relative water content, nitrogen pools, and growth of *Phaseolus vulgaris* L. and *P. acutifolius* A. Gray during water deficit, Crop Sci. 39 (1999) 467–475.
[7] M.A. O'Connell, The role of drought-responsive genes in drought resistance, Ag. Biotechnol. News Inform. 7 (1995) 143N–147N.
[8] A. Maggio, J.-K. Zhu, P.M. Hasegawa, R.A. Bressan, Osmogenetics: Aristotle to *Arabidopsis*, Plant Cell 18 (2006) 1542–1557.
[9] L. Rodriguez-Uribe, M.A. O'Connell, A root-specific bZIP transcription factor is responsive to water deficit stress in tepary bean (*Phaseolus acutifolius*) and common bean (*P. vulgaris*), J. Exp. Bot. 57 (2006) 1391–1398.
[10] K. Shinozaki, K. Yamaguchi-Shinozaki, Gene networks involved in drought stress response and tolerance, J. Exp. Bot. (2007) 221–227.
[11] P. Ramirez-Vallejo, J.D. Nelly, Traits related to drought resistance in common bean, Euphytica 99 (1998) 127–136.
[12] D.R. Laing, P.G. Jones, J.H.C. Davis, Common bean (*Phaseolus vulgaris*), in: P.R. Goldsworthy, N.M. Fisher (Eds.), The Physiology of Tropical Field Crops, John Wiley & Sons, NY, 1984, pp. 305–351.
[13] C. Lizana, M. Wentworth, J.P. Martinez, D. Villegas, R. Meneses, E.H. Murchie, C. Pastenes, B. Lerzri, P. Vernieri, P. Horton, M. Pinto, Differential adaptation of two varieties of common bean to abiotic stress. I. Effects of drought on yield and photosynthesis, J. Exp. Bot. 57 (2006) 685–697.
[14] C.G. Muñoz-Perea, H. Terán, R.G. Allen, J.L. Wright, D.T. Westermann, S.P. Singh, Selection for drought resistance in dry bean landraces and cultivars, Crop Sci. 46 (2006) 2111–2120.
[15] P. McClean, J. Kami, P. Gepts, Genomics and genetic diversity in common bean, in: Wilson Rf, H.T. Stalker, E.C. Brummer (Eds.), Legume Crop Genomics, AOCS Press, Champaign, IL, 2004, pp. 60–82.
[16] M. Ramírez, M.A. Graham, L. Blanco-López, S. Silvente, A. Medrano-Soto, M.W. Blair, G. Hernández, C.P. Vance, M. Lara, Sequencing and analysis of common bean ESTs. Building a foundation for functional genomics, Plant Physiol. 137 (2005) 1211–1227.
[17] A.H. Markhart, Comparative water relations of *Phaseolus vulgaris* L. and *Phaseolus acutifolius* Gray, Plant Physiol. 77 (1985) 113–117.
[18] D.F. Garvin, N.F. Weeden, Isozyme evidence supporting a single geographic origin for domesticated tepary bean, Crop Sci. 34 (1994) 1390–1395.
[19] P.N. Miklas, J.C. Rosas, L. Beaver, X. Telek, G.F. Freytag, Field performance of select tepary bean germplasm in the tropics, Crop Sci. 34 (1994) 1639–1644.
[20] C.V. Thomas, Physiological and genetics studies of drought resistance in Phaseolus *vulgaris* L. and *P. acutifolius* A. Gray. Ph.D. dissertation. University of California, Riverside (1983).
[21] P.L. Sanders, A.H. Markhart, Interspecific grafts demonstrate root system control of leaf water status in water-stressed *Phaseolus*, J. Exp. Bot. 43 (1992) 1563–1567.
[22] Brown Lab Microarray Protocols (http://cmgm.stanford.edu/pbrown/protocols).
[23] A. Zien, T. Aigner, R. Zimmer, T. Lengauer, Centralization: a new method for the normalization of gene expression data, Bioinform 17 (2001) S323–S331.
[24] D.M. Rocke, S. Lorenzato, A two-component model for measurement error in analytical chemistry, Technometrics 37 (1995) 176–184.
[25] Silicon Genetics. GeneSpring GX 7.3 http://www.sigenetics.com.
[26] S.F. Altschul, T.T. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller, D.J. Lipman, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, Nucleic Acids Res. 25 (1997) 3389–4302.
[27] A. Ruepp, A. Zollner, D. Maier, K. Albermann, J. Hani, M. Mokrejs, I. Tetko, U. Güldener, G. Mannhaupt, M. Münsterkötter, H.W. Mewes, The FunCat, a functional annotation scheme for systematic classification of proteins from whole genomes, Nucleic Acids Res. 32 (2004) 5539–5545. http://mips.gsf.de/projects/funcat.
[28] Y. Castonguay, A.H. Markhart, Saturated rates of photosynthesis in water-stressed leaves of *Phaseolus vulgaris* and *P. acutifolius*, Crop Sci. 31 (1991) 1605–1611.
[29] R.E. Sharp, V. Poroyko, L.G. Hejlek, W.G. Spollen, G.K. Springer, H.J. Bohnert, H.T. Nguyen, Root growth maintenance during water deficits: physiology to functional genomics, J. Exp. Bot. 55 (2004) 2343–2351.
[30] Z. Gao, W.H. Loescher, NADPH supply and mannitol biosynthesis, characterization, cloning, and regulation of the non-reversible glyceraldehyde-3-phosphate dehydrogenase in celery leaves, Plant Physiol. 124 (2000) 321–330.
[31] E.A. Bray, Genes commonly regulated by water-deficit stress in *Arabidopsis thaliana*, J. Exp. Bot. 55 (2004) 2331–2341.
[32] R. Sunkar, D. Bartels, H.H. Kirch, Overexpression of a stress-inducible aldehyde dehydrogenase gene from *Arabidopsis thaliana* in transgenic plants improves stress tolerance, Plant J. 35 (2003) 452–464.
[33] A. Oberschall, M. Deak, K. Torok, L. Sass, I. Vass, I. Kovacs, A. Feher, D. Dudits, G.V. Horvath, A novel aldose/aldehyde reductase protects transgenic plants against lipid peroxidation under chemical and drought stresses, Plant J. 24 (2000) 437–446.
[34] J.D. Cosgrove, Loosening of plant cell walls by expansins, Nature 407 (2000) 321–326.
[35] J. Sampedro, D.J. Cosgrove, The expansin superfamily, Genome Biol. 6 (2005) 242.

[36] R.A. Gaxiola, R. Rao, A. Sherman, P. Grisafi, S.L. Alper, G.R. Fink, The *Arabidopsis thaliana* proton ATPase, AtNhx1 and Avp1, can function in cation detoxification in yeast, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 1480–1485.

[37] N.C. Carpita, D.M. Gibeau, Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth, Plant J. 3 (1993) 1–30.

[38] B.A. Arpat, M. Waugh, J.P. Sullivan, M. Gonzales, D. Frisch, D. Main, T. Wood, A. Leslie, R.A. Wing, T.A. Wilkins, Functional genomics of cell elongation in developing cotton fibers, Plant Mol. Biol. 54 (2004) 911–929.