ORIGINAL ARTICLE

Exploring drought stress-regulated genes in senna (*Cassia angustifolia* Vahl.): a transcriptomic approach

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Abstract De novo assembly of reads produced by next-generation sequencing (NGS) technologies offers a rapid approach to obtain expressed gene sequences for non-model organisms. Senna (*Cassia angustifolia* Vahl.) is a drought-tolerant annual undershrub of Caesalpiniaceae, a subfamily of Fabaceae. There are insufficient transcriptomic and genomic data in public databases for understanding the molecular mechanism underlying the drought tolerance of senna. Therefore, the main purpose of this study was to know the transcriptome profile of senna, with special reference to drought stress. RNA from two different stages of leaf development was extracted and sequenced separately using the Illumina technology. A total of 200 million reads were generated, and a de novo assembly of processed reads in the pooled transcriptome using Trinity yielded 43,413 transcripts which were further annotated using NCBI BLAST with "green plant database (txid 33090)," Swiss Prot, Kyoto Encyclopedia of Genes and Genomes (KEGG), Clusters of Orthologous Groups (COG), and Gene Ontology (GO). Out of the total transcripts, 42,280 (95.0 %) were annotated by BLASTX against the green plant database of NCBI. Senna transcriptome showed the highest similarity to *Glycine max* (41 %), followed by *Phaseolus vulgaris* (16 %), *Cicer arietinum* (15 %), and *Medicago trancatula* (5 %). The highest number of GO terms were enriched for the molecular functions category; of these "catalytic activity" (GO: 0003824) (25.10 %) and "binding activity" (GO: 0005488) (20.10 %) were most abundantly represented. We used InterProscan to see protein similarity at domain level; a total of 33,256 transcripts were annotated against the Pfam domains. The transcripts were assigned with various KEGG pathways. Coding DNA sequences (CDS) encoding various drought stress-regulated pathways such as signaling factors, protein-modifying/degrading enzymes, biosynthesis of phytohormone, phytohormone signaling, osmotically active compounds, free radical scavengers, chlorophyll metabolism, leaf cuticular wax, polyamines, and protective proteins were identified through BLASTX search. The lucine-rich repeat kinase family was the most abundantly found group of protein kinases. Orphan, bHLH, and bZIP family TFs were the most abundantly found in senna. Six genes encoding MYC2 transcription factor, *9-cis-epoxycarotenoid dioxygenase* (NCED), *L-ascorbate peroxidase* (APX), *aminocyclopropane carboxylate oxidase* (ACO), *abscisic acid 8'-hydroxylase* (ABA), and WRKY transcription factor were confirmed through reverse transcriptase-PCR (RT-PCR) and Sanger sequencing for the first time in senna. The potential drought stress-related transcripts identified in this study provide a good start for further investigation into the drought adaptation in senna. Additionally, our transcriptome sequences are the valuable resource for accelerated genomics-assisted genetic improvement programs and facilitate manipulation of biochemical pathways for developing drought-tolerant genotypes of crop plants.

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Introduction

Plants are exposed to numerous stresses, abiotic and biotic. Both of these stresses have substantial impacts on plant growth and productivity. Abiotic stress caused by several factors including supra-optimal (high temperature) or sub-optimal (low temperature), excess water or water deficient, increased salt levels, increased chemicals, increased light incidence, and increased levels of pollutants. Often, stress conditions are compounded in the field due to occurrence of more than one stress at the same time. Among the many abiotic stresses, drought stress is one of the most important environmental stresses affecting the productivity of most crop plants. About 26 % of world's arable land is affected by drought stress (Blum 1988). The effects of drought on crop plants are complex, variable, and accentuated by a number of interacting factors. Drought delays plant development and affects morphology, as well as physiological processes such as photosynthesis, respiration, and translocation of assimilates (Do et al. 2013). Hence, it is important to understand the effects of drought stress on plants and identify crops tolerant to stressful environments in order to increase crop productivity and to mitigate food crisis without expending cultivated lands.

Plant species have developed diverse strategies to adapt and thrive in all kinds of climates. Drought avoidance and drought tolerance are the strategies by which a crop can minimize the loss in yield under drought stress. Drought avoidance can be achieved through morphological changes in plants, such as decreased stomatal conductance, reduced leaf area, and extensive root systems (Levitt 1980; Budak et al. 2013; Rama Reddy et al. 2014). Drought tolerance is achieved by physiological and molecular mechanisms, including osmotic adjustment, antioxidant, and scavenger compounds (Bartels and Sunkars 2005). These strategies are supported by rich and complex metabolic and gene networks that enable the plant to synthesize a wide range of compounds (Yamaguchi-Shinozaki and Shinozaki 2006; Shanker et al. 2014). Plant responses to drought stress involve interactions and crosstalk between many molecular pathways (Kantar et al. 2011). High-throughput screening techniques such as transcriptome sequencing have been used to study the adaptability of plants to drought (Ergen and Budak 2009; Raney et al. 2014; Thumma et al. 2012; Bowman et al. 2013; do Amara et al. 2016). This has led to identification of many genes related to drought stress (Zhang et al. 2013; Zhou et al. 2012; Akpinar et al. 2013; Dong et al. 2014). However, few natural allelic variants have been cloned for drought-related traits, so transcriptome analysis, quantitative trait loci (QTL), and other trait isolation methods are much essential to improve methodology for exploring drought tolerance which is a complex trait. Elucidation of the complex molecular mechanisms underlying drought tolerance in crops will accelerate the development of new varieties with enhanced drought tolerance.

The Caesalpiniaceae, a subfamily under Fabaceae, is a large family with several economically important species produceing economically important products, such as drugs (*Cassia angustifolia*, *Cassia tora*, *Cassia italica*, *Saraca asoca*), sour preparation (*Tamarindus indica*), dyes (*Caesalpina sappan*), timber (*Cassia fistula*), and ornamentals (*Bauhinia purpuria*, *Delonix regia*, *Poinciana pulchrrima*). Senna (*Cassia angustifolia*) ($2n = 28$) is a drought-tolerant annual undershrub of Caesalpiniaceae (Ayoub 1977; Khalid et al. 2012). It can survive arid environments by maintaining its water content under severe stress conditions. It is one of the important medicinal crops in the world and included in the pharmacopeia of USA, Germany, UK, India, and many other countries mainly for its cathartic properties (Lemli 1986; Folkard 1995). Medicinal properties are due to presence of sennosides in the leaves and pods (Hammouda et al. 2005). Senna is native of Yemen and Hardramaunt province of Saudi Arabia (Abulafatih 1987; Ghazanfar and Al-Sabahi 1993). The species grows in arid areas of Sudan and Egypt (Hammouda et al. 2005) and cultivated commercially in arid parts of Rajasthan, Gujarat, and Tamil Nadu in India.

Senna is a plant suitable to study the genes related to drought tolerance ultimately leading to enhancement of drought tolerance in legume crops through genetic manipulation. Physiological, developmental, and morphological characteristics collectively confer drought stress tolerance in senna (Ratnayaka and Kincaid 2005). These include maintenance of high carbon gain and water efficiency over a wide range of stress levels, drought deciduousness with re-allocation of resources to apical leaves, plasticity of stomatal and trichome numbers on a given leaflet surface, densely deposited epicuticular wax, isobilateral leaf anatomy with large bundle sheath extensions, and paraheliotropic leaflet movement. Also, production of various drought stress-related proteins and enzymes probably aids in the tolerance to drought stress in senna (Ratnayaka and Kincaid 2005). Abiotic stress-related enzymes like glutathione reductase, catalase, superoxide dismutase, and osmoprotectant proline production were enhanced in response to drought stress (Khammari et al. 2012), salt stress (Agarwal and Pandey 2004), and increased heavy metals (Qureshi et al. 2007) in senna.

Abiotic stress-tolerant plants are a potential source of genes for further research and breeding of stress-tolerant plants. Senna is considered as an excellent model plant to study plant adaptation to abiotic stress and have a deeper understanding of genetic control and strategies for adaptation to dry environment in Fabaceae. However, this objective is difficult to achieve due to lack of genomic sequence information of senna. Fortunately, availability of next-generation sequencing (NGS) has made sequencing affordable and reliable and has led to availability of transcriptomes of non-model organisms as well. RNA-Seq is a method of choice for study of drought adaptation and biological features in non-model plants (Hirayama and Shinozaki 2010; Van Eck et al. 2014; Singh et al. 2016). RNA-seq has been used

to study abiotic stress response genes in many plants such as parsley (Li et al. 2014a, b), common bean (Hiz et al. 2014), chrysanthemum (Xu et al. 2013), tall fescue (Hu et al. 2014), and grapevine (Rocheta et al. 2014).

In the present study, leaf transcriptome of senna was sequenced by next-generation sequencing (NGS) technology to implicate specific pathways putatively associated with drought stress tolerance. This study for the first time provides a whole dataset of genes expressed in senna along with the glimpse of biochemical pathways in this uniquely drought-tolerant member of Fabaceae and thus will provide a base for understanding the molecular mechanism underlying drought stress tolerance.

Material and methods

Total RNA isolation, library construction, and deep sequencing

Developing leaf samples were collected at flowering stage from *Cassia angustifolia* (var. Sona) plants grown in the experimental farm at the ICAR-Directorate of Medicinal and Aromatic Plants Research (ICAR-DMAPR), Anand, Gujarat, India, in the year 2013. RNA from the each sample was isolated using RaFlex Total RNA isolation kit (Merck Millipore, MA, USA) by using the standard protocol described by the manufacturer. The yield and quality of RNA were determined using a Nanodrop-8000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). For RNA library construction and deep sequencing, equal quantity of RNA from young and mature leaves was used. The samples were sequenced using Illumina Miseq platform (Illumina®, San Diego, CA, USA).

Raw data processing

Next-generation sequencing using illumina Miseq generated raw data in FASTQ format. Quality of the raw data was validated based on Phred quality score (Q). This raw data was filtered and trimmed for low-quality score reads; adaptor and primer sequences were removed and reads less than 40 bp was removed using Trimmomatic v0.30 (Bolger et al. 2014). Sequencing data with Phred quality score $Q \geq 20$ was further used assembly of raw data.

De novo assembly

High-quality data obtained after filtration from young and mature leaf samples were pooled to serve as a representative transcriptome and were provided as an input for transcriptome assembly using a Trinity RNA-Sequence assembler (Version 2013) (Grabherr et al. 2011) on optimized parameters and K-mer size set to 25. CLC

Genomics workbench (CLC Bio, Boston, MA 02108 USA) was used to validate the assembled transcript contigs by mapping high-quality reads back to the assembled transcript contigs. To identify the coding DNA sequences (CDS) from assembled transcript contigs, an online tool ORF-Predictor (Min et al. 2005) (http://proteomics.ysu.edu/tools/OrfPredictor.html) with the default parameters was used.

Annotation

All the predicted CDS were functionally annotated by aligning to green plant database (txid 33090) of NCBI using basic local alignment search tool (BLASTX) (Altschul et al. 1990) with an E value threshold of $1e^{-06}$. Functions of predicted CDS were classified and each CDS was provided with the ontology of defined terms using gene ontology (GO) assignment and mapping. GO terms for all the BLASTX functionally annotated CDS was retrieved using GO mapping. CDS were categorized into 45 functional groups by WEGO analysis which involved sketching a WEGO plot based on GO hits. To retrieve GO terms for annotated CDS, the GO mapping used defined criteria. This included use of (i) BLASTX result accession IDs to retrieve gene names or symbols, (ii) UniProt IDs, and (iii) direct search in the dbxref table of the GO database. Gene names or symbols thus identified were then searched against the species-specific entries of the gene-product tables in GO database. To retrieve UniProt IDs, Protein Information Resource (PIR) was used. PIR includes protein sequence database (PSD), UniProt, SwissProt, TrEMBL, RefSeq, GenPept, and PDB databases. Using GO analysis, all the annotated nodes comprising GO functional groups were specified.

All predicted CDS were annotated against protein database so as to assign putative function of the transcriptome after translation into protein. CDS were searched against the non-redundant protein sequences available in the Uni-ProtKB/SwisProt database using BLASTX with an E value threshold of $1e^{-06}$. The CDS were categorized in 24 functional clusters of phylogenetically widespread domain families of proteins by comparing the CDS to Clusters of Orthologus Groups (COG) protein database. For higher-level groupings of related protein families, also known as clans and the identification of domains that occurs within proteins, transcripts were compared against Pfam database. For the identification of transcription factors, predicted CDS were searched against all the transcription factor and protein kinases at Plant transcription factor database (http://planttfdb.cbi.pku.edu.cn/) using BLASTX with an E value cut-off of $<1e^{-05}$ (Jin et al. 2014).

KEGG mapping

KEGG automatic annotation server (KAAS) (Moriya et al. 2007) was used for ortholog assignment and mapping of

CDS to metabolic pathways. BLASTX with threshold bit-score value of 60 (default) was used to search all CDS against the KEGG database. The CDS mapped in 24 different functional KASS pathway categories represented different enzymes involved in different metabolic pathways. KAAS (version 1.6) (http://www.genome.jp/tools/kaas/) with default parameters was used to perform the KEGG orthology (KO) assignment reconstructions. KASS carry out functional annotation of genes by BLAST comparison of CDS against the manually curated KEGG genes database. Thus, CDS were annotated by KEGG Orthology (KO) assignments and also were mapped to various KEGG metabolic pathways.

Mining genes involved in drought stress response

To identify putative CDS/transcripts involved in drought stress response, the literature was searched for functional genes, transcription factors and enzymes involved directly or indirectly in drought stress regulation. Orthologs of such genes were identified in the annotated transcripts and CDS of senna using various databases.

RT-PCR and Sanger sequencing

Sequences of CDS of six genes known to be involved in drought stress tolerance were used to design gene specific primers so as to assess the quality as well as precession of assembly and annotation. cDNA was used as a template for amplification using these primers. Total RNA was extracted from leaf using plant RNA-isolation kit (GeNei, Bangalore, India) according to manufacturer's protocol. To check the quality of RNA, 8 μl of isolated RNA sample was loaded after adding 2 μl of 5× formaldehyde RNA loading dye (Thermo Scientific) and heat denaturation at 65 °C for 10 min, was loaded on 1 % denaturing agarose gel prepared in 1X MOPS buffer and formaldehyde. Gel electrophoresis was carried out using 1X MOPS buffer prepared in DEPC treated water. First strand cDNA was synthesized using M-MuLV RT-PCR kit (GeNei, Bangalore, India) with oligo-dT primer and 4 μl of isolated RNA as template according to manufacturer's protocol. Gene specific primers were designed using transcriptome sequence was used to amplify six drought related genes using first strand cDNA as template in a 50 μl system. Touchdown PCR using conditions of 95 °C for 5 min, 95 °C for 15 s, 61 °C for 20 s (−1 °C/cycle), 72 °C for 1 min 30 s, 95 °C for 10 s, 54 °C for 20 s, 72 °C for 30 s repeated for 30 cycles and final extension 72 °C for 20 min was carried out. Amplified product was loaded on 1.5 % agarose gel. Band of amplified product was extracted from gel using Gel extraction kit (GeNei, Bangalore, India) according to manufacturer's protocol. Extracted amplified products were sequenced using Sanger sequencing (Eurofins Scientific, India). The sequence

obtained was compared to predicted CDS sequence. Sequence was BLAST searched against existing sequence on NCBI to confirm the identity of amplified sequence. Sequences were deposited in GeneBank of NCBI.

Results and discussion

Sequencing and assembly

We sequenced two cDNA library from two different stages of leaf development using Illumina Mi-Seq platform. Sequencing data included 2,20,26,329 raw reads containing 6,34,59,92,202 nucleotide bases (Table 1). The raw paired-end sequencing data in FASTQ format was deposited in the National Center for Biotechnology Information (NCBI) BioProject database (as Short Read Archive) under accession number PRJNA273534. A pooled data set was created by combining the reads from two libraries and subjected to *de novo* assembly. The transcriptome shotgun assembly project was deposited at DDBJ/EMBL/GenBank under the accession GEEB00000000. The version described in this paper is the first version, GEEB01000000. The assembly using Trinity yielded 43,413 non-redundant transcript contigs after filtering out those shorter than 200 bases. The details of the pooled transcriptome are provided in Table 1. The total transcript length was 72,058,285 bases (72.0 Mb), with average transcript length of 1659 bases. The GC content of transcripts was 41.96 %, which was marginally lower than

Table 1 Transcriptome reads, assembly, and CDS statistics

Read statistics	Number
Total reads	22,026,329
Total nucleotides (bp)	6,345,992,202
Mean read length (bp)	143.5
Max read length (bp)	150
Assembly statistics	
Number of transcript contigs	43,413
Maximum length of transcript contig	8715
N$_{50}$ value	1697
GC%	41.96
Total transcript contig length (in bases)	72,058,285
Average transcript contig length (in bases)	1659
Total number of Ns	0
Transcript contig > 500b	43,413
Transcript contig > 1Kb	43,362
Transcript contig > 10Kb	0
CDS statistics	
Total number of CDS	43,413
Maximum length of CDS (in bases)	8691
Minimum length of CDS (in bases)	201

AT content of 58.04 %. The size distribution of transcripts ranged from more than 1000 bp to 3500 bp and above (Table 1), wherein the maximum number of transcripts i.e., 21,192 transcripts had size in the range of 1000–1499 followed by 12,950 transcripts, size of which was in the range of 1500–1999 bp (Figure S1). Maximum contig length was 8715 bp with an average contig length of 1659 bp (Table 1). The N_{50} of assembly was 1697 bp which is slightly higher than that reported in senna (Rama Reddy et al. 2015) and is higher than most of the recently published plant transcriptome assemblies like *Phaseolus vulgaris* L. (1449 bp) (Hiz et al. 2014), *Salvia hispanica* L. (1338 bp) (Sreedhar et al. 2015), *Raphanus sativus* (773 bp) (Wu et al. 2015), *Gentiana rigescens* (1384 bp) (Zhang et al. 2015a, b, c), *Camelia sinensis* (521 bp) (Wang et al. 2016) and *Codonopsis pilosula* (1243 bp) (Gao et al. 2015) indicating good transcriptome assembly. In medicinal plant *Cassia obtusifolia*, the transcriptome sequencing of seed using Illumina and its assembly yielded 40,102 unigenes with average length of 681 bases (Liu et al. 2014). The assembly was validated by mapping high quality reads back to the assembled transcript contigs using CLC Bio Genomics workbench, 82.72 % of reads, leaf library was mapped to the transcript thereby suggesting that the assembly was highly valid. However, all but 17.28 % of reads from the leaf transcriptome library were not mapped to transcript contigs, which might be due to the presence of certain very low expressing transcripts, the reads for which might either be partially assembled or left out completely during the assembly process. This might lead to small portion of reads that are not used in the transcript contigs assembly. Identification of coding DNA sequences (CDS) for assembled transcript contigs lead to prediction of a total of 43,413 CDS from the total transcripts. The maximum CDS length was found to be 8691 bp, whereas minimum CDS length was 201 bp (Table 1). The size distribution CDS ranged from 200 bp to 1000 bp and above, wherein, the maximum number of CDS were in range of 1000 and above bp (19,312 CDS) which was followed by 4079 CDS and 4065 CDS in range of 800–899 and 900–900 respectively (Figure S2).

Functional annotation

We first annotated the assembled senna unique transcripts through homologous search against green plant database (txid 33090) of NCBI using BLASTX search and threshold E-value as $1e^{-06}$. A total of 42,280 CDS (97.39 %) which had significant BLAST hits. Based on BLASTX annotation, top hit species with senna transcriptome showed highest similarity to *Glycine max* (41 %), followed by *Phaseolus vulgaris* (16 %), *Cicer arietinum* (15 %), and *Medicago trancatula* (5 %) (Fig. 1). Gene ontology (GO) was used for classification of CDS into functional categories by Blast2GO analysis. Function of predicted CDS were classified and each CDS were provided with ontology of defined terms. GO terms were enriched for 26,326 CDS and were grouped into three main domains: Biological process, Molecular function and Cellular component (Table 2 and Fig. 2). For the annotated CDS, a total of 55,318 GO terms were enriched using GO assignment. The number of enriched terms were more than that total number of CDS which is due to overlapping i.e., multiple CDS assigned to one GO term and the single CDS can have multiple GO terms. In biological processes category, 20,776 CDS were enriched, while 21,763 CDs were enriched in molecular functions category and 12,779 CDS were enriched in cellular components category (Table 2). CDS were categorized into 47 functional groups by WEGO analysis which involved making of WEGO plots based on GO hits (Fig. 2). In the biological process category, highest number of CDS were enriched in metabolic process (GO:0008152) (21.77 %) group, followed by cellular process (GO:0009987) (20.12 %) group. In the molecular function category, "catalytic activity" (GO: 0003824) (25.10 %) and "binding activity" (GO: 0005488) (20.10 %) were most abundantly represented. This indicates that the diverse metabolic processes are active in the *C. angustifolia* leaf, and a variety of metabolites synthesized. Under the cellular component category, the highest number of CDS were associated with "cell" (GO: 0005623) (26.10 %) and "cell part" (GO: 0044464) (21.10 %). Extremely low percentage of genes were classified in terms of "protein tag" (GO: 0031386), "locomotion" (GO: 0040011), "metallochaperone" (GO: 0016530) and "viral reproduction" (GO: 0016032).

We searched CDS against the non-redundant protein sequences available in the Uni-ProtKB/SwisProt database using BLASTX with E-value threshold of $1e^{-06}$ and their putative protein functions were predicted. A total of 33,256 (76.6 %) CDS showed significant hits thus indicated overall gene conservation across species. In addition, many CDS were annotated as unknown, hypothetical and expressed proteins as the CDS showed homology to uncharacterised proteins.

A total of 17,966 CDS were categorized in 24 functional clusters of phylogenetically wide spread domain families of proteins by comparing the CDS to the Clusters of Orthologous Groups (COG) protein database (Fig. 3). The highest number of CDS were represented under "General Functional Prediction only [R]" followed by "Secondary Structure [O]" and "Carbohydrate metabolism and transport [G]." The lowest number of CDS was represented under "Cell motility [N]" and "Cytoskeleton [Y]."

InterProscan was used to see protein similarity at domain level. Transcripts were annotated against Pfam domains and 37,872 transcripts were annotated (Fig. 4). The pentatricopeptide repeat (PPR) (PF01535.15) domain represented the most (4880 transcripts) which was followed by PPR_3 (PF13812.1) (4526 transcripts), PPR_2 (PF13041.1) (4293 transcripts), and PPR_1 (PF12854.2) (3985 transcripts). Other domains frequently represented in the leaf library

Fig. 1 BLASTX top hit species distribution of transcript contigs in th leaf transcriptome of senna
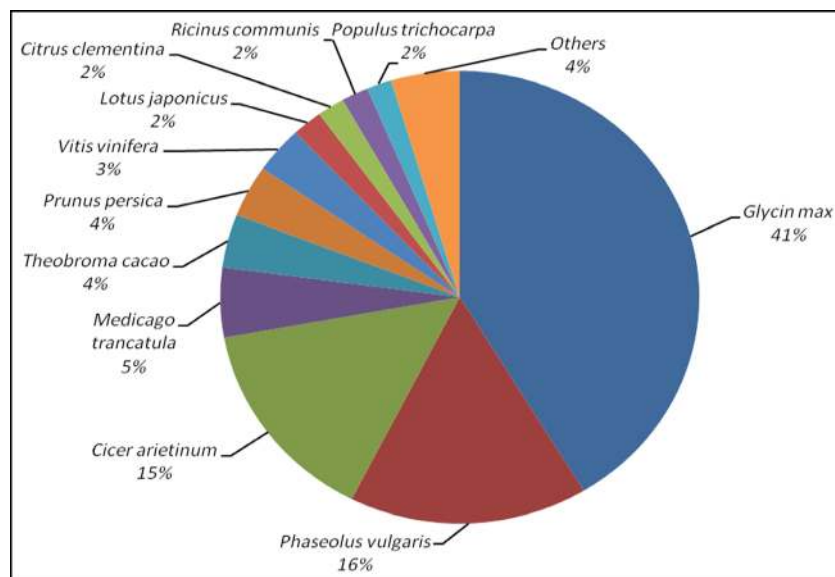
include LRR_1 (PF00560.28) (3705 transcripts), LRR_6 (PF13516.1) (3469 transcripts) TPR_14 (PF13428.1) (3382 transcripts), LRR_7 (PF13504.1) (3307 transcripts), WD40 (PF00400.27) (3203 transcripts), and LRR_4 (PF12799.2) (2952 transcripts) in the transcripts indicating strong signal transduction mechanisms.

KEGG pathway mapping

KEGG automatic annotation server (KAAS) was used for ortholog assignment and mapping of CDS to metabolic pathways. BLASTX with threshold bit-score value of 60 (default) was used to search all CDS against the KEGG database. The CDS mapped in 24 different functional KASS pathway categories represented different enzymes involved in metabolic pathways. A total of 8250 CDS were enriched in functional KASS pathway categories (Table 3). All CDS were assigned to 191 KEGG pathways (Table S1). The highest number of CDS was represented under Translation (846) and Carbohydrate metabolism (744) pathway category indicating that many active metabolic processes occurred in senna leaves, while the least number of CDS was represented under "signal molecules and interaction" and membrane transport. Under environmental adaptation category, 201 CDS were represented.

Genes encoding enzymes involved in drought stress response

Drought stress triggers a wide variety of plant responses, including alterations in gene expression, the accumulation of secondary metabolites or osmotically active compounds, and the synthesis of specific proteins and others (Ramchandra Reddy et al. 2004; Ergen et al. 2009). The ecotypic expression or

Table 2 Distribution of BLAST results of senna CDS

| Description	Number of CDS
Number of CDS with BLAST hits	42,280
Number of CDS without BLAST hits	1133
GO distribution of BLAST hits—biological processes	20,776
GO distribution of BLAST hits—molecular functions	21,763
GO distribution of BLAST hits—cellular component	12,779
Database	
Green plant database	42,280
Uni-ProtKB/SwisProt	33,256
COG	17,966
Pfam	37,872
PlantTFDB	10,833
KEGG	8250
iTAK	2060
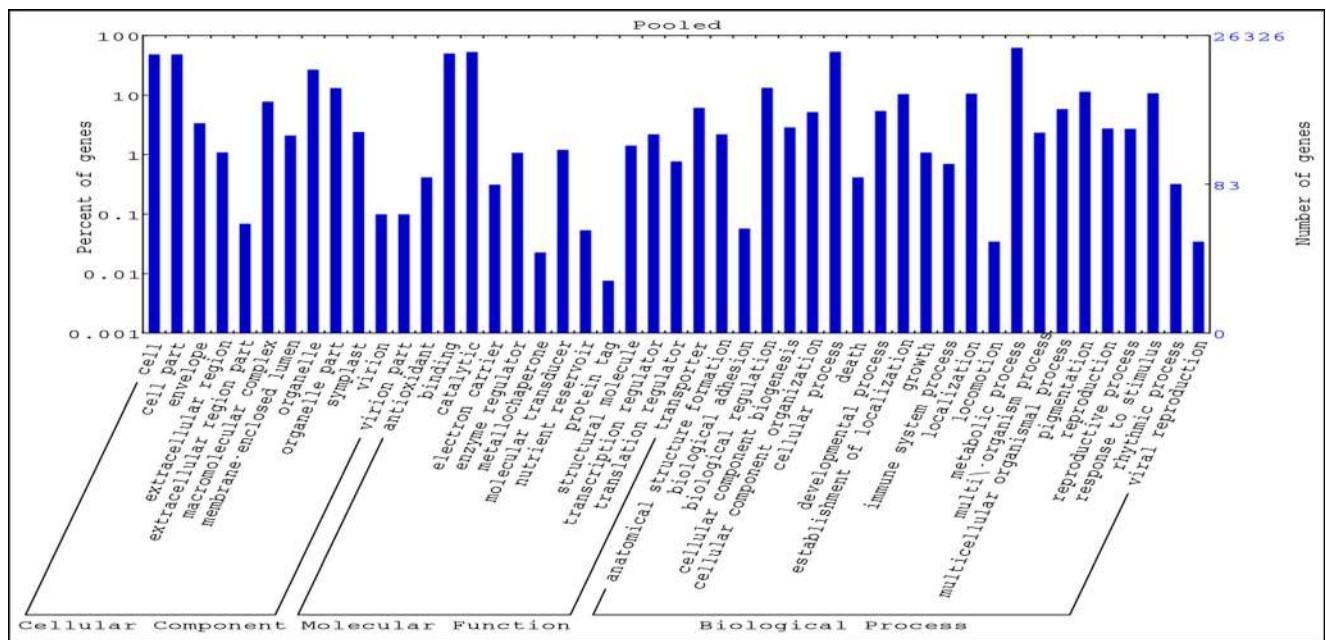
Fig. 2 GO Classification. GO terms were derived based on the similarity search within leaf CDS in the transcriptome of *Cassia angustofolia*

suppression of regulatory genes could potentially activate multiple mechanisms of drought tolerance. Signaling factors, protein-modifying/degrading enzymes, biosynthesis of phytohormones (abscisic acid, ethylene, jasmonate, salicylic acid, brassinosteroid, gibberellic acid, and nitric oxide), phytohormone signaling (ABA, abscisic acid, ethylene, jasmonate, salicylic acid, brassinosteroid, gibberellic acid, and auxin),

biosynthesis of osmotically active compounds (proline, glycin betaine, trehalose, sorbitol, mannitol, and galactinol), synthesis of free radical scavengers (catalase, peroxidase, antioxidant enymes, glutathionine, ascorbic acid, and phytochelatin), chlorophyll biosynthesis and degradation, leaf cuticular wax biosynthesis, polyamine biosynthesis, protective proteins (heat shock proteins, LEA proteins, chaprones, osmatin, and aquaporin), and
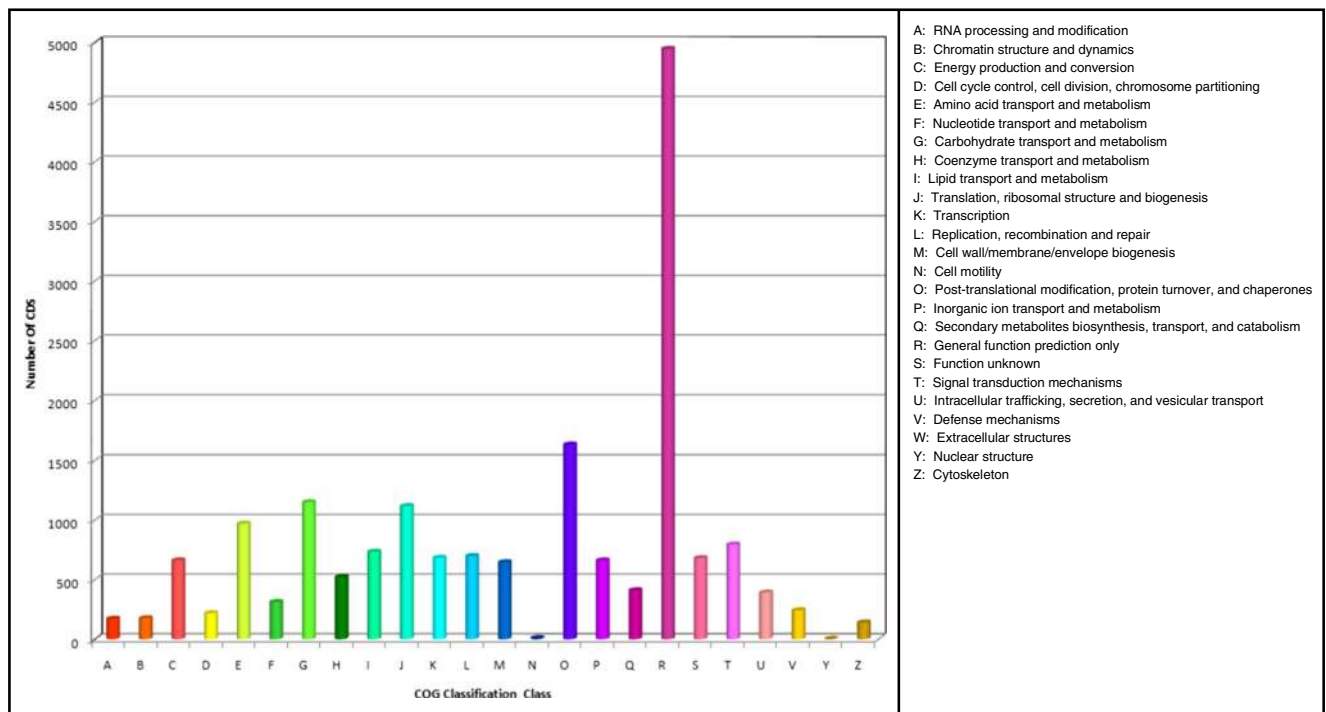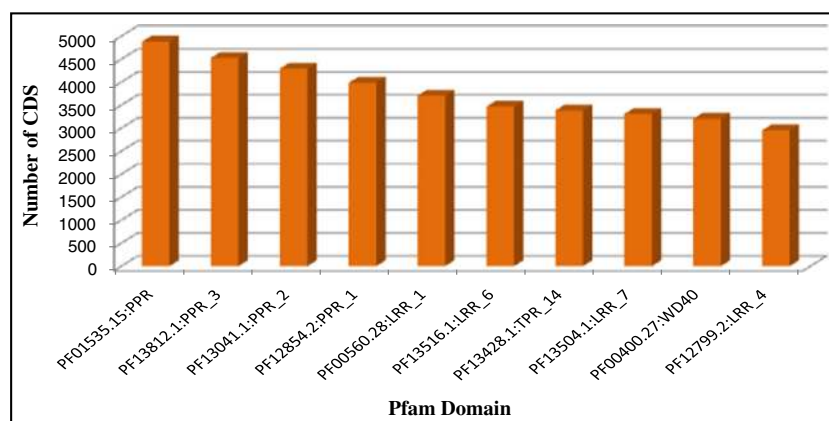

Fig. 3 Clusters of orthologous groups (COG) functional classification of CDS predicted in the senna transcriptome

Fig. 4 Top 10 Pfam domains represented in InterProScan transcript annotations of the *Cassia angustifolia* leaf transcriptome

others have been exploited for engineering drought tolerance in plants (Hu and Xiong 2014; Umezawa et al. 2006).

Drought signaling factors in senna

Plants have developed complex and efficient signal transduction networks to cope with the continual challenges of an unfavorable environment especially during drought stress. Protein kinases, transcription factors, and others such as cyclin-dependent protein kinase, ATPase/hydrogen-

translocating pyrophosphatase (AVP1), and ABA catabolism (Chl-NADP-ME) are major signaling factors involved in drought stress signaling (Akpinar et al. 2012).

Protein kinases

Protein kinases play essential roles in developmental and environmental signal transduction in plants (Rodriguez et al. 2010; Liu et al. 2016). Protein kinases activate transcription factors and drought-responsive proteins through post-

Table 3 KEGG categories of CDS in the leaf transcriptome

KEGG process	Category	Number of CDS
Metabolism	Carbon metabolism	627
	Carbohydrate metabolism	744
	Energy metabolism	489
	Lipid metabolism	398
	Nucleotide metabolism	270
	Amino acid metabolism	570
	Metabolism of other amino acids	211
	Glycan biosynthesis and metabolism	165
	Metabolism of cofactors and vitamins	351
	Metabolism of terpenoids and polyketides	185
	Biosynthesis of other secondary metabolites	145
	Xenobiotics biodegradation and metabolism	105
Genetic information processing	Transcription	525
	Translation	846
	Folding, sorting and degradation	701
	Replication and repair	184
Environmental information processing	Membrane transport	34
	Signal transduction	673
	Signaling molecules and interaction	2
Cellular processes	Transport and catabolism	436
	Cell motility	72
	Cell growth and death	215
	Cell communication	101
Organismal systems	Environmental adaptation	201

transcriptional modification, thus important candidates for improving drought tolerance. Mitogen-activated protein kinase (MAPK) cascades, calcineurin B-like protein-interacting protein kinase (CIPK), calcium-dependent protein kinase (CDPK or CPK), receptor-like kinases, and others have roles in drought stress signaling and regulation pathways. Genes encoding various PK have been identified in plants as they are involved in stress signaling (Long et al. 2014; Yang et al. 2008; Wei et al. 2014; Zhao et al. 2013). In the present study, BLASTX search of senna transcripts against protein kinase family database (http://bioinfo.bti.cornell.edu/tool/itak/) resulted in 2060 PKs which were classified into 41 known PK families based the kinase domins (Table 4 , Fig. 5). The most abundant group of protein kinases was leucine-rich repeat kinase family (346; 17 %), receptor like cytoplasmic kinase family (316; 15 %), domain of unknown function 26 (DUF26) kinase (126; 6 %), CDC2 like kinase family (114; 6 %), GmPK6/AtMRK1 family (109; 5 %), and IRE/NPH/PI dependent/S6 kinase (104; 5 %) in senna. Genes from these families have been reported to play significant roles in plant responses to drought stress in plants (Hu and Xiong 2014; Sun et al. 2015a, b). For example, there were CDS detected for MAPK cascades (74) and CDPK (84), receptor-like kinases (42) involved in drought signaling forms important candidates for improvement of drought tolerance (Hu and Xiong 2014). Other PKs were present in lower number.

Transcription factors

Transcription factors (TFs) are the important upstream regulatory proteins and play critical roles in various plant developmental processes and plant responses to abiotic and biotic stresses. Genes encoding various TF family members were identified as involved in regulating drought tolerance and are promising in improving the drought tolerance in plants (Ciftci-Yilmaz and Mittler 2008; Fang et al. 2008; Lucas et al. 2011a, b; Sun et al. 2016). The BLASTX search of senna transcripts against Plant Transcription Factor database resulted in 10,833 transcription factors which were classified into 78 known transcription factor families based on their DNA binding domains. The largest group of transcription factors included was the Orphan family (263, 2.40 %), followed by bHLH (235, 2.10 %), C3H (193, 1.78 %), and C2C2 Family (190, 1.75 %) (Table 4). Genes from these families have been reported to play significant roles in plant responses to drought stress in plants (Hu and Xiong 2014). NAM-ATAF-CUC2 (NAC) is a plant-specific TF family with a highly conserved DNA-binding domain, and many genes belonging to this family are responsive to drought stress (Fang et al. 2008). There were 183 CDS encoding NAC TF family (Table 4). Over expression of SNAC1 a NAC family TF, in rice and wheat showed improved drought resistance (Nakashima et al. 2012). Similarly, CDS for TF families such as MYB-related (Xiong et al. 2014), MYB

(Su et al. 2014), MYC (Abe et al. 1997), C2H2 type zinc Finger (Shi et al. 2014), bZIP (Zhong et al. 2015), HB (Jain et al. 2008), WRKY (Ren et al. 2010; Van Eck et al. 2014), AP2-EREBP (Liu et al. 1998; Wu et al. 2016),CCAAT/NF-Y (Li et al. 2008), HSF (Li et al. 2014a, 2014b), CAMTA (Pandey et al. 2013), and zf-HD (Tran et al. 2007) were identified which have been reported to play important roles in plant responses to drought stress, and were also highly abundant in our transcriptome dataset. CDS encoding other signaling factors such as Cyclin-dependent protein kinase (95), ATPase/hydrogen-translocating pyrophosphatase (AVP1) (5), and ABA catabolism (Chl-NADP-ME) (65) were also identified in the senna leaf transcriptome.

Protein-modifying/degrading enzymes

Protein modifications and degrading enzymes play important roles in ABA signaling, hence used for engineering drought tolerance in crop plants. The cytosolic enzymes viz., E3 ubiquitin-protein ligase, ubiquitin-conjugating enzyme E2, and ubiquitin-activating enzyme E1 play an important role in degradation of ubiquitinated proteins, are demonstrated to function as a positive regulator of ABA-dependent response to drought stress (Ryu et al. 2010). Genes encoding various protein-modifying/degrading enzymes have been identified in plants (Gao et al. 2011; Kuzuoglu-Ozturk et al. 2012; Song et al. 2016) and were use in improving drought tolerance in plants (Ning et al. 2011; Park et al. 2010). In the present study, CDS encoding for E3 ubiquitin-protein ligase (609), ubiquitin-conjugating enzyme E2 (57), and ubiquitin-activating enzyme E1 (14) were identified through BLASTX search which forms important resource to understand the drought adaptation in senna (Table 4). Farnesylation is a posttranslational modification in which a farnesyl group is added to inactive target proteins so that they are targeted to membranes. Suppressing the rice farnesyltransferase SQS by RNA interference greatly enhanced drought resistance (Manavalan et al. 2012). There were nine CDS encoding farnesyltransferase/squalene synthase in senna which may be exploited for improving drought tolerance in crop plants. Ski-Interacting protein/SNW domain-containing protein and ribosome-inactivating proteins are involved in drought signaling (Lim et al. 2010; Jiang et al. 2012). There were six CDS encoding Ski-Interacting protein/SNW domain-containing protein in senna which form additional candidates for developing drought-tolerant transgenic plants.

Biosynthesis of phytohormone

Plant hormones play a major role in abiotic stress response in plants (Khan et al. 2012) and regulate developmental processes and signaling networks under abiotic stress. In plants, accumulation of abscisic acid (ABA) plays an important role in drought stress signaling and transduction pathways, mediating many

Table 4 Putative genes encoding signaling factors and protein modifying/degrading enzymes involved in drought stress response identified in the leaf transcriptome of senna

Functional category	Pathway	Sr. no.	Enzyme	E.C. no.	Number of CDS
Signaling factors	Protein kinase	1	Leucine Rich Repeat Kinase Family		346
		2	Receptor Like Cytoplasmic Kinase Family		316
		3	Domain of Unknown Function 26 (DUF26) Kinase		126
		4	CDC2 Like Kinase Family		114
		5	GmPK6/AtMRK1 Family		109
		6	IRE/NPH/PI dependent/S6 Kinase		104
		7	Calcium Dependent Protein Kinase (CDPK)		84
		8	SNF1 Related Protein Kinase (SnRK)		84
		9	LAMMER Kinase Family		80
		10	S Domain Kinase Family		76
		11	MAP Kinase Family (MAPK)		74
		12	Unknown Function Kinase		58
		13	CTR1/EDR1 Kinase		45
		14	Receptor like protein kinase		42
		15	Casein Kinase		40
		16	Other Protein Kinase		32
		17	STE20-PAK Like Protein Kinase		31
		18	GSK3/Shaggy Like Protein Kinase Family		27
		19	Ankyrin Repeat Domain Kinase		22
		20	Others		250
	Transcription factors	1	Orphans		263
		2	bHLH		235
		3	bZIP		193
		4	C2C2 Family		190
		5	C2H2		132
		6	G2-like		132
		7	C3H		121
		8	MYB-related		113
		9	Trihelix		101
		10	NAC		94
		11	WRKY		94
		12	HB		88
		13	ERF		78
		14	GRAS		74
		15	NF-Y family		55
		16	HSF		52
		17	TCP		51
		18	B3		44
		19	MYB		42
		20	Others		372
	Others	1	Cyclin dependent protein kinase		95
		2	ATPase/ hydrogen-translocating pyrophosphatase (AVP1)	–	5
		3	ABA catabolism (Chl-NADP-ME)	[EC:1.1.1.40]	65

Table 4 (continued)

Functional category	Pathway	Sr. no.	Enzyme	E.C. no.	Number of CDS
Protein-modifying/ degrading enzymes	Protein degradation	1	E3 ubiquitin-protein ligase	[EC:6.3.2.19]	609
		2	Ubiquitin-conjugating enzyme E2	[EC:6.3.2.19]	57
		3	Ubiquitin-activating enzyme E1	[EC:6.3.2.19]	14
	Protein modification	1	Farnesyltransferase/ Squalene synthase	–	9
	Other nuclear proteins	1	Ski-Interacting Protein/ SNW domain-containing protein 1	–	6
		2	Ribosome-inactivating Protein	–	0

responses. Plants perceive and respond adaptively to drought stress controlled mainly by the phytohormone abscisic acid (ABA). Other plant hormones involved in drought stress response include ethylene, jasomnate and methyl jasmonate, salicylic acid, brasinosteroids, gibberlic acid, and nitric oxide.

ABA biosynthesis

ABA is synthesized and accumulated in guard cells where it triggers stomatal closer under drought (Schroeder et al. 2001). ABA is synthesized from the C_{40} carotenoid precursor beta-carotene in plants (Cutler and Krochko 1999). In senna, 38 CDS encoding eight enzymes involved in ABA biosynthesis, i.e., beta-carotene 3-hydroxylase, beta-ring hydroxylase, zeaxanthin epoxidase, violaxanthin de-epoxidase, 9-*cis*-epoxycarotenoid dioxygenase, xanthoxin dehydrogenase, abscisic-aldehyde oxidase, and abscisic acid 8′-hydroxylase were identified (Table 5 and Fig. 6). Beta-carotene 3-

hydroxylase (BCH) has been shown to be critical for drought tolerance and oxidative stress (Du et al. 2010). There were the CDS encoding BCH in senna. Recently, a key ABA biosynthesis gene ABA8H encoding abscisic acid 8′-hydroxylase plays a critical role in regulating ABA levels during seed imbibition and dehydration stress (Saito et al. 2004; Kushiro et al. 2004; Xu et al. 2014a, b). There were seven CDS encoding abscisic acid 8′-hydroxylase in senna, which form important resource for improvement of drought tolerance.

Ethylene biosynthesis

Under drought conditions, ethylene causes leaf abscission and consequently reduced water loss (Zhu et al. 2011). Several studies in model plants have evaluated the importance of ethylene hormone in crosstalk signaling with different metabolic pathways, in addition to responses to biotic stresses (Arraes et al. 2015; Shang et al. 2014; Kantar et al. 2011). Ethylene is

Fig. 5 Transcript annotated to different protein kinases family in the transcriptome of senna
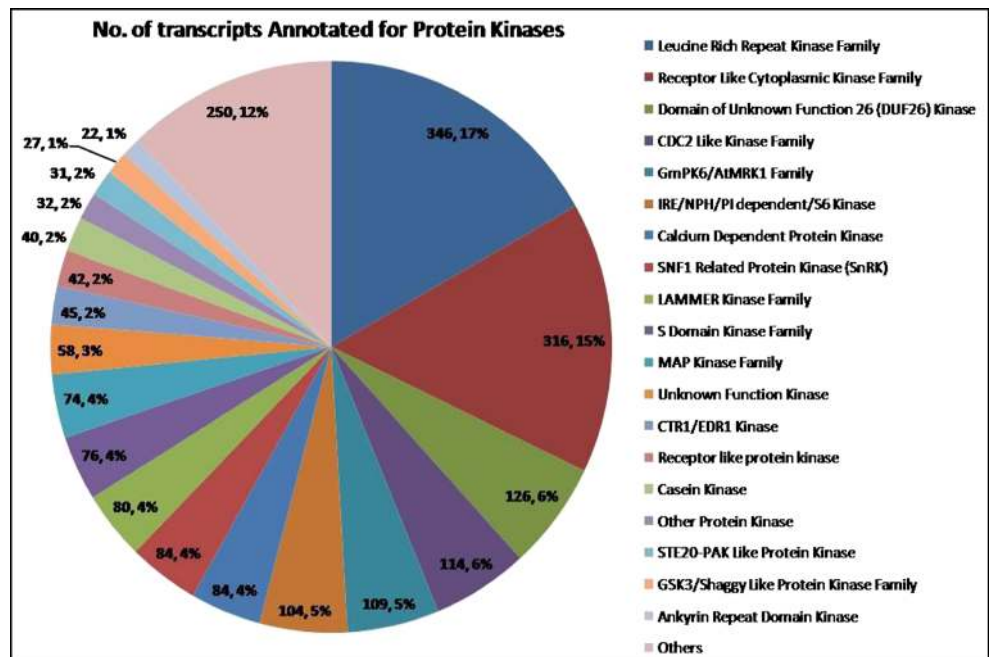
Table 5 Putative genes encoding enzymes involved in biosynthesis of phytohormones identifed in the senna leaf transcriptome

Functional category	Pathway	Sr. no.	Enzyme	E.C. no.	Number of CDS
Biosynthesis of phytohormones	Abscisic acid biosynthesis	1	Beta-carotene 3-hydroxylase	[EC:1.14.13.129]	3
		2	Beta-ring hydroxylase	[EC:1.14.-.-]	3
		3	Zeaxanthin epoxidase	[EC:1.14.13.90]	5
		4	Violaxanthin de-epoxidase	[EC:1.10.99.3]	7
		5	9-cis-epoxycarotenoid dioxygenase	[EC:1.13.11.51]	4
		6	Xanthoxin dehydrogenase	[EC:1.1.1.288]	5
		7	Abscisic-aldehyde oxidase	[EC:1.2.3.14]	4
		8	Abscisic acid 8′-hydroxylase	[EC:1.14.13.93]	7
	Ethylene biosynthesis	1	S-Adenosylmethionine synthetase	[EC:2.5.1.6]	8
		2	1-Aminocyclopropane-1-carboxylate synthase	[EC:4.4.1.14]	2
		3	Aminocyclopropanecarboxylate oxidase	[EC:1.14.17.4]	39
	Jasmonate and methyl jasmonate biosynthesis	1	Phospholipase A2/TAG lipase/steryl ester hydrolase/LPA acyl-transferase	[EC:3.1.1.4]	3
		2	Lipoxygenase	[EC:1.13.11.12]	24
		3	Hydroperoxide dehydratase	[EC:4.2.1.92]	5
		4	Allene oxide cyclase	[EC:5.3.99.6]	4
		5	12-Oxophytodienoic acid reductase	[EC:1.3.1.42]	4
		6	OPC-8:0 CoA ligase 1	[EC:6.2.1.-]	3
		7	Acyl-CoA oxidase	[EC:1.3.3.6]	9
		8	Enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase/long-chain-3-hydroxyacyl-CoA dehydrogenase	[EC:4.2.1.17]	13
		9	Acetyl-CoA acyltransferase 1	[EC:2.3.1.16]	4
		10	Jasmonate O-methyltransferase	[EC:2.1.1.141]	2
	Salicylic acid biosynthesis	1	Isochorismate synthase	[EC:5.4.4.2]	9
		2	Phenylalanine ammonia-lyase	[EC:4.3.1.24]	7
		3	trans-Cinnamate 4-monooxygenase	[EC:1.14.13.11]	5
		4	Benzoic acid 2-hydroxylase	[EC:1.14.13.-]	0
	Brassinosteroid biosynthesis	1	steroid 22-alpha-hydroxylase CYP90B1, DWF4	[EC:1.14.13.-]	3
		2	3-epi-6-Deoxocathasterone 23-monooxygenase CYP90C1, ROT3	[EC:1.14.13.112]	2
		3	Cytochrome P450, family 90, subfamily A, polypeptide 1 CYP90A1, CPD	[EC:1.14.-.-]	6
		4	Steroid 5-alpha-reductase DET2	[EC:1.3.1.22]	4
		5	Steroid 3-oxidase CYP90D2, D2	[EC:1.14.-.-]	0
		6	Brassinosteroid-6-oxidase 1 CYP85A1, BR6OX1	[EC:1.14.-.-]	5
		7	PHYB activation tagged suppressor 1 CYP734A1, BAS1	[EC:1.14.-.-]	8
	Gibberellic acid biosynthesis	1	1-Deoxy-D-xylulose-5-phosphate synthase	[EC:2.2.1.7]	8
		2	1-Deoxy-D-xylulose-5-phosphate reductoisomerase	[EC:1.1.1.267]	8
		3	2-C-Methyl-D-erythritol 4-phosphate cytidylyltransferase	[EC:2.7.7.60]	2
		4	4-Diphosphocytidyl-2-C-methyl-D-erythritol kinase	[EC:2.7.1.148]	5
		5	2-C-Methyl-D-erythritol 2,4-cyclodiphosphate synthase	[EC:4.6.1.12]	2
		6	(E)-4-Hydroxy-3-methylbut-2-enyl-diphosphate synthase	[EC:1.17.7.1]	4
		7	4-Hydroxy-3-methylbut-2-enyl diphosphate reductase	[EC:1.17.1.2]	2
		8	Acetyl-CoA C-acetyltransferase	[EC:2.3.1.9]	4
		9	Hydroxymethylglutaryl-coa synthase	[EC:2.3.3.10]	6
		10	Hydroxymethylglutaryl-coa reductase (NADPH)	[EC:1.1.1.34]	4
		11	Mevalonate kinase	[EC:2.7.1.36]	2
		12	Phosphomevalonate kinase	[EC:2.7.4.2]	0
		13	Diphosphomevalonate decarboxylase	[EC:4.1.1.33]	3
		14	Isopentenyl-diphosphate delta-isomerase	[EC:5.3.3.2]	2

Table 5 (continued)

Functional category	Pathway	Sr. no.	Enzyme	E.C. no.	Number of CDS
		15	Geranyl diphosphate synthase	[EC:2.5.1.1]	4
		16	Farnesyl diphosphate synthase	[EC:2.5.1.10]	6
		17	Geranylgeranyl diphosphate synthase	[EC:2.5.1.29]	6
		18	Ent-copalyl diphosphate synthase	[EC 5.5.1.13]	0
		19	Ent-kaurene synthase	[EC:4.2.3.19]	2
		20	Ent-kaurene oxidase	[EC 1.14.13.78]	0
		21	Ent-kaurenoic acid oxidase/ ent-kaurenoic acid hydroxylase	[EC:1.14.13.79]	1
		22	Gibberellin 20-oxidase	[EC:1.14.11.12]	10
		23	Gibberellin 3-beta-dioxygenase	[EC:1.14.11.15]	3
		24	Gibberellin 2-oxidase	[EC:1.14.11.13]	7
		25	GA4 desaturase, DES	–	0
		26	P450-1	–	0
		27	P450-2	–	0
		28	P450-3	–	0
	Nitric oxide biosynthesis	1	Nitric oxide synthase	[EC:1.14.13.39]	8
		2	Nitric oxide synthase-interacting protein	–	2

Fig. 6 ABA biosynthetic pathway in senna (*numbers in brackets* represent number of CDS)
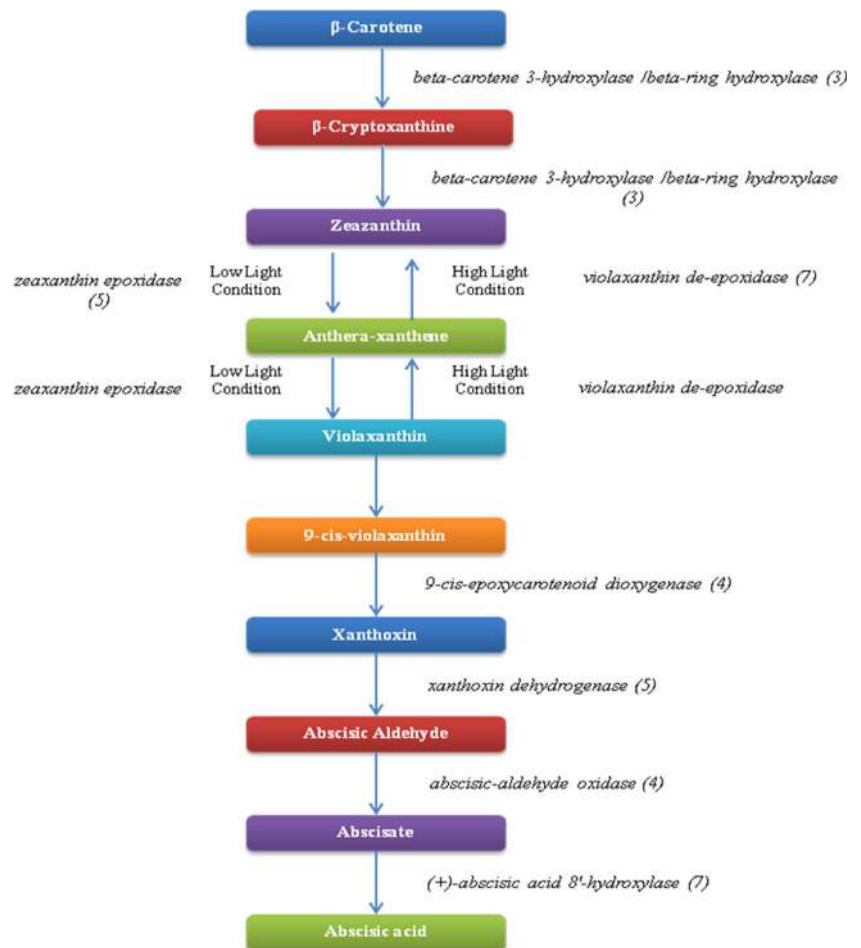
derived from the amino acid methionine provided by the Yang cycle (Roje 2006) in which the precursor *S*-adenosylmethionine (AdoMet or SAM) is synthesized from ATP and methionine by *S*-adenosylmethionine synthetase (SAMS; EC 2.5.1.6). AdoMet is then converted into 1-aminocyclopropane-1-carboxylic acid (ACC) and 5-methylthioadenosine (MTA) by the enzyme 1-aminocyclopropane-1-carboxylase synthase (ACS, EC 4.4.1.14). MTA is recycled through a series of Yang cycle reactions back to methionine (Argueso et al. 2007). In the present study, 49 CDS encoding for three enzymes involved in ethylene biosynthesis namely *S*-adenosylmethionine synthetase (8), 1-aminocyclopropane-1-carboxylate synthase (ACS) (2), and aminocyclopropanecarboxylate oxidase (39) were identified (Table 5) thus providing important candidates for engineering drought tolerance in crops.

Jasmonate and methyl-jasmonate biosynthesis

Plant responses to abiotic stresses particularly drought stress are orchestrated locally and systemically by signaling molecules known as the jasmonates (JAs), a class of polyunsaturated fatty acid-derived phytohormones (Turner et al. 2002). The biosynthesis of JA initiates in chloroplasts, involving the release of α-linolenic acid (α-LeA, 18:3 or 18:2) from the lipid membrane by phospholipases (PLDs). In this study, from the leaf transcriptome of senna, 71 CDS encoding for 10 enzymes involved in jasmonate and methyl-jasmonate biosynthesis, i.e., phospholipase A2 (3), lipoxygenase (24), hydroperoxide dehydratase (5), allene oxide cyclase (4), 12-oxophytodienoic acid reductase (4), OPC-8:0 CoA ligase (3), acyl-CoA oxidase (9), enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (13), acetyl-CoA acyltransferase (4), and jasmonate *O*-methyltransferase (2) were identified (Table 5) which will form on invaluable resource to understand the JA pathway which in turn lead to practical biotechnological applications in plants.

Salicylic acid biosynthesis

Salicylic acid (SA) is a seven carbon-containing, naturally occurring phenolic compound and endogenously synthesized signaling molecule in plants (Wang et al. 2015). The most common pathway in plants for SA synthesis is phenylalanine pathway; however, SA biosynthesis may also be accomplished by isochorismate pathway (Kawano et al. 2004; Mustafa et al. 2009). SA is produced after a series of chemical reactions catalyzed by many enzymes. There were 21 CDS identified encoding for three enzymes involved in salicylic acid biosynthesis namely isochorismate synthase (9), phenylalanine ammonia-lyase (7), and *trans*-cinnamate 4-monooxygenase (5) in our dataset (Table 5). We did not find CDS for benzoic acid 2-hydroxylase involved in catalyzing the biosynthesis of salicylic acid from benzoic acid. This might be due to the

presence of very low levels of transcripts in the sample or possibility of being a key enzyme, and thus, its expression might be tightly regulated and thus absence of the transcript. Similarly, the CDS encoding enzymes involved in biosynthesis of brassinosteroids, gibbarlic acid, and nitric oxide were identified (Table 5) in the leaf transcriptome of senna.

Plant hormone signaling in senna

Plant hormones regulate plant responses under biotic and abiotic stresses through specific signaling networks (Kohli et al. 2013). Plant hormones such as abscisic acid, ethylene, jasmonic acid, salicyclic acid, brassinosetroids, gibbarlic acid, and auxin were studied for their role in abiotic stress responses (Peleg and Blumwald 2011; Santner et al. 2009). Putative plant hormone signaling genes detected in senna during the drought response are listed in Table 6. Abscisic acid (ABA), acts as an endogenous messenger in the regulation of the plant's water status (Swamy and Smith 1999). ABA-dependent stress signaling involves many enzymes. The soluble PYR/PYL/RCAR receptors function at the apex of a negative regulatory pathway to directly regulate PP2C phosphatases, which in turn directly regulate SnRK2 kinases (Cutler et al. 2010). A maximum number of CDS (253) encoding for protein phosphatase 2C PP2C, a negative regulator of ABA response in plants, were detected in senna. Similarly, CDS for abscisic acid receptor PYR/PYL family (11), serine/threonine-protein kinase SRK2 SNRK2 (25), and ABA responsive element binding factor ABF (21) were also detected in the leaf transcriptome of senna which forms candidates for understanding and improvement of drought stress in plants. Ethylene, a gaseous plant hormone, regulates growth and development as well as responses to biotic and abiotic stresses in plants (Bleecker and Kende 2000). Over the last few decades, key elements involved in ethylene signal transduction have been identified in plants (Shakeel et al. 2013). Almost all of the ethylene-signaling homologous members was detected in the *C. agustifolia* transcriptome. Serine/threonine-protein kinase CTR1 was well-known for mediating stress responses and development in plants (Xu et al. 2014a). JAs also regulate such diverse processes as pollen maturation and wound responses in *Arabidopsis*. Several core factors in the JA signaling pathway are listed in Table 6. MYC transcription factors and Coronatine-insensitive protein 1 (COI1) are key regulator of genes involved in wound- and methyl jasmonate-induced secondary metabolism, defense, and hormone interactions (Devoto et al. 2005 and Zhang et al. 2015a, b, c). In the salicyclic acid signaling pathway, NPR1 (non-specific disease resistance 1) is a key regulator in SA-dependent defense signaling (Boatwright and Pajerowska-Mukhtar 2013). Similarly, WRKY and TGA play major roles as transcriptional regulators in the SA pathway. We detected three NPR1-related proteins and 22 TGAs in *C. angustifolia*, which might function in the SA signaling pathway in *C. angustifolia*. Similarly,

Table 6 Putative genes encoding plant hormone signaling, osmotic adjustment, and free radical scavengers detected in the leaf transcriptome of senna

Functional category	Pathway	Sr. no.	Enzyme	E.C. no.	Number of CDS
Plant hormone signaling	Abscisic acid	1	Abscisic acid receptor pyr/pyl family	–	11
		2	Protein phosphatase 2c pp2c	[EC:3.1.3.16]	253
		3	Serine/threonine-protein kinase srk2 snrk2	[EC:2.7.11.1]	25
		4	Aba responsive element binding factor abf	–	21
	Ethylene	1	Ethylene receptor ets/etr	[EC:2.7.13.-]	11
		2	Serine/threonine-protein kinase ctr1	[EC:2.7.11.1]	36
		3	Mitogen-activated protein kinase kinase simkk	[EC:2.7.12.2]	12
		4	Mitogen-activated protein kinase 6	[EC:2.7.11.24]	2
		5	Ethylene-insensitive protein 2 ein2	–	10
		6	Ethylene-insensitive protein 3 ein3	–	11
		7	Ein3-binding f-box protein ebf1_2	–	7
		8	Ethylene-responsive transcription factor 1/2	–	0
	Jasmonic acid	1	Jasmonic acid-amino synthetase jar1	–	2
		2	Coronatine-insensitive protein 1 coi-1	–	9
		3	Jasmonate zim domain-containing protein jaz	–	8
		4	Transcription factor myc2	–	10
		5	Ap2-domain dna-binding protein orca2/3	–	0
	Salicylic acid	1	Regulatory protein npr1	–	3
		2	Transcription factor tga	–	22
		3	Pathogenesis-related protein 1	–	0
	Brassinosteroid	1	Brassinosteroid insensitive 1-associated receptor kinase 1 or leu-rich receptor serine/threonine protein kinase bak1	–	6
		2	Protein brassinosteroid insensitive 1	–	6
		3	Bri1 kinase inhibitor 1	–	2
		4	Br-signaling kinase	–	12
		5	Serine/threonine-protein phosphatase bsu1	–	0
		6	Protein brassinosteroid insensitive 2	–	5
		7	Brassinosteroid resistant 1/2	–	9
		8	Xyloglucan:xyloglucosyl transferase tch4	2.4.1.207	25
		9	Cyclin d3	–	8
	Gibberellic acid	1	Gibberellin receptor gid	[EC:3.-.-.-]	14
		2	Della protein	–	9
		3	F-box protein gid2	–	2
		4	Phytochrome-interacting factor 3	–	13
	Auxin	1	Auxin influx carrier lax3	–	11
		2	Protein transport inhibitor response 1	–	8
		3	Auxin-responsive protein iaa	–	36
		4	Auxin response factor	–	73
		5	Auxin responsive gh3 gene family	–	9
		6	Saur family protein	–	8
Osmotic adjustment	Proline biosynthesis	1	Delta-1-pyrroline-5-carboxylate synthetase	[EC:2.7.2.11]/ [EC:1.2.1.41]	14
		2	Pyrroline-5-carboxylate reductase	[EC:1.5.1.2]	3
	Glycin betaine biosynthesis	1	Choline monooxygenase	[EC 1.14.15.7]	1
		2	Betaine-aldehyde dehydrogenase	[EC:1.2.1.8]	3
	Trehalose biosynthesis	1	Hexokinase	[EC:2.7.1.1]	12
		2	Glucokinase	[EC:2.7.1.2]	0
		3	Phosphoglucomutase	[EC:5.4.2.2]	18
		4	Utp–glucose-1-phosphate uridylyltransferase	[EC:2.7.7.9]	9

Table 6 (continued)

Functional category	Pathway	Sr. no.	Enzyme	E.C. no.	Number of CDS
		5	Alpha,alpha-trehalose-phosphate synthase	[EC:2.4.1.15]	17
		6	Trehalose-phosphate phosphatase	[3.1.3.12]	7
	Sorbitol and mannitol biosynthesis	1	Aldehyde reductase	[EC:1.1.1.21]	2
		2	Xylose isomerase	[EC:5.3.1.5]	4
		3	L-Iditol 2-dehydrogenase	[EC:1.1.1.14]	4
		4	D-Sorbitol dehydrogenase	[EC:1.1.1.15]	2
		5	D-Sorbitol dehydrogenase (acceptor)	[E1.1.99.21]	0
		6	Mannitol 2-dehydrogenase	[E1.1.1.67]	4
		7	Mannitol 2-dehydrogenase (NADP+)	[EC 1.1.1.138]	0
	Galactinol biosynthesis	1	Aldose 1-epimerase	[EC:5.1.3.3]	10
		2	Galactokinase	[EC:2.7.1.6]	4
		3	Galactose-1-phosphate uridylyltransferase	[EC 2.7.7.10]	0
		4	UDP-sugar pyrophosphorylase	[EC:2.7.7.64]	3
		5	UDPglucose–hexose-1-phosphate uridylyltransferase/galactose-1-phosphate uridyltransferase	[EC:2.7.7.12]	3
		6	Inositol 3-alpha-galactosyltransferase/galactinol synthase	[EC 2.4.1.123]	2
		7	Alpha-galactosidase	[EC 3.2.1.22]	11
		8	Raffinose synthase/galactinol–sucrose galactosyltransferase	[EC:2.4.1.82]	12
Free radical scavengers	Catalase and peroxidase synthesis	1	Ferrochelatase HemH	[EC:4.99.1.1]	9
	Antioxidant enzyme	1	Superoxide dismutase	[EC:1.15.1.1]	10
		2	Catalase	[EC:1.11.1.6]	2
		3	Peroxidase	[EC:1.11.1.7]	29
	Glutathione biosynthesis	1	Glutamate–cysteine ligase	[EC:6.3.2.2]	2
		2	Glutathione synthase	[EC:6.3.2.3]	2
		3	Glutathione reductase (NADPH)	[EC:1.8.1.7]	9
		4	Glutathione peroxidase	[EC:1.11.1.9]	5
		5	Glutathione S-transferase	[EC:2.5.1.18]	48
	Ascorbic acid biosynthesis	1	GDP-D-mannose 3′, 5′-epimerase	[EC:5.1.3.18]/ [EC: 5.1.3.-]	5
		2	GDP-L-galactose phosphorylase	[EC:2.7.7.69]	2
		3	Inositol-phosphate phosphatase/L-galactose 1-phosphate phosphatase	[EC:3.1.3.25]/ [EC:3.1.3.-]	3
		4	L-Galactose dehydrogenase	[EC:1.1.1.316]	2
		5	L-Galactono-1,4-lactone dehydrogenase	[EC:1.3.2.3]	6
		6	L-Ascorbate peroxidase	[EC:1.11.1.11]	22
		7	Monodehydroascorbate reductase (NADH)	[EC:1.6.5.4]	20
		8	L-Ascorbate oxidase	[EC:1.10.3.3]	15
	Phytochelatin biosynthesis	1	Phytochelatin synthase/glutathione gamma-glutamylcysteinyltransferase	[EC:2.3.2.15]	9

brassinosteroids (BRs) are growth-promoting steroid hormones that regulate diverse physiological processes in plants. The BR signal is transduced by a receptor kinase-mediated signal transduction pathway, which is distinct from animal steroid-signaling systems. CDS for majority of the BR signaling homologous members were detected in the *C. angustifolia* transcriptome. We detected 14 gibberellin receptor GIBBERELLIN INSENSITIVE DWARF1 (GID1), nine DELLA growth inhibitors (DELLAs), two F-box proteins (GID2), and 13 phytochrome-interacting factor 3, which play important roles in GA signaling pathways (Daviere and Achard 2013; Richards et al. 2001) in senna. Auxin regulates transcription by rapidly modulating levels of Aux/IAA proteins throughout development. Auxin binds to TIR1, the F-box

subunit of the ubiquitin ligase complex SCF (TIR1), and stabilizes the interaction between TIR1 and Aux/IAA substrates (Mockaitis and Estelle 2008). The auxin response factor (ARF) family contains transcription factors that bind to auxin-responsive elements (AREs) in the promoters of primary auxin-responsive genes (Sun et al. 2015a, b; Wang et al. 2010). All of the major auxin signaling factors are found in the senna transcriptome (Table 6) which will enrich our knowledge on molecular basis of phytohormone signaling in plants during drought stress.

Osmolytes in drought stress response

Plants respond to stress by production of low molecular weight, non-toxic solutes which are known as osmolytes or osmoprotectants. Osmolytes stabilize cell proteins and structures under stress. The major osmolytes accumulated by plants under abiotic stress include proline, glycine betaine (GB), trehalose, sorbitol and mannitol and galactinol. Plants accumulate proline which functions as an osmolyte to stabilize cell proteins and structures under drought stress. Proline is considered as a scavenger of free radicals, an energy sink, and a stress-regulated signal (Seki et al. 2007). Tissue-specific proline synthesis and catabolism have been found promoting growth and maintain a higher NADP/NADPH ratio at lower water potential (Sharma et al. 2011; Zhang et al. 2015a, b, c). In the present study, there were 17 CDS encoding for two enzymes involved in proline biosynthesis of which 14 were encoding delta-1-pyrroline-5-carboxylate synthetase (P5CS) and three encoding pyrroline-5-carboxylate reductase (P5CR) enzymes (Table 6 and Fig. 7). Similarly, for the biosynthesis of glycine betaine, four CDS encoding for two enzymes namely choline monooxygenase (one CDS) and betaine-aldehyde dehydrogenase (three CDS) were identified. In addition, we also found unique transcripts in the trehalose, sorbitol, and mannitol and galactinol biosynthesis in senna. The accumulation of these osmolytes could be critical to improve stress tolerance especially osmotic stress of senna.

Analysis of genes for free radical scavengers in senna

Plants, when subjected to drought stress, increase the production of reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide, and hydroxyl radical. To protect cells and subcellular systems from the effect of these ROS, plants produce free radical scavengers (D'Autréaux and Toledano 2007; Kumari et al. 2015). These include enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (APX), and non-enzyme compounds such as ascorbic acid, phytochelatin, and glutathione. In the present study, ten CDS encoding for superoxide dismutase (SOD), two CDS encoding for catalase (CAT), and 29 CDS encoding for
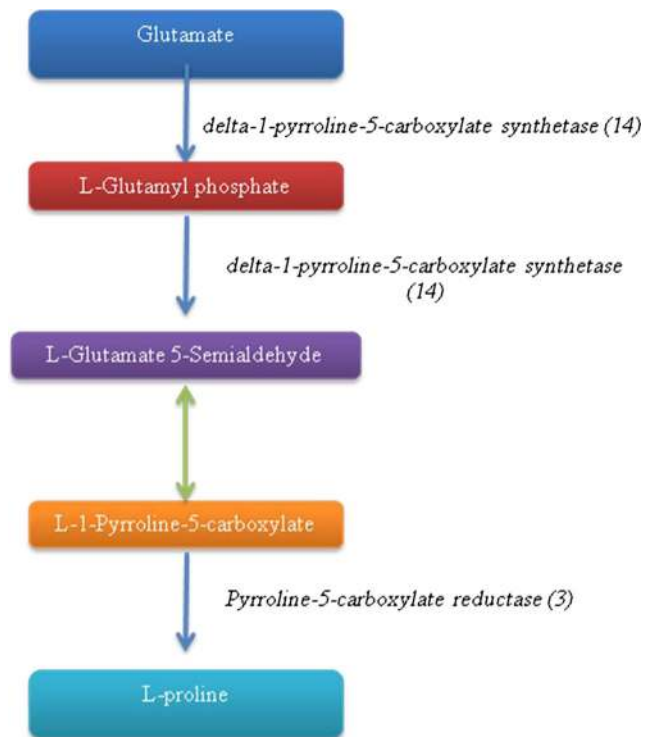

Fig. 7 Proline biosynthetic pathway in senna (*numbers in brackets* represent number of CDS)

peroxidase (APX) were identified. Glutathione reductase (GR, EC 1.6.4.2) and tripeptide glutathione (GSH, γ-glutamyl-cysteinyl-glycine) are two major components of the ascorbate-glutathione (AsA-GSH) pathway which play a significant role in protecting cells against ROS and its reaction products-accrued potential anomalies (Gill et al. 2013). There were CDS encoding for glutathione S-transferase (48), glutathione reductase (9), glutathione peroxidase (5), glutamate cysteine ligase (2), and glutathione synthase (2) in the transcriptome of senna which are involved in the biosynthesis of glutathione, a ubiquitous intracellular peptide with diverse functions (Table 6). L-Ascorbic acid (vitamin C) is a major antioxidant in plants and plays a significant role in mitigation of excessive cellular reactive oxygen species activities caused by a number of abiotic stresses (Venkatesh and Park 2014). There were 75 CDS encoding for eight enzymes involved in ascorbic acid biosynthesis which were identified namely GDP-D-mannose 3′, 5′-epimerase, GDP-L-galactose phosphorylase, inositol-phosphate phosphatase/L-galactose 1-phosphate phosphatase, L-galactose dehydrogenase, L-galactono-1,4-lactone dehydrogenase, L-ascorbate peroxidase, monodehydroascorbate reductase, and L-ascorbate oxidase. We also found nine CDS encoding for phytochelatin synthase also known as glutathione gamma-glutamylcysteinyltransferase which is responsible for phytochelatin synthesis in senna which suggests drought stress may trigger the complex antioxidant network, and finely

tuned ROS accumulation to facilitate appropriate signaling functions (Munné-Bosch et al. 2013).

Chlorophyll metabolism

Delayed leaf senescence or stay green is an important drought adaptation in crop plants (Rama Reddy et al. 2014). The stay-green trait reflects impaired or delayed chlorophyll catabolism (Thomas and Ougham 2014). Plants are engineered to over-produce chlorophyll—for example by overexpression of the gene encoding chlorophyllide a oxygenase (Kusaba et al. 2013). In chlorophyll metabolism, there are 15 enzymes cata-lyzing chlorophyll biosynthesis and five enzymes catalyzing its degradation and the genes coding these enzymes have been cloned from this model plant (Beale 2005; Hortensteiner 2006). We searched the orthologs of these genes in the leaf transcriptome of senna and found CDS for all the enzymes except ferredoxin:protochlorophyllide reductase involved in the chlorophyll biosynthesis. Similarly, out of five enzymes, CDS were identified for four enzymes viz., chlorophyll(ide) b

reductase (11), chlorophyllase (6), Mg-dechelatase, pheophor-bide a oxygenase (7), red chlorophyll catabolite reductase (16) involved in chlorophyll degradation (Table 7 and Fig. 8) and form important candidates to understand chlorophyll metabo-lism in senna.

Gene involved in cuticular wax biosynthesis

Cuticular wax covers outer organs of plants and functions as the outermost barrier against non-stomatal water loss and UV light. Cuticular waxes are composed of very-long-chain fatty acids (VLCFAs) and their derivatives, such as aldehydes, al-kanes, esters, and primary and secondary alcohols. Many genes involved in cuticular wax biosynthesis and export have been characterized by forward and reverse genetic approaches in plants (Hooker et al. 2002; Kim et al. 2013) and their role in conferring water stress tolerance has been reported (Zhou et al. 2015; Krugman et al. 2010). There were 51 CDS encoding for three enzymes of leaf cuticular wax biosynthesis namely fatty acyl-CoA reductase (6), aldehyde decarbonylase

Table 7 Putative genes encoding enzymes involved in chlorophyll metabolism and leaf cuticular wax biosynthesis

| Functional category	Pathway	Sr. no.	Enzyme name	E.C. no.	Number of CDS
Chlorophyll metabolism	Chlorophyll biosynthesis and degradation	1	Glutamyl-trna reductase	[EC:1.2.1.70]	5
		2	Glutamate-1-semialdehyde 2,1-aminomutase	[EC:5.4.3.8]	2
		3	Porphobilinogen synthase	[EC:4.2.1.24]	3
		4	Hydroxymethylbilane synthase/porphobilinogen deaminase	[EC:2.5.1.61]	2
		5	Uroporphyrinogen-iii synthase	[EC:4.2.1.75]	2
		6	Uroporphyrinogen decarboxylase	[EC:4.1.1.37]	4
		7	Coproporphyrinogen iii oxidase	[EC:1.3.3.3]/ [EC:1.3.99.22]	4
		8	Oxygen-dependent protoporphyrinogen oxidase	[EC:1.3.3.4]	8
		9	Magnesium chelatase	[EC:6.6.1.1]	6
		10	Magnesium-protoporphyrin o-methyltransferase	[EC:2.1.1.11]	1
		11	Magnesium-protoporphyrin ix monomethyl ester (oxidative) cyclase	[EC:1.14.13.81]	2
		12	Divinyl chlorophyllide a 8-vinyl-reductase/ [4-vinyl]chlorophyllide a reductase	[EC 1.3.1.75]	2
		13	Ferredoxin:protochlorophyllide reductase (atp-dependent)/ light-independent protochlorophyllide reductase	[EC 1.3.7.7]	0
		14	Chlorophyll synthase	[EC:2.5.1.62]	5
		15	Chlorophyllide a oxygenase	[EC:1.14.13.122]	2
	Chlorophyll degradation	1	Chlorophyll(ide) b reductase	[EC:1.1.1.294]	11
		2	Chlorophyllase	[EC:3.1.1.14]	6
		3	Mg-dechelatase	–	0
		4	Pheophorbide a oxygenase	[EC:1.14.12.20]	7
		5	Red chlorophyll catabolite reductase	[EC:1.3.1.80]	16
Leaf cuticular wax	Leaf cuticular wax biosynthesis	1	Fatty acyl-CoA reductase	[EC:1.2.1.-]	6
		2	Aldehyde decarbonylase	[EC:4.1.99.5]	6
		3	Diacylglycerol O-acyltransferase 1	[EC:2.3.1.20]/ [EC:2.3.1.75]/ [EC:2.3.1.76]	39
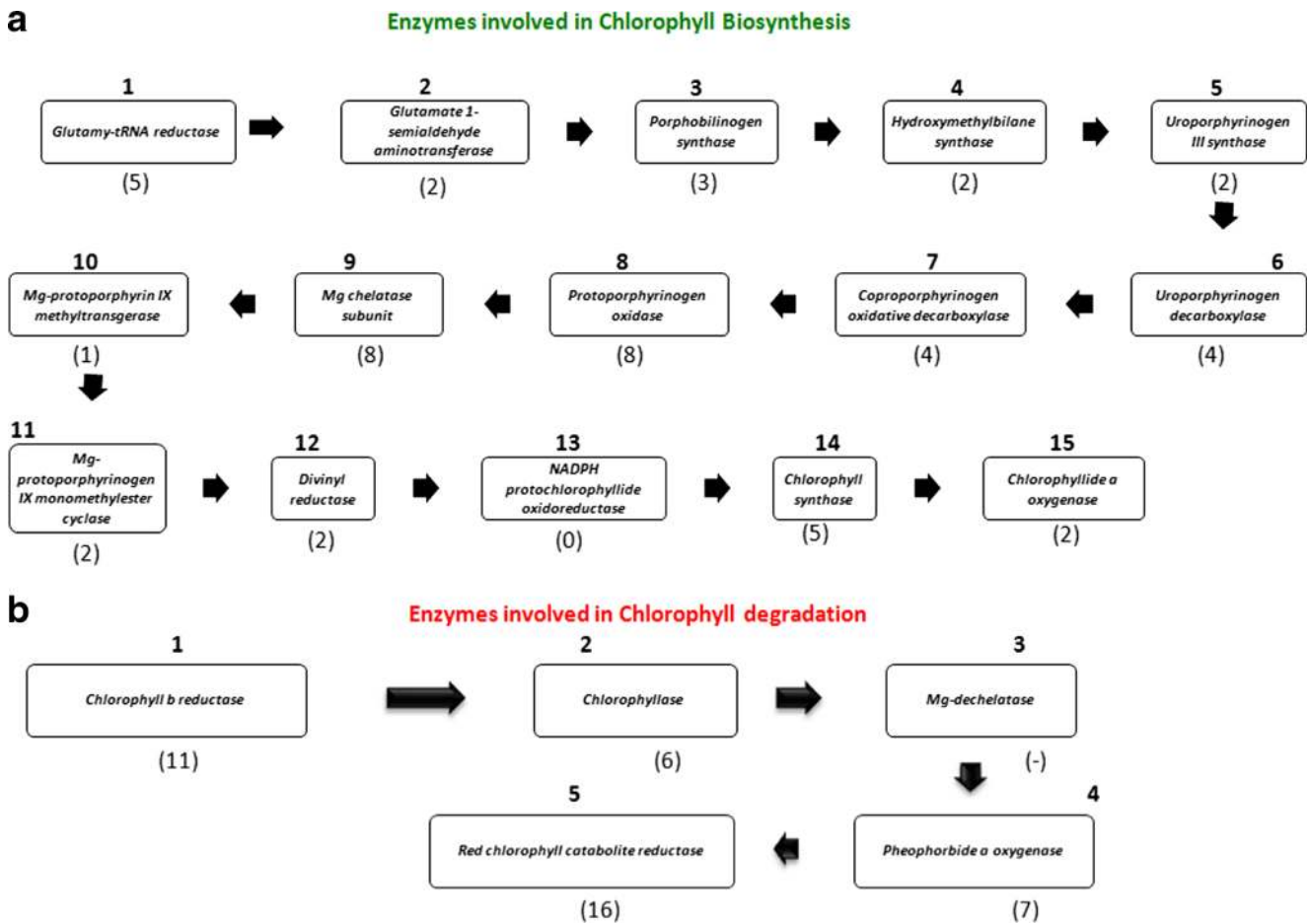
Fig. 8 Chlorophyll metabolism pathway under stress in senna (*numbers in brackets* indicate number of CDS encoding the enzyme in the pathway

(6), and diacylglycerol *O*-acyltransferase (39) which were identified which form important candidates for improvement of drought tolerance in senna (Table 7).

Genes involved in biosynthesis of polyamines

Polyamines (PAs) (putrescine, spermidine, and spermine) are a group of phytohormone-like aliphatic amine natural compounds with aliphatic nitrogen structure involved in cell growth and development, and respond to stress tolerance to various environmental factors (Gill and Tuteja 2010). We explored the gene encoding the enzymes in the production of polyamines and found that there were 64 CDS encoding for eight enzymes, arginine dacarboxylase (2), agmatine deiminase (2), *S*-adenosylmethionine decarboxylase (8), spermidine synthase (15), spermine oxidase (2), polyamine oxidase (25), and thermospermine synthase (8) regulating the production of PAs in senna (Table 8). However, none were found for agmatinase and *N*-carbamoylputrescine amidase may due to presence very low levels of transcripts in the transcriptome or possibility of being a key enzyme and thus its expression might be tightly regulated.

Protective proteins and transporters

Protective proteins (PP) include heat shock proteins (HSPs), late embryogenesis abundant proteins (LEA), chaperones, osmatin and aquaporins produced in response to drought stress in plants. HSPs are the family of proteins produced in response to stress (Song et al. 2014; Leng et al. 2015). HSPS protect cells from injury and facilitate recovery and survival after a return to normal growth conditions. HSPs function as chaperones involved in protein folding, assembly, translocation, and degradation, and also help to stabilize proteins and membranes (Boston et al. 1996; Lucas et al. 2011a, b). The well-characterized HSPs belong to the HSP70 family. We explored the senna transcriptome and found 79 CDS putatively encoding for heat shock protein. However, the role of these HSPs needs further study. We also identified many CDS encoding protective proteins such as LEA proteins (6), cheperones (236), osmatin (1), and aquaporins (34) and transporter such as Na(+)/H(+) antiporter (17) and auxin efflux carrier (18) (Table 8). Constitutive overexpression of these candidates genes could improve defense against drought stress in plants.

Table 8 Putative genes encoding enzymes involved in biosynthesis of polyamine and protective proteins

Functional category	Pathway	Sr. no.	Enzyme	E.C. no.	No. of CDS
Polyamine	Polyamine biosynthesis	1	Arginine decarboxylase	[EC:4.1.1.19]	2
		2	Agmatinase	[EC:3.5.3.11]	0
		3	Agmatine deiminase	[EC:3.5.3.12]	2
		4	*N*-Carbamoylputrescine amidase	[EC:3.5.1.53]	0
		5	*S*-Adenosylmethionine decarboxylase	[EC:4.1.1.50]	8
		6	Spermidine synthase	[EC:2.5.1.16]	15
		7	Spermine Synthase	[EC:2.5.1.22]	2
		8	Spermine oxidase	[EC:1.5.3.16]/ [EC:1.5.3.-]	2
		9	Polyamine oxidase	[EC:1.5.3.14]/ [EC:1.5.3.16]/ [EC:1.5.3.-]	25
		10	Thermospermine synthase	EC 2.5.1.79	8
Protective proteins	Heat shock	1	Heat Shock Protein	–	79
	Late embryogenesis abundant proteins	1	Late embryogenesis abundant protein	–	6
	Other transporters	1	Na(+)/H(+) antiporter	–	17
		2	Auxin efflux carrier	–	18
		3	choline dehydrogenase	–	0
	Chaperones	1	Chaperones	–	236
	Osmotin	1	Osmotin	–	1
	Aquaporin	1	Aquaporin	–	34

Table 9 List of gene specific primers used for validation of assembly

Gene name	GenBank accession	Primer	Sequence (5′ → 3′)	Location in the gene	Tm (°C)	Expected product size (bp)	Observed product size (bp)	Size of the sanger sequence (bp)
Transcription factor MYC2	KU533732	MycIDCA02-F	TTCTTCAACTCGAG CCCTGT	694–2154	57.3	1461	1500	1329
		MycIDCA02-R	ATCGCTAACCTTGG ACGAAA		55.3			
9-*cis*-Epoxycarotenoid dioxygenase	KU533733	NcedIDCa02-F	TGTCAGATCATACA GGGCTCAC	404–1651	60.3	1248	1200	1140
		NcedIDCa02-R	CACATACCCATCGT CTTCCTCT		60.3			
ʟ-Ascorbate peroxidase	KU533731	ApxIDCa01-F	CTTATCAGGGCTTC CACCAA	403–1775	57.3	1373	1300	1269
		ApxIDCa01-R	CTTATCAGGGCTTC CACCAA		55.3			
1-Aminocyclopropane-1-carboxylate oxidase	KU533730	AccoIDCa01-F	CAGTGGAGAGGTTG ACCAAAGA	143–739	60.3	597	590	577
		AccoIDCa01-R	TGGACATTCTGGTT CCATCAGT		58.4			
(+)-Abscisic acid 8′-hydroxylase	KU366613	Aba8hIDCa02-F	TCAAGACGCATATA TTGGGATG	185–1282	56.5	1098	1100	1043
		Aba8hIDCa02-R	TGTAAGAAATGGTG AGGTGGTG		58.4			
WRKY transcription factor 33	KU533734	WrkyIDCa01-F	TGGACAGAAGCAAG TGAAAGC	316–1529	57.9	1214	1200	1105
		WrkyIDCa01-R	TGACGCATTCATCA ACCAACA		55.9			

Validation of transcriptome

Since transcript assembly needs to be validated and as our interest was in drought stress metabolism, we choose to validate using six genes involved in drought stress (Table 9). The transcripts of all the six abiotic stress genes, viz., transcription factor MYC2, 9-*cis*-epoxycarotenoid dioxygenase, L-ascorbate peroxidase, aminocyclopropanecarboxylate oxidase, (+)-abscisic acid 8′-hydroxylase, and WRKY transcription factor were confirmed by reverse transcription polymerase chain reaction (RT-PCR), as was observed in an agarose gel. The amplicon sizes matched with the expected size of the gene based on assembled transcripts (Fig. 9). Transcription factor MYC2, 9-*cis*-epoxycarotenoid dioxygenase, L-ascorbate peroxidase, aminocyclopropanecarboxylate oxidase, (+)-abscisic acid 8′-hydroxylase, and WRKY transcription factor PCR products were further Sanger sequenced for confirmation, giving ~100 % identity with the assembled transcriptome sequences (File S1). This is the first time that these genes have been identified in *Cassia angustifolia*. The sequences have been deposited at NCBI as mRNA sequences.
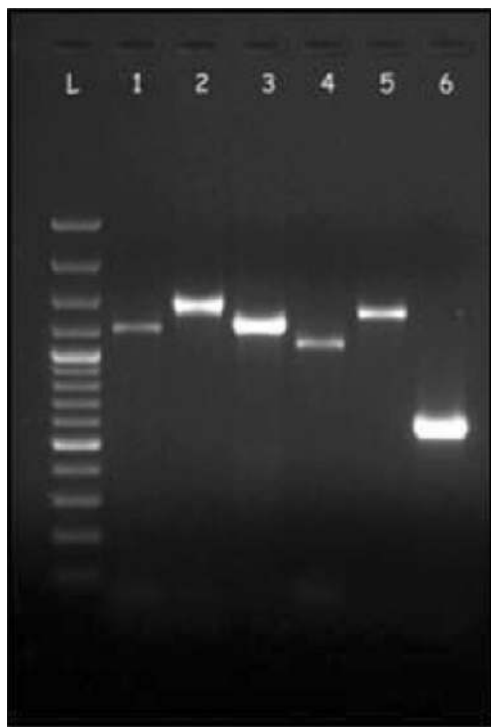
Fig. 9 Detection and validation of different drought-related genes in *Cassia angustifolia*. cDNA was used as template to amplify drought stress-related genes. The amplified fragments were analyzed by 1.5 % agarose gel electrophoresis. DNA ladder containing 14 bands (from down 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1500, 2000, 3000; O'gene ruler 100 bp plus DNA ladder SM1153) was used. Lane *L*: DNA ladder; lane *1*: WrkyIDCa01 (~1200 bp); lane *2*: MycIDCa02 (~1500 bp); lane *3*: NcedIDCa02 (~1300 bp); lane *4*: Aba8hIDCa02 (~1100 bp); lane *5*: ApxIDCa01 (~1400 bp); lane *6*: AccoIDCa01 (~600 bp)

Conclusions

In this study, we performed large-scale transcriptome sequencing of senna, an important drought-tolerant herb cultivated in arid and semi-arid tropics. Insufficient transcriptomic and genomic data in public databases has limited our understanding of the molecular mechanism underlying the drought stress tolerance of senna. More than 200 million reads were generated and assembled into 43,413 unique transcripts which were further extensively annotated by comparing sequences with different databases. Coding DNA sequences (CDS) encoding various drought stress-regulated pathways such as signaling factors, protein-modifying/degrading enzymes, biosynthesis of phytohormone, phytohormone signaling, osmotically active compounds, free radical scavengers, chlorophyll metabolism, leaf cuticular wax, polyamines, and protective proteins were identified through BLASTX search. Six genes (transcription factor MYC2, *9-cis-epoxycarotenoid dioxygenase*, L-*ascorbate peroxidase*, *aminocyclopropane carboxylate oxidase*, (+)-*abscisic acid* 8′-*hydroxylase*, and WRKY transcription factor) encoding enzymes involved in drought stress regulation were confirmed through RT-PCR and Sanger sequencing for the first time in senna. The potential drought stress-related transcripts identified in this study provide a good start for further investigation into the drought adaptation in senna. Additionally, our transcriptome sequences can be a valuable resource for accelerated genomics-assisted genetic improvement programs and facilitate a better understanding and more effective manipulation of biochemical pathways for developing drought-tolerant crop plants.

Authors' contributions Conceived and designed the experiments: NRRR. Performed the experiments: NRRR RHM. Analyzed the data: NRRR MP JK. Contributed reagents/materials/analysis tools: NRRR MP JK. Wrote the paper: RHM NRRR MP JK

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Availability of supporting data The datasets supporting the results of this article are available at the National Center for Biotechnology Information (NCBI) BioProject database (Short Read Archive) under

accession number PRJNA273534 and the Transcriptome Shotgun Assembly (TSA) at DDBJ/EMBL/GenBank under the accession GEEB00000000. The version described in this paper is the first version, GEEB01000000.

References

Abe H, Yamaguchi-Shinozaki K, Urao T, Iwasaki T, Hosokawa D, Shinozaki K (1997) Role of Arabidopsis MYC and MYB homologs in drought- and abscisic acid-regulated gene expression. Plant Cell 9(10):1859–1868

Abulafatih HA (1987) Medicinal plants of Southern Arabia. Econ Bot 41:354–360

Agarwal S, Pandey V (2004) Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Biol Plantarum 48(4):555–560

Akpinar BA, Avsar B, Lucas SJ, Budak H (2012) Plant abiotic stress signalling. Plant Signal Behav 7(11):1450–1455

Akpinar BA, Lucas SJ, Budak H (2013) Genomics approaches for crop improvement against abiotic stress. Sci World J 15:361921

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215(3):403–410

Argueso CT, Hansen M, Kieber JJ (2007) Regulation of ethylene biosynthesis. J Plant Growth Regul 26(2):92–105

Arraes FB, Beneventi MA, Lisei de Sa ME, Paixao JF, Albuquerque EV, Marin SR, Purgatto E, Nepomuceno AL, Grossi-de-Sa MF (2015) Implications of ethylene biosynthesis and signaling in soybean drought stress tolerance. BMC Plant Biol 15:213

Ayoub AT (1977) Some primary features of salt tolerance in senna (*Cassia angastifolia*). J Exp Bot 28:484–492

Bartels D, Sunkar R (2005) Drought and salt tolerance in plants. Cr Rev Plant Sci 24(1):23–58

Beale SI (2005) Green genes gleaned. Trends Plant Sci 10(7):309–312

Bleecker AB, Kende H (2000) Ethylene: a gaseous signal molecule in plants. Annu Rev Cell Dev Biol 16:1–18

Blum A (1988) Plant breeding for stress environments. CRC Press, Inc., Boca Raton

Boatwright JL, Pajerowska-Mukhtar K (2013) Salicylic acid: an old hormone up to new tricks. Mol Plant Pathol 14(6):623–634

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for illumina sequence data. Bioinformatics 30:2114–2120

Boston RS, Viitanen PV, Vierling E (1996) Molecular chaperones and protein folding in plants. In: Filipowicz W, Hohn T (eds) Posttranscriptional control of gene expression in plants. Springer, Dordrecht, pp 191–222

Bowman MJ, Park W, Bauer PJ, Udall JA, Page JT, Raney J, Scheffler BE, Jones DC, Campbell BT (2013) RNA-Seq transcriptome profiling of upland cotton (*Gossypium hirsutum* L.) root tissue under water-deficit stress. PLoS One 8(12):e82634

Budak H, Kantar M, Kurtoglu KY (2013) Drought tolerance in modern and wild wheat. Sci World J 15:548246

Ciftci-Yilmaz S, Mittler R (2008) The zinc finger network of plants. Cell Mol Life Sci 65(7–8):1150–1160

Cutler AJ, Krochko JE (1999) Formation and breakdown of ABA. Trends Plant Sci 4(12):472–478

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: emergence of a core signaling network. Annu Rev Plant Biol 61:651–679

D'Autréaux B, Toledano MB (2007) ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. Nat Rev Mol Cell Biol 8(10):813–824

Daviere JM, Achard P (2013) Gibberellin signaling in plants. Development 140(6):1147–1151

Devoto A, Ellis C, Magusin A, Chang HS, Chilcott C, Zhu T, Turner JG (2005) Expression profiling reveals COI1 to be a key regulator of genes involved in wound- and methyl jasmonate-induced secondary metabolism, defence, and hormone interactions. Plant Mol Biol 58(4):497–513

do Amaral MN, Arge LW, Benitez LC, Danielowski R, Silveira SF, Farias DD, de Oliveira AC, da Maia LC, Braga EJ (2016) Comparative transcriptomics of rice plants under cold, iron, and salt stresses. Funct Integr Genom 16(5):567–579

Do PT, Degenkolbe T, Erban A, Heyer AG, Kopka J, Köhl KI, Hincha DK, Zuther E (2013) Dissecting rice polyamine metabolism under controlled long-term drought stress. PLoS One 8(4):e60325

Dong Y, Fan G, Deng M, Xu E, Zhao Z (2014) Genome-wide expression profiling of the transcriptomes of four *Paulownia tomentosa* accessions in response to drought. Genomics 104(4):295–305

Du H, Wang N, Cui F, Li X, Xiao J, Xiong L (2010) Characterization of the beta-carotene hydroxylase gene DSM2 conferring drought and oxidative stress resistance by increasing xanthophylls and abscisic acid synthesis in rice. Plant Physiol 154(3):1304–1318

Ergen NZ, Budak H (2009) Sequencing over 13 000 expressed sequence tags from six subtractive cDNA libraries of wild and modern wheats following slow drought stress. Plant Cell Environ 32(3):220–236

Ergen NZ, Thimmapuram, Bohnert J, Hans J, Budak H (2009) Transcriptome pathways unique to dehydration tolerant relatives of modern wheat. Funct Integr Genomics 9(3):377–396

Fang Y, You J, Xie K, Xie W, Xiong L (2008) Systematic sequence analysis and identification of tissue-specific or stress-responsive genes of NAC transcription factor family in rice. Mol Genet Genomics 280(6):547–563

Folkard C (1995) Encyclopedia of herbs and their uses. Herb Society of America, Dorling Kindersley Publishing Inc., New York

Gao T, Wu Y, Zhang Y, Liu JL, Ning Y, Wang D, Tong H, Chen S, Chu C, Xie Q (2011) OsSDIR1 overexpression greatly improves drought tolerance in transgenic rice. Plant Mol Biol 76(1–2):145–156

Gao JP, Wang D, Cao LY, Sun HF (2015) Transcriptome sequencing of Codonopsis pilosula and identification of candidate genes involved in polysaccharide biosynthesis. PLoS One 10(2):e0117342

Ghazanfar SA, Al-Sabahi AA (1993) Medicinal plants of northern and central Oman (Arabia). Econ Bot 41:89–98

Gill SS, Tuteja N (2010) Polyamines and abiotic stress tolerance in plants. Plant Signal Behav 5(1):26–33

Gill SS, Anjum NA, Hasanuzzaman M, Gill R, Trivedi DK, Ahmad I, Pereira E, Tuteja N (2013) Glutathione and glutathione reductase: a boon in disguise for plant abiotic stress defense operations. Plant Physiol Biochem 70:204–212

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A (2011) Full length transcriptome assembly from RNA-seq data without a reference genome. Nat Biotechnol 29:644–652

Hammouda FM, Ismail SI, Abdel-Azim NS, Shams KA (2005) A guide to medicinal plants in North Africa. In: Batanouny KH (eds) IUCN Centre for Mediterranean Cooperation, Malaga, Andalusia, Spain, pp 217–218

Hirayama T, Shinozaki K (2010) Research on plant abiotic stress responses in the postgenome era: past, present and future. Plant J 61(6):1041–1052

Hiz MC, Canher B, Niron H, Turet M (2014) Transcriptome analysis of salt tolerant common bean (*Phaseolus vulgaris* L.) under saline conditions. PLoS One 9(3):e92598

Hooker TS, Millar AA, Kunst L (2002) Significance of the expression of the CER6 condensing enzyme for cuticular wax production in Arabidopsis. Plant Physiol 129(4):1568–1580

Hortensteiner S (2006) Chlorophyll degradation during senescence. Annu Rev Plant Biol 57:55–77

Hu H, Xiong L (2014) Genetic engineering and breeding of drought-resistant crops. Annu Rev Plant Biol 65:715–741

Hu T, Sun X, Zhang X, Nevo E, Fu J (2014) An RNA sequencing transcriptome analysis of the high-temperature stressed tall fescue reveals novel insights into plant thermotolerance. BMC Genomics 15:1147

Jiang SY, Bhalla R, Ramamoorthy R, Luan HF, Venkatesh PN, Cai M, Ramachandran S (2012) Over-expression of OSRIP18 increases drought and salt tolerance in transgenic rice plants. Transgenic Res 21(4):785–795

Jin JP, Zhang H, Kong L, Gao G, Luo JC (2014) PlantTFDB 3.0: a portal for the functional and evolutionary study of plant transcription factors. Nucleic Acids Res 42(D1):1182–1187

Kantar M, Lucas SJ, Budak H (2011) Drought stress: molecular genetics and genomics approaches. Adv Bot Res 57:445–493

Kawano T, Furuichi T, Muso S (2004) Controlled free salicylic acid levels and corresponding signaling mechanisms in plants. Plant Biotechnol 21:319–335

Khalid H, Abdalla WE, Abdelgadir H, Opatz T, Effert T (2012) Gems from traditional north-African medicine: medicinal and aromatic plants from Sudan. Nat Prod Bioprospect 2(3):92–103

Khammari I, Galavi M, Ghanbari A, Solouki M, Poorchaman MRA (2012) The effect of drought stress and nitrogen levels on antioxidant enzymes, proline and yield of Indian Senna (*Cassia angustifolia* L.). J Med Plants Res 6(11):2125–2130

Khan NA, Nazar R, Iqbal N, Anjum NA (2012) Phytohormones and abiotic stress tolerance in plants. Springer, Berlin

Kim J, Jung JH, Lee SB, Go YS, Kim HJ, Cahoon R, Markham JE, Cahoon EB, Suh MC (2013) Arabidopsis 3-ketoacyl-coenzyme a synthase9 is involved in the synthesis of tetracosanoic acids as precursors of cuticular waxes, suberins, sphingolipids, and phospholipids. Plant Physiol 162(2):567–580

Kohli A, Sreenivasulu N, Lakshmanan P, Kuman PP (2013) The phytohormone crosstalk paradigm takes center stage in understanding how plants respond to abiotic stresses. Plant Cell Rep 32(7):945–957

Krugman T, Chagué V, Peleg Z, Balzergue S, Just J, Korol AB, Nevo E, Saranga Y, Chalhoub B, Fahima T (2010) Multilevel regulation and signalling processes associated with adaptation to terminal drought in wild emmer wheat. Funct Integr Genomics 10(2):167–186

Kumari S, Joshi R, Singh K, Roy S, Tripathi AK, Singh P, Singla-Pareek SL, Pareek A (2015) Expression of a cyclophilin OsCyp2-P isolated from a salt-tolerant landrace of rice in tobacco alleviates stress via ion homeostasis and limiting ROS accumulation. Funct Integr Genomics 15(4):395–412

Kusaba M, Tanaka A, Tanaka R (2013) Stay-green plants: what do they tell us about the molecular mechanism of leaf senescence. Photosynth Res 117(1–3):221–234

Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T, Hirai N, Koshiba T, Kamiya Y, Nambara E (2004) The Arabidopsis cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. EMBO J 23(7):1647–1656

Kuzuoglu-Ozturk D, Cebeci Yalcinkaya O, Akpinar BA, Mitou G, Korkmaz G, Gozuacik D, Budak H (2012) Autophagy-related gene, TdAtg8, in wild emmer wheat plays a role in drought and osmotic stress response. Planta 236(4):1081–1092

Lemli J (1986) The chemistry of senna. Fitoterapia 57:33–40

Leng X, Mu Q, Wang X, Li X, Zhu X, Shangguan L, Fang J (2015) Transporters, chaperones, and P-type ATPases controlling grapevine copper homeostasis. Funct Integr Genomics 15(6):673–684

Levitt J (1980) Responses of plants to environmental stress: chilling, freezing and high temperature stresses, vol 1, 2nd edn. Academic, New York

Li WX, Oono Y, Zhu J, He XJ, Wu JM, Iida K, Lu XY, Cui X, Jin H, Zhu JK (2008) The Arabidopsis NFYA5 transcription factor is regulated transcriptionally and post-transcriptionally to promote drought resistance. Plant Cell 20(8):2238–2251

Li MY, Tan HW, Wang F, Jiang Q, Xu ZS, Tian C, Xiong AS (2014) *De novo* transcriptome sequence assembly and identification of AP2/ERF transcription factor related to abiotic stress in parsley (*Petroselinum crispum*). PLoS One 9(9):e108977

Li PS, Yu TF, He GH, Chen M, Zhou YB, Chai SC, Xu ZS, Ma YZ (2014) Genome-wide analysis of the Hsf family in soybean and functional identification of GmHsf-34 involvement in drought and heat stresses. BMC Genom 15:1009

Lim GH, Zhang X, Chung MS, Lee DJ, Woo YM, Cheong HS, Kim CS (2010) A putative novel transcription factor, AtSKIP, is involved in abscisic acid signalling and confers salt and osmotic tolerance in Arabidopsis. New Phytol 185(1):103–113

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. Plant Cell 10(8):1391–1406

Liu Z, Song T, Zhu Q, Wang W, Zhou J, Liao H (2014) *De novo* assembly and analysis of *Cassia obtusifolia* seed transcriptome to identify genes involved in the biosynthesis of active metabolites. Biosci Biotechnol Biochem 78(5):791–799

Liu H, Che Z, Zeng X, Zhou X, Sitoe HM, Wang H, Yu D (2016) Genome-wide analysis of calcium-dependent protein kinases and their expression patterns in response to herbivore and wounding stresses in soybean. Funct Integr Genom 16(5):481–493

Long L, Gao W, Xu L, Liu M, Luo X, He X, Yang X, Zhang X, Zhu L (2014) GbMPK3, a mitogen-activated protein kinase from cotton, enhances drought and oxidative stress tolerance in tobacco. Plant Cell Tiss Organ Cult 116:153–162

Lucas S, Dogan E, Budak H (2011a) TMPIT1 from wild emmer wheat: first characterisation of a stress-inducible integral membrane protein. Gene 483(1):22–28

Lucas S, Durmaz E, Akpınar BA, Budak H (2011b) The drought response displayed by a DRE-binding protein from *Tritium dicoccoides*. Plant Physiol Biochem 49(3):346–351

Manavalan LP, Chen X, Clarke J, Salmeron J, Nguyen HT (2012) RNAi-mediated disruption of squalene synthase improves drought tolerance and yield in rice. J Exp Bot 63(1):163–175

Min XJ, Butler G, Storms R, Tsang A (2005) OrfPredictor: predicting protein-coding regions in EST-derived sequences. Nucleic Acids Res. Web Server Issue W677–W680. (http://bioinformatics.ysu.edu/tools/OrfPredictor.html)

Mockaitis K, Estelle M (2008) Auxin receptors and plant development: a new signaling paradigm. Annu Rev Cell Dev Biol 24:55–80

Moriya Y, Itoh M, Okuda S, Yoshizawa A, Kanehisa M (2007) KAAS: an automatic genome annotation and pathway reconstruction server. Nucleic Acids Res 35:W182–W185

Munné-Bosch S, Queval G, Foyer CH (2013) The impact of global change factors on redox signaling underpinning stress tolerance. Plant Physiol 161(1):5–19

Mustafa NR, Kim HK, Choi YH, Erkelens C, Lefeber AW, Spijksma G, van der Heijden R, Verpoorte R (2009) Biosynthesis of salicylic acid in fungus elicited *Catharanthus roseus* cells. Phytochemistry 70(4):532–539

Nakashima K, Takasaki H, Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2012) NAC transcription factors in plant abiotic stress responses. Biochim Biophys Acta 1819(2):97–103

Ning Y, Jantasuriyarat C, Zhao Q, Zhang H, Chen S, Liu J, Liu L, Tang S, Park CH, Wang X, Liu X, Dai L, Xie Q, Wang GL (2011) The SINA E3 ligase OsDIS1 negatively regulates drought response in rice. Plant Physiol 157(1):242–255

Pandey N, Ranjan A, Pant P, Tripathi RK, Ateek F, Pandey HP, Patre UV, Sawant SV (2013) CAMTA 1 regulates drought responses in *Arabidopsis thaliana*. BMC Genomics 14:216

Park GG, Park JJ, Yoon J, Yu SN, An G (2010) A RING finger E3 ligase gene, *Oryza sativa* delayed seed germination 1 (OsDSG1), controls seed germination and stress responses in rice. Plant Mol Biol 74(4–5):467–478

Peleg Z, Blumwald E (2011) Hormone balance and abiotic stress tolerance in crop plants. Curr Opin Plant Biol 14(3):290–295

Qureshi MI, Abdin MZ, Qadir S, Iqbal M (2007) Lead-induced oxidative stress and metabolic alterations in *Cassia angustifolia* Vahl. Biol Plantarum 51(1):121–128

Rama Reddy NR, Ragimasalawada M, Sabbavarapu MM, Nadoor S, Patil JV (2014) Detection and validation of stay-green QTL in post-rainy sorghum involving widely adapted cultivar, M35-1 and a popular stay-green genotype B35. BMC Genom 18(15):909

Rama Reddy NR, Mehta RH, Soni PH, Makasana J, Gajbhiye NA, Ponnuchamy M, Kumar J (2015) Next generation sequencing and transcriptome analysis predicts biosynthetic pathway of sennosides from senna (*Cassia angustifolia* Vahl.), a non-model plant with potent laxative properties. PLoS One 10(6):e0129422

Ramchandra Reddy A, Chaitanya KV, Vivekanandan M (2004) Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. J Plant Physiol 161(11):1189–1202

Raney JA, Reynolds DJ, Elzinga DB, Page J, Udall JA, Jellen EN, Bonfacio A, Fairbanks DJ, Maughan PJ (2014) Transcriptome analysis of drought induced stress in *Chenopodium quinoa*. Am J Plant Sci 5(3):338–357

Ratnayaka HH, Kincaid D (2005) Gas exchange and leaf ultrastructure of tinnevelly senna, *cassia angustifolia*, under drought and nitrogen stress. Crop Sci 45(3):840–847

Ren X, Chen Z, Liu Y, Zhang H, Zhang M, Liu Q, Hong X, Zhu J, Gong Z (2010) ABO3, a WRKY transcription factor, mediates plant responses to abscisic acid and drought tolerance in Arabidopsis. Plant J 63(3):417–429

Richards DE, King KE, Ait-Ali T, Harberd NP (2001) How gibberellin regulates plant growth and development: a molecular genetic analysis of gibberellins signaling. Annu Rev Plant Physiol Plant Mol Biol 52:67–88

Rocheta M, Becker JD, Coito JL, Carvalho L, Amâncio S (2014) Heat and water stress induce unique transcriptional signatures of heat-shock proteins and transcription factors in grapevine. Funct Integr Genomics 14(1):135–148

Rodriguez MC, Petersen M, Mundy J (2010) Mitogen-activated protein kinase signaling in plants. Annu Rev Plant Biol 61:621–649

Roje S (2006) S-adenosyl-L-methionine: beyond the universal methyl group donor. Phytochemistry 67(15):1686–1698

Ryu MY, Cho SK, Kim WT (2010) The Arabidopsis C3H2C3-type RING E3 ubiquitin ligase AtAIRP1 is a positive regulator of an abscisic acid-dependent response to drought stress. Plant Physiol 154(4):1983–1997

Saito S, Hirai N, Matsumoto C, Ohigashi H, Ohta D, Sakata K, Mizutani M (2004) Arabidopsis CYP707As encode (+)-abscisic acid 8′-hydroxylase, a key enzyme in the oxidative catabolism of abscisic acid. Plant Physiol 134(4):1439–1449

Santner A, Calderon-Villalobos LI, Estelle M (2009) Plant hormones are versatile chemical regulators of plant growth. Nat Chem Biol 5(5):301–307

Schroeder JI, Allen GJ, Hugouvieux V, Kwak JM, Waner D (2001) Guard cell signal transduction. Annu Rev Plant Physiol Plant Mol Biol 52:627–658

Seki M, Umezawa T, Urano K, Shinozaki K (2007) Regulatory metabolic networks in drought stress responses. Curr Opin Plant Biol 10(3):296–302

Shakeel SN, Wang X, Binder BM, Schaller GE (2013) Mechanisms of signal transduction by ethylene: overlapping and non-overlapping signalling roles in a receptor family. AoB Plants 5:plt010

Shang J, Song P, Ma B, Qi X, Zeng Q, Xiang Z, He N (2014) Identification of the mulberry genes involved in ethylene biosynthesis and signaling pathways and the expression of MaERF-B2-1 and MaERF-B2-2 in the response to flooding stress. Funct Integr Genomics 14(4):767–777

Shanker AK, Maheswari M, Yadav SK, Desai S, Bhanu D, Attal NB, Venkateswarlu B (2014) Drought stress responses in crops. Funct Integr Genomics 14(1):11–22

Sharma S, Villamor JG, Verslues PE (2011) Essential role of tissue-specific proline synthesis and catabolism in growth and redox balance at low water potential. Plant Physiol 157(1):292–304

Shi H, Wang X, Ye T, Chen F, Deng J, Yang P, Zhang Y, Chan Z (2014) The cysteine2/histidine2-type transcription factor ZINC FINGER OF ARABIDOPSIS THALIANA6 modulates biotic and abiotic stress responses by activating salicylic acid-related genes and C-REPEAT-BINDING FACTOR Genes in Arabidopsis. Plant Physiol 165(3):1367–1379

Singh R, Kumar R, Mahato AK, Paliwal R, Singh AK, Kumar S, Marla SS, Kumar A, Singh NK (2016) *De novo* transcriptome sequencing facilitates genomic resource generation in *Tinospora cordifolia*. Funct Integr Genomics 16(5):581–591

Song A, Zhu X, Chen F, Gao H, Jiang J, Chen S (2014) A chrysanthemum heat shock protein confers tolerance to abiotic stress. Int J Mol Sci 15(3):5063–5078

Song L, Jiang L, Chen Y, Shu Y, Bai Y, Guo C (2016) Deep-sequencing transcriptome analysis of field-grown *Medicago sativa* L. crown buds acclimated to freezing stress. Funct Integr Genom 16(5):495–511

Sreedhar RV, Kumari P, Rupwate SD, Rajasekharan R, Srinivasan M (2015) Exploring triacylglycerol biosynthetic pathway in developing seeds of Chia (*Salvia hispanica* L.): a transcriptomic approach. PLoS One 10(4):e0123580

Su LT, Li JW, Liu DQ, Zhai Y, Zhang HJ, Li XW, Zhang QL, Wang Y, Wang QY (2014) A novel MYB transcription factor, GmMYBJ1, from soybean confers drought and cold tolerance in *Arabidopsis thaliana*. Gene 538(1):46–55

Sun R, Wang K, Guo T, Jones DC, Cobb J, Zhang B, Wang Q (2015a) Genome-wide identification of auxin response factor (ARF) genes and its tissue-specific prominent expression in *Gossypium raimondii*. Funct Integr Genomics 15(4):481–493

Sun W, Chen H, Wang J, Sun HW, Yang SK, Sang YL, Lu XB, Xu XH (2015b) Expression analysis of genes encoding mitogen-activated protein kinases in maize provides a keylink between abiotic stress signaling and plant reproduction. Funct Integr Genomics 15(1):107–120

Sun X, Xie Z, Zhang C, Mu Q, Wu W, Wang B, Fang J (2016) A characterization of grapevine of GRAS domain transcription factor gene family. Funct Integr Genomics 16(4):347–363

Swamy PM, Smith BN (1999) Role of abscisic acid in plant stress tolerance. Curr Sci 76:1220–1227

Thomas H, Ougham H (2014) The stay-green trait. J Exp Bot 65(14):3889–3900

Thumma BR, Sharma N, Southerton SG (2012) Transcriptome sequencing of *Eucalyptus camaldulensis* seedlings subjected to water stress reveals functional single nucleotide polymorphisms and genes under selection. BMC Genomics 13:364

Tran LS, Nakashima K, Sakuma Y, Osakabe Y, Qin F, Simpson SD, Maruyama K, Fujita Y, Shinozaki K, Yamaguchi-Shinozaki K (2007) Co-expression of the stress-inducible zinc finger homeodomain ZFHD1 and NAC transcription factors enhances expression of the ERD1 gene in Arabidopsis. Plant J 49(1):46–63

Turner JG, Ellis C, Devoto A (2002) The jasmonate signal pathway. Plant Cell 14(Suppl):S153–S164

Umezawa T, Fujita M, Fujita Y, Yamaguchi-Shinozaki K, Shinozaki K (2006) Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. Curr Opin Biotechnol 17(2):113–122

Van Eck L, Davidson RM, Wu S, Zhao BY, Botha AM, Leach JE, Lapitan NL (2014) The transcriptional network of WRKY53 in cereals links

oxidative responses to biotic and abiotic stress inputs. Funct Integr Genomics 14(2):351–362

Venkatesh J, Park SW (2014) Role of L-ascorbate in alleviating abiotic stresses in crop plants. Botan Stud 55:38

Wang S, Bai Y, Shen C, Wu Y, Zhang S, Jiang D, Guilfoyle TJ, Chen M, Qi Y (2010) Auxin-related gene families in abiotic stress response in *Sorghum bicolor*. Funct Integr Genomics 10(4):533–546

Wang Z, Jia C, Li J, Huang S, Xu B, Jin Z (2015) Activation of salicylic acid metabolism and signal transduction can enhance resistance to Fusarium wilt in banana (*Musa acuminata* L. AAA group, cv. Cavendish). Funct Integr Genomics 15(1):47–62

Wang YN, Tang L, Hou Y, Wang P, Yang H, Wei CL (2016) Differential transcriptome analysis of leaves of tea plant (*Camellia sinensis*) provides comprehensive insights into the defense responses to *Ectropis oblique* attack using RNA-Seq. Funct Integr Genomics 16(4):383–398

Wei S, Hu W, Deng X, Zhang Y, Liu X, Zhao X, Luo Q, Jin Z, Li Y, Zhou S, Sun T, Wang L, Yang G, He G (2014) A rice calcium-dependent protein kinase OsCPK9 positively regulates drought stress tolerance and spikelet fertility. BMC Plant Biol 14:133

Wu G, Zhang L, Yin Y, Wu J, Yu L, Zhou Y, Li M (2015) Sequencing, *de novo* assembly and comparative analysis of *Raphanus sativus* transcriptome. Front Plant Sci 6:198

Wu ZJ, Li XH, Liu ZW, Li H, Wang YX, Zhuang J (2016) Transcriptome-based discovery of AP2/ERF transcription factors related to temperature stress in tea plant (*Camellia sinensis*). Funct Integr Genomics 15(6):741–752

Xiong H, Li J, Liu P, Duan J, Zhao Y, Guo X, Li Y, Zhang H, Ali J, Li Z (2014) Overexpression of OsMYB48-1, a novel MYB-related transcription factor, enhances drought and salinity tolerance in rice. PLoS One 9(3):e92913

Xu Y, Gao S, Yang Y, Huang M, Cheng L, Wei Q, Fei Z, Gao J, Hong B (2013) Transcriptome sequencing and whole genome expression profiling of chrysanthemum under dehydration stress. BMC Genomics 14:662

Xu A, Zhang W, Wen CK (2014a) Enhancing ctr1-10 ethylene response2 is a novel allele involved in constitutive triple-response 1-mediated ethylene receptor signaling in Arabidopsis. BMC Plant Biol 14:48

Xu DB, Gao SQ, Ma YZ, Xu ZS, Zhao CP, Tang YM, Xy L, Li LC, Chen YF, Chen M (2014b) ABI-like transcription factor gene TaABL1 from wheat improves multiple abiotic stress tolerances in transgenic plants. Funct Integr Genomics 14(4):717–730

Yamaguchi-Shinozaki K, Shinozaki K (2006) Transcriptional regulatory networks in cellular response and tolerance to dehydration and cold stresses. Annu Rev Plant Biol 57:781–803

Yang W, Kong Z, Omo-Ikerodah E, Xu W, Li Q, Xue Y (2008) Calcineurin B-like interacting protein kinase OsCIPK23 functions in pollination and drought stress responses in rice (*Oryza sativa* L.). J Genet Genomics 35(9):531–543, S1-2

Zhang J, Mao Z, Chong K (2013) A global profiling of uncapped mRNAs under cold stress reveals specific decay patterns and endonucleolytic cleavages in *Brachypodium distachyon*. Genome Biol 14(8):R92

Zhang F, Yao J, Ke J, Zhang L, Lam VQ, Xin XF, Zhou XE, Chen J, Brunzelle J, Griffin PR, Zhou M, Xu HE, Melcher K, He SY (2015a) Structural basis of JAZ repression of MYC transcription factors in jasmonate signalling. Nature 525(7568):269–273

Zhang L, Hu W, Wang Y, Feng R, Zhang Y, Liu J, Jia C, Miao H, Zhang J, Xu B, Jin Z (2015b) The MaASR gene as a crucial component in multiple drought stress response pathways in Arabidopsis. Funct Integr Genomics 15(2):247–260

Zhang X, Allan AC, Li C, Wang Y, Yao Q (2015c) *De novo* assembly and characterization of the transcriptome of the Chinese medicinal herb, *Gentiana rigescens*. Int J Mol Sci 16(5):11550–11573

Zhao J, Gao Y, Zhang Z, Chen T, Guo W, Zhang T (2013) A receptor-like kinase gene (GbRLK) from *Gossypium barbadense* enhances salinity and drought-stress tolerance in Arabidopsis. BMC Plant Biol 13:110

Zhong L, Chen D, Min D, Li W, Xu Z, Zhou Y, Li L, Chen M, Ma Y (2015) AtTGA4, a bZIP transcription factor, confers drought resistance by enhancing nitrate transport and assimilation in *Arabidopsis thaliana*. Biochem Biophys Res Commun 457(3):433–439

Zhou Y, Gao F, Liu R, Feng J, Li H (2012) *De novo* sequencing and analysis of root transcriptome using 454 pyrosequencing to discover putative genes associated with drought tolerance in *Ammopiptanthus mongolicus*. BMC Genomics 13:266

Zhou X, Li L, Xiang J, Gao G, Xu F, Liu A, Zhang X, Peng Y, Chen X, Wan X (2015) OsGL1-3 is involved in cuticular wax biosynthesis and tolerance to water deficit in rice. PLoS One 10(1):e116676

Zhu H, Dardick CD, Beers EP, Callanhan AM, Xia R, Yuan R (2011) Transcriptomics of shading-induced and NAA-induced abscission in apple (*Malus domestica*) reveals a shared pathway involving reduced photosynthesis, alterations in carbohydrate transport and signaling and hormone crosstalk. BMC Plant Biol 11:138