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RESEARCH ARTICLE

Expression of Drought Responsive Genes in Pigeonpea and *In Silico* Comparison with Soybean cDNA Library

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Abstract

Pigeonpea, a drought tolerant, semi-arid pulse crop has been investigated for the expression of differentially expressed genes (DEGs) under drought stress. The cDNA library of soybean leaf tissue retrieved from the Unigene database of the NCBI, were compared for in silico expression using IDEG6 web statistical tool. A list of 52 non-redundant DEGs consisting of 11 up-regulated and 41 down-regulated was obtained. Among these, more photosynthesis and light harvesting proteins were down-regulated in drought stress conditions. Pathways were assigned based on KEGG database, revealing 32 genes involved in 17 metabolic pathways. Homologous sequences of six up-regulated genes namely, *ADF3, APB, ASR, DLP, LTP1,* and *UGE5* were then used for quantitative reverse transcription PCR (qRT-PCR) in pigeonpea. The qRT-PCR result revealed the significant up-regulation of *dehydrin-like protein* (*DLP*) (5.02 log2 fold) and down-regulation of *acid phosphatase class B family protein* (*APB*) (9.43 log2 fold) and non-specific lipid transfer protein 1-like (LTP1) (18.81 log2 fold) in pigeonpea water-stressed leaf sample compared to well-watered leaf samples. No significant difference was observed in the stressed root compared to the stressed pigeonpea leaf sample except that *APB* showed an up-regulation of 11.35 log2 fold change.

Key words: differentially expressed genes, drought stress, pigeonpea, qRT-PCR

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) belongs to subtribe *Cajanine* of tribe *Phaseoleae* under sub-family *Papilionoideae* of the family *Leguminosae* (Varshney et al. 2010). It is a semi-arid, drought-tolerant pulse crop grown in a wide variety of soil textures ranging from sandy to heavy clays and usually cultivated under rainfed conditions in hot humid climates (Keller and Ludlow 1993; Saxena et al. 2010). India is the largest producer with a production area of 4.42 million hectares (FAOSTAT 2011). Reports indicate that pigeonpea genes hold promise for engineering crop plants bestowed with tolerance to major abiotic stresses or in multiple abiotic stresses (Priyanka et al. 2010; Sekhar et al. 2010). However, genes responsible for the drought resistant characteristics have to be evaluated in detail for the crop improvement. In contrast, detailed cDNA libraries have been studied in soybean (*Glycine max* L.) a closely related species in the phylogeny of *Papilionoideae* (Zhu et al. 2005). The reports also indicate that cDNA sequences of pigeonpea showed more homology with soybean sequences because of their phylogenetic relationship (Priyanka et al. 2010; Raju et al. 2010). Through *in silico* comparative study, it is possible to get an insight of the mechanism of drought tolerance in pigeonpea.

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Drought is one of the major abiotic stress conditions limiting the crop productivity all over the world and future climate change is predicted to exacerbate its frequency and severity due to altered rainfall patterns and higher temperatures (Setter et al. 2010). Several metabolic pathways and signaling molecules are involved in drought stress (Gong et al. 2010; Nakashima et al. 2009) that leads to stress tolerance in plants.

The quantitative reverse transcription PCR (qRT-PCR) has been widely used by the researchers (Gachon et al. 2004) for validation of suppression subtractive hybridization, cDNA library, microarray, and other high throughput studies. The differential expression of expressed sequence tags (ESTs) or genes under drought conditions have been demonstrated by qRT-PCR in many of the crop plants (Barozai and Husnain 2011; Chen et al. 2010; Lata et al. 2010). In the present investigation, *in silico* soybean cDNA libraries have been analyzed to identify the drought-responsive genes. It revealed a total of 11 up-regulated and 41 down-regulated genes. Among them, six up-regulated genes were selected and qRT-PCR was performed in pigeonpea for their response to drought stress.

Materials and Methods

In silico cDNA libraries of soybean

The Unigene database of the NCBI (http://www.ncbi.nlm.nih.gov/unigene) has a tissue-specific grouping system which is used for accessing drought stressed and unstressed libraries. Presently, 321 cDNA libraries of soybean are available in the Unigene database, of which 26 libraries belong to the leaf tissue. Six leaf cDNA libraries having more than 1,000 ESTs in each were selected and among these, five unstressed libraries was compared with a drought stressed library of soybean (Supplementary Table 1).

Identification of DEGs and tracing of metabolic pathways

The identification of DEGs in the drought stressed cDNA library was done based on ESTs count in each Unigene entry using the online IDEG6 web statistical tool (Romualdi et al. 2003; http://telethon.bio.unipd.it/bioinfo/IDEG6_form). Each Unigene entry represents a single gene in the cDNA library and singleton is a Unigene entry containing a single EST. Audic and Claverie test, Fisher exact test, and Chi-square (X^2) test (P = 0.05)were employed for the identification of DEGs. The non-redundant genes of soybean identified by IDEG6 were used for the tracing of metabolic pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/) which is a collection of online databases dealing with genomes, enzymatic pathways, and biological chemicals (Ogata et al. 1999).

Drought induction and tissue harvest

Pigeonpea seeds of cultivar Asha (ICPL 87119) variety obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India was used for the study. Plants were raised in pots containing 2:1 proportion of coarse sand and clay, maintained under greenhouse conditions (26 ± 2°C and 30-40% relative humidity).

After 20 days of sowing, water was withheld to induce water stress conditions in the testing plants while the control pots were irrigated normally. The plant water status was evaluated by the relative water content (RWC) method (Barrs and Weatherley 1962). The fully expanded leaves were weighed to get fresh weight (FW) and hydrated for full turgidity in Petri dishes containing deionized water at room temperature for 6 hours in dark conditions. After full turgidity, surface water was blot dried and weighed immediately to get the turgid weight (TW). Turgid leaf samples were then dried overnight at 65°C and weighed for dry weight (DW). Leaf RWC was calculated using the equation: RWC (%) = [(FW-DW) / (TW-DW)] × 100. The RWC was monitored on alternate days until it reached 60% with visual stress symptoms in plants. The leaves and roots from the stress-induced and control plants were harvested separately, flash frozen in liquid nitrogen, and stored at -80°C for future use.

Isolation of total RNA and first strand cDNA synthesis

Total RNA was isolated from pigeonpea samples using plant total RNA mini kit (cat#YRP50, Real Biotech Corporation, Taiwan) as per the manufacturer's protocol. Finally, the total RNA was eluted with 50 µL of RNase-free water and stored at -80°C. RNA was then assessed for quality and quantity using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, USA) with RNase-free water as blank.

The single stranded cDNA was prepared by using First Strand cDNA synthesis Kit (cat#K1611, Fermentas, USA) as per the manufacturer's protocol with Oligo[dT]$_{18}$ primers. About 2 µg of total RNA in a single 20 µL reaction was quantitatively converted to single-stranded cDNA using standard thermal conditions.

Primer design

The primer pairs were designed for pigeonpea gene-specific sequences using Primer3plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and cross validated with NetPrimer (http://www.premierbiosoft.com/netprimer/index.html) for secondary structures. All primers were synthesized at Sigma-Aldrich Pvt. Ltd. (Bangalore, India) with optimum parameters set as melting temperature (Tm) of 60 ± 2°C, primer size of 20-24 nucleotides, GC content of 45-55%, and product size of 100-150 base pairs. The specificity of primer pairs was confirmed by using BLAST analysis in NCBI against pigeonpea sequences.

qRT-PCR reaction

The reaction mixture of 10 µL containing 5 µL of VeriQuest SYBR Green qPCR Master Mix (2×) (Product number 75600, Affymetrix, USA), 1 µL of diluted cDNA, 500 nM of each gene-specific primer, and appropriate amount of sterile

ddH$_2$O was freshly mixed for the qRT-PCR experiment. PCR reactions were performed in 0.2 mL, MicroAmp® Optical 8-Tube Strips (Product number 4316567, Applied Biosystems, USA) and was done on the Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, USA) to monitor the DNA synthesis. Individual components of the reaction mixtures were standardized for 10 µL volumes and were carried out in triplicates. The qRT-PCR standard thermal cycling program of initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing and extension at 60°C for 60 s was run. Melting curve analysis was done for all the reactions and expression level was calculated by $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak 2008). The gene actin was used as a reference for normalizing the expression data to measure the response of predicted drought-responsive genes.

Results

In silico soybean cDNA library analysis

The six cDNA libraries of soybean leaf tissue selected for analysis are summarized in Supplementary Table 1. In the Gm-c1068 library, 5802 ESTs was analyzed and in the rest of the five normal libraries, the EST concentrations decreased. Comparison of soybean leaf cDNA libraries using IDEG6 revealed the differential expression of 105 genes (Fig. 1) of which 11 were up-regulated and 41 were down-regulated, non-redundant genes as shown in Table 1.

The non-redundant set of 52 genes was subjected to the KEGG database for pathway annotation. It revealed the presence of 32 genes in three major signaling pathways of which 42% were involved in photosynthesis: 29% in light harvesting/antenna molecules and 10% in carbon metabolism. In addition, 19% of genes involved in miscellaneous pathways were evidenced (Fig. 2). Among the 52 (11+ 41) DEGs predicted in soybean, six up-regulated genes were selected based on their possible involvement in drought stress and their homologous sequences were searched in pigeonpea. The reported soybean reference gene actin (GenBank accession no. GQ339774) was also subjected to Blast analysis against the pigeonpea data available in NCBI. Primer pairs were designed to pigeonpea Blast-hit sequences to verify the expression of genes in qRT-PCR (Supplementary Table 2).

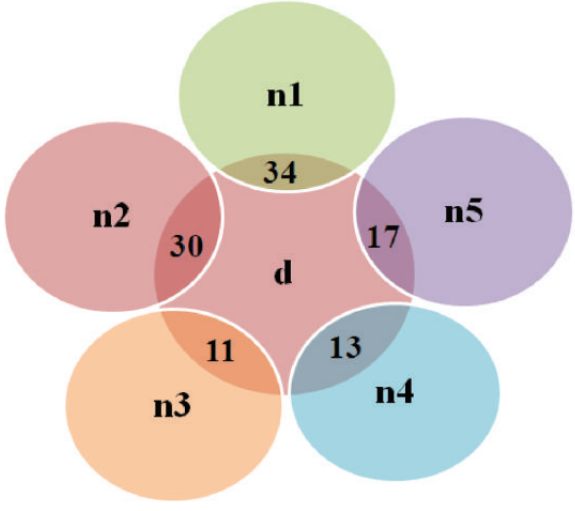

Fig. 1. Radial Venn diagram showing number of DEGs in soybean leaf cDNA libraries obtained by IDEG6. d = drought stressed library, n = normal unstressed library Gm-c1068 (d); Gm-c1054 (n1); Gm-c1050 (n2); Gm-c1014 (n3); Gm-c1037 (n4); Gm-c1018 (n5)

Stress induction and qRT-PCR

To measure the water content and stress imposed on plants, RWC was monitored up to the harvesting of tissue samples. RWC of > 90% was recorded before the induction of water stress and prior to tissue harvest of the control plant. RWC of 60% and visual symptoms of stress after withholding of water is considered as optimum stress for the collection of tissue samples. Total RNA extracted from pigeonpea samples using the RNA mini kit yielded $OD_{260/280}$ nm absorption ratio of 2.0 ± 0.1 and $OD_{260/230}$ nm absorption ratio of 1.5-2.0 in Nanodrop ND-1000 spectrophotometer and showed good integrity and purity when loaded on agarose gel. The specificity of the PCR reactions was determined by amplification plot, melting curve, and loading the qRT-PCR products on a 3.5% agarose gel. All genes yielded specific sized amplicons as predicted.

Expression of selected genes

The six up-regulated genes, namely *Actin depolymerizing factor 3 (ADF3), Acid phosphatase class B family protein (APB), Abscisic acid stress ripening-like protein (ASR), Dehydrin-like protein (DLP), non-specific lipid transfer protein 1-like (LTP1), and UDP-D-glucose/UDP-D-galactose 4-epimerase 5 (UGE5)* were selected for *in silico* analysis and synthesis of primers. The expression of these genes was analyzed in pigeonpea leaf and root samples by qRT-PCR (Table 2). *DLP* showed 5.02 log2 fold up-regulation in water-stressed leaf samples compared to well-watered leaf samples, whereas, *APB* and *LTP1* showed down-regulation of 9.43 and 18.81 log2 fold change in stressed conditions, respective-

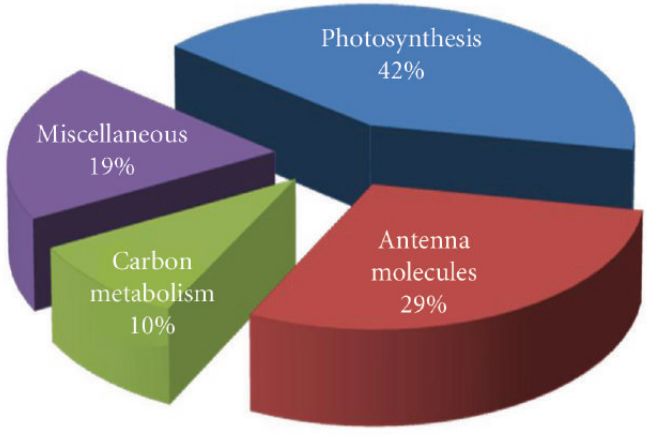

Fig. 2. Metabolic pathways of DEGs based on KEGG database.

Table 1. Differentially expressed genes predicted by statistical tool IDEG6

Sl.No	Gene	IDEG6 expression value		KEGG
		Drought	Control	
	Up-regulated genes			
1	Abscisic acid stress ripening-like protein (Asr, Gma.22054)	41.40	0.90	LOC100250281
2	Actin depolymerizing factor 3 (ADF3, Gma.30809)	24.10	0.00	AT5G59880
3	UDP-D-glucose/UDP-D-galactose 4-epimerase 5 (UGE5, Gma.5150)	24.10	0.00	AT4G10960
4	Transcribed locus (Gma.7880)	34.50	0.00	LOC100794293
5	Acid phosphatase class B family protein (APB, Gma.30703)	101.70	19.53	AT4G29260
6	Ribosomal protein L23AA (RPL23AA, Gma.5082)	58.60	7.10	AT2G39460
7	Hypothetical protein LOC100306563 (Gma.52867)	96.50	0.36	LOC100306563
8	Dehydrin-like protein LOC547842 (DLP, Gma.33428)	120.60	0.00	LOC547842
9	Maturation-associated protein (MAT9, Gma.2044)	74.10	0.00	No hits
10	Hypothetical protein LOC100306661 (Gma.37125)	32.70	0.00	LOC100306661
11	Non-Specific Lipid transfer protein 1(LTP1, Gma.16710)	153.40	35.00	AT2G38540
	Down-regulated genes			
	Photosynthesis pathway (14 genes)			
1	DNA-damage-repair/toleration (DRT112 (PETE2), Gma.2731)	5.20	63.70	AT1G20340
2	Photosynthetic electron transfer c (PETC, Gma.10843)	5.20	52.10	AT4G03280
3	Photosystem I subunit D-2 (PSAD-2, Gma.31528)	1.70	34.20	AT1G03130
4	Photosystem I subunit H2 (PSAH2, Gma.15376)	1.70	52.75	AT1G52230
5	Photosystem I subunit K (PSAK, Gma.1992)	0.00	28.50	AT1G30380
6	Photosystem I subunit L (PSAL, Gma.1316)	3.40	72.73	AT4G12800
7	Photosystem I subunit O (PSAO, Gma.22583)	5.20	40.20	AT1G08380
8	Photosystem I P subunit (PSI P, Gma.11189)	0.00	38.93	AT2G46820
9	Photosystem II subunit O-2 (PSBO2, Gma.31764)	0.00	29.35	AT3G50820
10	Photosystem II subunit P-1 (PSBP-1, Gma.31716)	10.30	60.20	AT1G06680
11	Photosystem II subunit Q (PSBQ-2, Gma.16800)	0.00	50.80	AT4G05180
12	Photosystem II subunit R (PSBR, Gma.30095)	22.40	107.95	AT1G79040
13	Non-photochemical quenching (NPQ4 (PsbS), Gma.14976)	1.70	39.15	AT1G44575
14	Photosystem II subunit BY-2 (PSBY-2, Gma.31724)	5.20	43.90	AT1G67740
	Antenna/Light harvesting molecules			
	Light-harvesting complex I (3 genes)			
15	Light-harvesting complex I encoded chlorophyll a/b binding protein 1 (LHCA1, Gma.30726)	3.40	51.30	AT3G54890
16	Light-harvesting complex I chlorophyll a/b binding protein 3 (LHCA3, Gma.31787)	5.20	58.60	AT1G61520
17	Light-harvesting chlorophyll-protein complex I subunit A4 (LHCA4, Gma.18151)	1.70	52.90	AT3G47470
	Light-harvesting complex II (7 genes)			
18	Chlorophyll a/b binding protein 1 (CAB1, Gma.4593)	15.50	132.00	AT1G29930
19	Light-harvesting chlorophyll a/b binding protein 1 (LHB1B1, Gma.12947),	3.40	110.43	AT2G34430
20	Light-harvesting chlorophyll a/b binding protein 1 (LHB1B1, Gma.31642)	1.70	45.60	AT2G34430
21	Light-harvesting chlorophyll b-binding 2.1 (LHCB2.1, Gma.16943)	3.40	57.70	AT2G05100
22	Light-harvesting chlorophyll b-binding protein 3 (LHCB3, Gma.18268)	0.00	56.90	AT5G54270
23	Light harvesting complex of photosystem II 5 (LHCB5, Gma.2360)	3.40	57.00	AT4G10340
24	Light harvesting complex of photosystem II 4.2 (LHCB4.2, Gma.31353)	15.50	82.55	AT3G08940
	Miscellaneous (17 genes)			
25	Fructose-bisphosphate aldolase (FBA, Gma.10892)	10.30	52.10	AT4G38970
26	Ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco) activase (RCA, Gma.10987)	5.20	55.40	AT2G39730
27	Glycine cleavage system (gcs) having gcs H protein (GCSH, Gma.12807)	1.70	50.80	AT1G32470
28	Chloroplast protein 12-2 (CP12-2, Gma.15488)	1.70	75.30	AT3G62410
29	Ribulose bisphosphate carboxylase small chain 3B / RuBisCO small subunit 3B (RBCS-3B, Gma.2798)	22.40	615.30	AT5G38410
30	Glyceraldehyde 3-phosphate dehydrogenase A subunit 2 (GAPA-2, Gma.31588)	8.60	52.65	AT1G12900
31	Thylakoid membrane protein of 14kDa (TMP14, Gma.31664)	3.40	44.80	AT4G01150
32	Carbonic anhydrase 1 (CA1, Gma.31761)	1.70	78.33	AT3G01500
33	Germin-like protein 1 (GER1, Gma.3208)	0.00	81.30	AT1G72610
34	(S)-2-hydroxy-acid oxidase, peroxisomal, putative ((S)-2-HAO, Gma.7309)	0.00	23.80	AT3G14420
35	Granule-bound starch synthase 1 (GBSS1, Gma.30481)	1.70	40.20	AT1G32900
36	Metallothionein-like protein-like isoform 1 (Gma.15844)	6.90	82.10	LOC100305954
37	Citryl-CoA lyase (CCL, Gma.52485)	1.70	111.35	AT3G26740
38	Glycine max cDNA, clone: GMFL01-31-E23 (Gma.11215)	1.70	41.40	LOC100796066
39	OB-fold nucleic acid binding domain-containing protein (Gma.3256)	0.00	37.30	AT1G23750
40	Thiamine4/Thiazole-requiring protein homodimerization (THI1, Gma.32369)	0.00	96.60	LOC100788953
41	Hypothetical protein LOC100306349 (Gma.7694)	0.00	35.60	LOC100306349

Color Scale: 0-5 | 5-10 | 10-25 | 25-30 | 50-100 | >100

Table 2. Expression of six genes in leaf and root samples of pigeonpea analyzed by qRT-PCR

Sl.No	Gene	Stressed leaf compared to control leaf (Log2-fold change)	Stressed root compared to stressed leaf (Log2-fold change)
1	ADF3	-3.49	-3.49
2	APB	-9.43	-9.43
3	ASR	-1.38	-1.38
4	DLP	5.02	5.02
5	LTP1	-18.81	-18.81
6	UGE5	-6.87	-6.87

('-' down-regulation)

ly. *APB* gene showed 11.35 log2 fold up-regulation in stressed roots compared to the stressed leaf sample and there was no significant difference observed in the rest of the genes in stressed roots.

Discussion

Drought resistance is a quantitative trait controlled by multiple genes and prediction of mechanism involved in drought tolerance is difficult. Due to the induction of drought stress, the aerial part of the plant shows the visual symptoms since photosynthesis and light harvesting molecules respond primarily at the larger proportion. Gene expression under abiotic stress is regulated by different elements and factors like DEGs, transcription factor encoding genes, promoters, microRNAs, chaperons etc., and finally gives rise to a composable trait which hence leads to stress tolerance. Pigeonpea is a major semi-arid pulse crop of India, consisting of many unexplored drought resistance genes for the development of drought resistant varieties. The cDNA sequences of pigeonpea showed more similarity with soybean and many investigators compared the cDNA sequences of pigeonpea with soybean sequences for the annotation purpose (Priyanka et al. 2010; Raju et al. 2010).

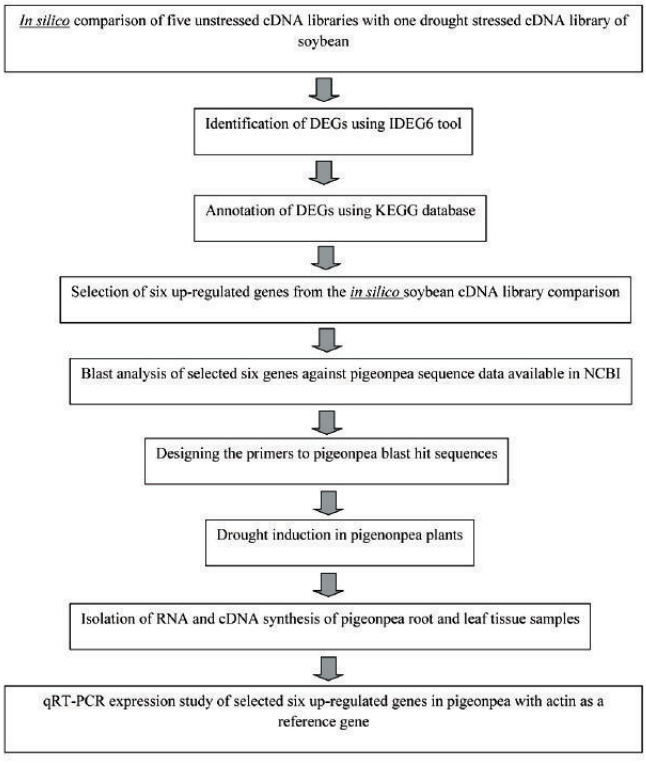

Fig. 3. Schematic workflow of the study.

In the present study, we used the *in silico* soybean cDNA library available in the Unigene database for the identification of DEGs under drought stress (Supplementary Table 1). The drought stressed Gm-c1068 and normal Gm-c1054 library (Supplementary Table 1) having nearly equal concentration of ESTs yielded 34 DEGS and some are shown to be repeated among the other libraries compared. We selected six up-regulated genes in a panel of 52 DEGs comprising 11 up-regulated and 41 down-regulated genes (Table 1). Asha (ICPL 87119) variety of pigeonpea resistant to *Fusarium* wilt and sterility mosaic disease has been used to isolate RNA from drought and well-watered leaf and root samples. RWC is a parameter often used to assess the water retention capacity of plants. This measurement was carried out by many researchers for evaluating the drought induction in crop plants including pigeonpea (Teulat et al. 1997). It is an important and often used criterion that indicates the effect or severity of water stress on plants. The Barrs and Weatherley (1962) method was followed to monitor the RWC in control and drought-stressed pigeonpea plants. The schematic representation of the workflow is shown in Fig. 3.

All six genes selected for qRT-PCR analysis showed more or less involvement in drought situations. The actin cytoskeleton is essential for a wide variety of cellular processes, ranging from cell division and morphogenesis to cell polarity, motility, and is required for polarized cell growth (Augustine et al. 2011). The ADFs are one of the groups of actin-binding proteins involved in the turnover of actin filaments that occurs via polymerization, depolymerization, severing, nucleation, as well as large-scale translocation events (Augustine et al. 2011). In *Arabidopsis*, ADF3 was induced following infection by plant nematodes and it persisted in feeding sites of the plant (Fuller et al. 2007). Proteomic analysis of rice leaves revealed up-regulation of *ADF* during drought stress (Ali and Komatsu 2006) indicating the involvement in drought stress response. In our study, the *ADF3* gene was down-regulated in pigeonpea leaf under drought stress showing its response to drought.

Phosphorus (P) is an essential macro-nutrient for plant growth which is catalyzed by Acid phosphatases (APases) through the breakdown of phosphomonoesters. The increase in secretion of APases is one of the ways that plants adapt to P deficiency. Higher expression of purple acid phosphatase in transgenics showed great potential for improving plant P acquisition and biomass yield in P-deficient agricultural soils (Ma et al. 2012). From our study, *APB* gene showed down-regulation under drought stress in pigeonpea that might be affected by stress induction. It also showed differential expression between drought-stressed leaf and root samples.

The *ASR* genes in various species are presumed to act as

part of a transcription-regulating complex involved in plant development processes such as senescence, fruit ripening, pollen maturation, and glucose metabolism. It also responds to different abiotic stress factors, including drought, salt, cold, and limited light (Liu et al. 2010). *ASR* orthologues have been cloned from a wide range of plant species including tomato, maize, pummelo, loblolly pine, apricot, pear, lily, rice, and grape (Carrari et al. 2004). Over-expression of lily *ASR* gene in *Arabidopsis* displayed a reduced sensitivity toward ABA during seed germination, dormancy, and stomatal closure. *ASR* transgenic plants exhibit markedly enhanced drought and salt resistance suggesting a dual role as a regulator as well as a protective molecule upon water deficit (Yang et al. 2005). Here it showed a slight reduction in expression under drought stress indicating less involvement during drought response.

Dehydrins are a class of hydrophilic thermostable stress proteins with a high number of charged amino acids that belong to the Group II Late Embryogenesis Abundant family, expressed during late embryogenesis. They also expressed in vegetative tissues subjected to drought, low temperature, and high salt conditions (Liang et al. 2012; Yang et al. 2012). These are key components of dehydration tolerance associated with the maintenance of protein structure and water-binding (Liang et al. 2012). Some of the members of the dehydrin gene family from grapevine species (Yang et al. 2012), barley (Tommasini et al. 2008), and *Arabidopsis* (Puhakainen et al. 2004) responded to abiotic and biotic stress. In the present investigation, *DLP* gene showed significant up-regulation in drought-stressed leaves in pigeonpea, which indicates its important role during stress adaptation.

The LTPs are a group of proteins found in plants originally identified by their ability to catalyze the transfer of lipids between membranes *in vitro*. These proteins have been attributed to transport cutin monomers, involvement in flowering, and in plant stress responses towards pathogens, drought, and temperature changes (Jung et al. 2005; Lindorff and Winther 2001). The *LTP*s were expressed in drought-tolerant plant *Prosopis juliflora* (George et al. 2007) and the transgenic plants expressing the pepper *lipid transfer protein 1* gene showed high levels of tolerance to NaCl and drought stresses at various vegetative growth stages (Jung et al. 2005). The significant down-regulation of *LTP1* in pigeonpea leaf under drought stress indicates its functional importance in drought situations.

Complex carbohydrate synthesis in plants requires enormous machinery that is mediated by the activities of different carbohydrate acting enzymes (Scheible and Pauly 2004). UDP-glucose 4-epimerase (UGE) is a family of five UGE isoforms encoded in the *Arabidopsis* genome which freely inter-converts UDP-glucose and UDP-galactose and shows *in vitro* variations in substrate affinity, cofactor requirement, and metabolite inhibition profile (Barber et al. 2006). The UGE5 is co-regulated with carbohydrate biosynthetic enzymes and contributes non-specifically to UGE activity and growth under unstressed conditions but might be more specifically involved in stress situations (Roesti et al. 2007). It showed down-regulation in drought-stressed leaf samples of pigeonpea specifying its involvement in drought conditions.

Conclusion

Water deficit has found to alter plant gene expression and led to specific gene induction (Ingram and Bartels 1996). In the present study, we performed an *in silico* analysis and investigated the expression of six genes in pigeonpea using qRT-PCR. The *DLP* gene showed up-regulation whereas *APB, LTP1*, and *UGE5* showed down-regulation in leaf samples. The *in silico* ESTs analysis between the drought-stressed and unstressed leaf library has shown significant decrease in efficiency with respect to light harvesting and carbon fixation pathways which results in reduced yield limiting the crop productivity. Furthermore, confirmation of the *DLP* gene by transgenic lines will determine its functional involvement in stress tolerance. The drought stress genes and their regulatory networks in pigeonpea have immense importance which can be analyzed in detail for stress resistance mechanism and its applications in crop improvement.

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Supplementary Table 1. Six cDNA libraries of soybean leaf tissue used for *in silico* analysis

Name	BioSample ID	Tissue	ESTs	Unigenes	Singletons
Gm-c1068[d]	LIBEST_007137	Leaf, drought stressed, 1-month old plants, greenhouse-grown plants	5,802	2,971	683
Gm-c1054[n]	LIBEST_006820	Leaf, 3-week old, greenhouse-grown plants	5,472	2,406	444
Gm-c1050[n]	LIBEST_006109	Leaf tissue at various developmental stages of greenhouse-grown plants	3,512	1,636	274
Gm-c1014[n]	LIBEST_001957	Leaves, 2-3-week old seedlings, greenhouse-grown plants	2,413	1,021	331
Gm-c1037[n]	LIBEST_004133	Fully expanded leaves of greenhouse-grown plants	1,967	1,109	202
Gm-c1018[n]	LIBEST_002233	Leaves of greenhouse-grown plants	1,461	867	228

[d] = drought stressed library, [n] = unstressed normal library

Supplementary Table 2. List of primer sequences used for qRT-PCR expression study

Name	Gene	Forward	Reverse	Product size (bp)
1	Abscisic acid stress ripening-like protein (ASR)	TGCCGATGACTACGACTCTG	CGGAAGGGTCATCAGAAGAA	113
2	Actin depolymerizing factor 3 (ADF3)	CATAAACAGCATAGCGGCACT	CAGAAGCAAGTCGTTGTGGA	100
3	UDP-D-glucose/galactose 4-epimerase 5 (UGE5)	TCACGGCACATCTCATCAAT	CGTAGACCTGGTGATGCTGA	101
4	Acid phosphatase class B family protein (APB)	GGTCTCCAATGTTTCCAATGAT	CCACTGTCACTACTGCCAACTT	168
5	Dehydrin-like protein (DLP)	GGTTATGGAGGCAACACTGG	TATCCCTTTCTTCTCGTGATCG	114
6	Lipid transfer protein 1 (LTP1)	ACCATAGTAGTGAGGGGTGTGC	GAAACCAGAAACACGCACAAT	120
7	Actin	TTGGACTCTGGTGATGGTGT	TCAGCAGAGGTGGTGAACAT	158

[d] = drought stressed library, [n] = unstressed normal library